A simple, rapid, reliable and accurate HPTLC method has been developed for the estimation of Nabumetone in pharmaceutical dosage form. The drug was extracted from (NILTIS* 500). Various aliquots of this sample solution were spotted automatically by means of Camag (Muttenz, Switzerland) Linomat V applicator on Merck HPTLC plates (10.2 mm thickness) precoated with silica gel 60 F254 on aluminum sheet as stationary phase prewashed with methanol using toluene:acetonitrile:triethylamine 9.5:0.5:0.1 (v/v/v) as mobile phase. The spots were scanned at λ =229 nm using Camag TLC scanner 3. The R value of Nabumetone was 0.54. Calibration curves were linear in the range of 200-1200 ng/spot with regression coefficient of 0.999. The limit of detection and quantitation were found to be 15.59 and 47.27 respectively. The suitability of this method for quantitative determination of compound was proved by validation in accordance with requirement of pharmaceutical regulatory standards. Therefore this proposed method was applied for routine analysis of this drug in pharmaceutical dosage form.

Keywords: Nabumetone, HPTLC, Pharmaceutical dosage form.

INTRODUCTION

Nabumetone (NAB), 4-(6-methoxy napthalen-2yl) butane 2-one is a non-steroidal anti-inflammatory drug from a class of 2, 6 disubstituted napthyl- alkanes. The real action is exerted by its active metabolite 6-methoxy 2-napthylacetic acid (6-MNA) which more strongly inhibits the action of cox-2 than that of cox-1. This metabolite is almost entirely conjugated and only ≤ 1 % of the active form is excreted with urine\(^1\). It is used for acute and chronic treatment of signs and symptoms of osteoarthritis and rheumatoid arthritis. The drug is official in USP and EP. Literature survey reveals a few HPLC methods reported for the determination of Nabumetone in biological fluids, tablet formulation and some other HPLC methods for stability studies\(^2\-10\). No HPTLC method has been reported for estimation of Nabumetone in pharmaceutical dosage form. In recent years, advances in HPTLC have not only resulted in the methods which are convenient, fast, robust and cost effective but also the methods which are reproducible, accurate and reliable. Keeping this point into consideration, an attempt was made to develop a simple, accurate HPTLC method for the analysis of Nabumetone in bulk and pharmaceutical dosage form.

MATERIALS AND METHODS

Instrumentation:

Camag (Muttenz, Switzerland) Linomat V applicator, Camag twin trough TLC chamber, Camag TLC scanner 3, Camag winCATS Software (Version 1.3.0) and Hamilton syringe (100 µl).

Material and Reagents:

A pure gift sample of nabumetone from Matrix Laboratories Ltd, Secundrabad, India was used as working standard. Toluene and acetonitrile of HPLC grade (Merck Chem.) and Triethylamine Analytical Reagent grade (S.D. Fine. chem. Ltd.) to prepare the mobile phase were used.

Standard Stock Solution:

Standard stock solution containing 1 mg/ml of nabumetone was prepared by dissolving 10 mg standard NAB in 10 ml of acetonitrile and used as working standard solution.

Sample Preparation:

Twenty NILTIS* 500 manufactured by IPCA Lab. containing 500 mg of Nabumetone were weighed and powdered. An amount of powder equivalent to 10 mg of NAB was transferred to 50 ml calibrated volumetric flask. After addition of 40 ml of acetonitrile and sonication (20 min), the solution was made up to volume with the same solvent and filtered. A sample solution (1 µl containing 200 ng of Nabumetone) was spotted for assay of Nabumetone.
**Chromatography:**

Chromatography was performed on 10 x 20 cm aluminum backed silica gel 60 F<sub>254</sub> HPTLC plates (Merck, Darmstadt, Germany). Before use, the plates were washed with methanol and dried in an oven at 50 °C for 5 min. Samples were applied as 6 mm bands by spraying at rate of 15 µl/s by means of Camag Linomat V sample applicator equipped with a 100 µl syringe (Hamilton, Reno, Nevada, USA), the distance between the bands was 15.4 mm. Ascending development of the plate, migration distance 80 mm, was performed at 25 ± 2 °C with toluene-acetonitrile-triethylamine (9.5:0.5:0.1 v/v/v) as mobile phase in a Camag twin-trough chamber previously saturated for 20 min. The average development time was 20 min. Densitometric scanning was then performed with a Camag TLC scanner 3 equipped with winCATS Software (Version 1.3.0) at λ<sub>max</sub> 229 nm using Deuterium light source, the slit dimensions were 6.00 x 0.45 mm.

**RESULTS AND DISCUSSION**

**Validation of HPTLC method**

**Linearity:**

Amount of standard solution equivalent to 200-1200 ng/spot of NAB was spotted on the prewashed TLC plates. The plates were developed, dried and scanned as described above. The calibration plot was constructed by plotting peak areas against the corresponding concentrations (ng/spot) of NAB. The linearity of response for NAB assessed in the concentration range 200-1200 ng/spot in terms of slope, intercept and correlation coefficient values. Each concentration was spotted six times on the TLC plate. The calibration plot showed the correlation coefficient (r<sup>2</sup> = 0.999), intercept was (5.838) and slope was (703.3) over the concentration range of 200-1200 ng/spot (Fig. 1).

**Sensitivity:**

The sensitivity of measurement of nabumetone by the use of proposed method was estimated in the terms of Limit of detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ were calculated by the use of equation LOD = 3.3 x ASD/S and LOQ = 10 x ASD/S, where S is slope of calibration curve and ASD is the average standard deviation of the peak areas of the drug taken as measure for noise. The LOD and LOQ for nabumetone were 15.59 and 47.27 respectively.

**Accuracy:**

The accuracy of the method was determined by the use of standard addition at three different levels. The pre analyzed sample solution of 400 ng/spot of NAB was spiked with extra amount equivalent to 80 %, 100 % and 120 % of the standard nabumetone and the mixtures were analyzed by the proposed method. The experiment was conducted in triplicate. Result of recovery was determined by taking the difference between the areas of unfortified and fortified samples. When these solutions were analyzed the recoveries were found to be within acceptable limits (Table 1).

**Specificity:**

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for nabumetone in sample was confirmed by comparing the R<sub>f</sub> and spectra of the spot.

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Initial amount (ng)</th>
<th>Amount added (%)</th>
<th>Amount added (ng)</th>
<th>Amount Recovered (ng) (Mean ± SD, % RSD)</th>
<th>% Recovery (Mean ± SD, % RSD)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>400</td>
<td>80</td>
<td>320</td>
<td>320.87±0.7121, 0.2219</td>
<td>100.27± 0.2221, 0.2215</td>
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<tr>
<td>2</td>
<td>400</td>
<td>100</td>
<td>400</td>
<td>401.67±0.5940, 0.1479</td>
<td>100.41± 0.1490, 0.1484</td>
</tr>
<tr>
<td>3</td>
<td>400</td>
<td>120</td>
<td>480</td>
<td>480.41±7.3433, 1.5284</td>
<td>100.09±1.5304, 1.5290</td>
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</tbody>
</table>

n is the number of repetitions, SD- Standard deviation, RSD- Relative standard deviation.
with that of standard (Fig. 2). The peak purity of nabumetone was assessed by comparing the spectra at three different levels, i.e. peak start, peak apex and peak end position of the spot.

**Precision:**
The intra-day and inter-day variation for the determination of NAB was carried out at three different concentration levels 400, 600, 800 ng/spot. Intra-day variations were assessed by analyzing these concentrations in triplicate within a day and inter-day variation was assessed by using the same concentration of drug and analyzing it for three different days, over a period of week as shown in (Table 2).

**Analysis of Marketed Formulation:**
One microlitre of sample solution of the marketed formulation was spotted on to the same plate followed by development scanning. The analysis was repeated five times. The content of drug was calculated from the peak areas recorded (Table 3). The low % RSD value indicated the suitability of this method for routine analysis of nabumetone in pharmaceutical dosage form.

**CONCLUSION**
The method was developed for the first time on HPTLC to determine the drug in formulation, in order to analyze more samples at a time. The method described for the determination of Nabumetone in pharmaceutical dosage form is very simple, rapid and provides accurate and precise results.

**ACKNOWLEDGEMENTS**
The authors express their gratitude to Matrix Laboratories Ltd. Secundrabad, India for providing gift sample of Nabumetone and thanks to the principal, R. C. Patel college of Pharmacy, Shirpur 425405, Dist.-Dhule, (M.S.) India for providing necessary facilities.

**REFERENCES**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc. (ng)</th>
<th>Mean of conc. found (ng)</th>
<th>SD %</th>
<th>RSD</th>
<th>Mean of conc. found (ng)</th>
<th>SD %</th>
<th>RSD</th>
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<tbody>
<tr>
<td>Nabumetone</td>
<td>400</td>
<td>399.4</td>
<td>1.2430</td>
<td>0.3112</td>
<td>398.9</td>
<td>1.7898</td>
<td>0.2955</td>
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<tr>
<td></td>
<td>600</td>
<td>604.5</td>
<td>1.3476</td>
<td>0.2221</td>
<td>603.2</td>
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<td></td>
<td>800</td>
<td>794.2</td>
<td>2.9178</td>
<td>0.3673</td>
<td>793.9</td>
<td>1.0535</td>
<td>0.1327</td>
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</table>

n is number of repetitions,

<table>
<thead>
<tr>
<th>Brand</th>
<th>Conc. (ng)</th>
<th>Conc. found (ng)</th>
<th>% of drug found</th>
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<tbody>
<tr>
<td>NILTIS* 500</td>
<td>200</td>
<td>199.86</td>
<td>99.93</td>
</tr>
<tr>
<td></td>
<td>200</td>
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<tr>
<td></td>
<td>200</td>
<td>199.82</td>
<td>99.91</td>
</tr>
</tbody>
</table>

Mean* assay (%) ± SD 99.93 ± 0.0397
% RSD, SE 0.0397, 0.0229

* Average of % of drug found, SE- Standard error.


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