

## Simultaneous Estimation of Naproxen Sodium and Domperidone Maleate in Bulk and Pharmaceutical Dosage Form by Modified RP-HPLC Method

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### ABSTRACT

A modified RP-HPLC method with UV detection has been developed and validated for the simultaneous estimation of Naproxen sodium (NAP) and Domperidone maleate (DOM) in bulk and pharmaceutical dosage form. Chromatography was carried out on a sunfire C<sub>18</sub> column (5 µm, 250 mm x 4.6 mm, i.d.) using a mixture of phosphate buffer (pH 6.5 adjusted with orthophosphoric acid) and acetonitrile in the ratio of 50:50 as the mobile phase at a flow rate of 1ml/min. The UV detection wavelength was 274 nm. The two drugs satisfactorily separated with retention time 2.63 min, and 4.27 min for DOM and NAP, respectively. The calibration curves were linear over the range of 2.54 - 15.264 µg/ml for DOM and 50 - 300 µg/ml for NAP, with significant high value of correlation coefficient (>0.999 for both drugs). The percentage recovery value for NAP 100.52% and for DOM was 98.12%.

**Keywords:** Naproxen Sodium, Domperidone Maleate, RP- HPLC, Method development, Validation.

### INTRODUCTION

Domperidone maleate (DOM) is chemically 5-chloro- 1-[1-[3-(2-oxo-1, 3-dihydro benzimidazole-1-yl) propyl]-4-piperidyl]-1, 3-dihydro benzimidazole-2-one maleate (Fig 1) is a potent dopamine antagonist used for treatment of nausea and vomiting. Domperidone does not cross the blood brain barrier and therefore has fewer adverse CNS effects than other dopamine antagonist<sup>1, 2</sup>. Naproxen sodium (NAP) is chemically 2-(6-methoxy naphthalen-2-yl) propanoic acid (Fig 2) is a potent non steroidal anti inflammatory drug (NSAID) commonly used for the reduction of moderate to severe pain, fever, inflammation and stiffness. It works by inhibiting the COX-1 and COX-2 enzymes<sup>3,4</sup>. A novel formulation commercially available in combination of NAP 250 mg and DOM 10 mg is commercially available in Indian market for treatment of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, fever and prevent some of the gastro-intestinal problems that NSAIDs can cause.

Literature survey reveals different methods have been reported for analysis of DOM by Spectrophotometry<sup>2,5,6</sup>, Spectrofluorimetry<sup>7</sup>, and HPLC<sup>8-14</sup> and HPTLC<sup>15-17</sup> and for NAP, the methods reported are Spectrophotometry<sup>18, 19</sup>, Spectrofluorimetry<sup>20</sup> HPLC<sup>21-23</sup> and HPTLC<sup>24</sup>, either alone or in combination with other drugs. There are fewer HPLC<sup>25, 26</sup> and HPTLC<sup>27</sup> methods are reported for the simultaneous analysis of NAP and DOM in their combined dosage form. However the existing analytical methods are time-consuming, inaccurate and do not allow the simultaneous determination of the drugs. For these reasons there is increasing need for the development of well validated, accurate, time-saving, economic, modern method for, multi component analysis.

### EXPERIMENTAL

#### Chemicals

Working standards of pharmaceutical grade DOM and NAP were obtained as generous gifts from Kaushikh Therapeutics Pvt., Ltd., Chennai

(Tamilnadu, India). It was used without further purification and certified to contain 99.80 % and 99.85 % (w/w) on dry weight basis of DOM and NAP respectively. Fixed dose combination tablet containing Domperidone maleate equivalent to Domperidone 10 mg and Naproxen Sodium 250 mg was purchased from local Indian market. Potassium dihydrogen phosphate, dipotassium hydrogen phosphate, HPLC grade water and acetonitrile were purchased from Merck chemicals, Mumbai, India.

### Instrumentation

The high performance liquid chromatographic experimentation were performed on waters LC E2629 auto sampler system equipped with 2489 UV - visible detector. Data acquisition and processing were performed using empower2 software. Chromatographic separation was achieved on sunfire C<sub>18</sub> (5 µm, 250 mm x 4.6 mm) analytical column.

### Preparation of Standard Stock Solutions

Reference standard of DOM 12.72 mg and NAP 250 mg was transferred to 250 ml of volumetric flask and dissolved with mobile phase (acetonitrile: phosphate buffer pH 6.5). The flask was sonicated for 30 min and made up the volume with mobile phase to obtain standard stock solution, the concentration was found to be 50.88 µg/ml and 1000 µg/ml of DOM and NAP respectively. Stock solution was filtered through 0.2 µm membrane filter.

### Preparation of working standard solution

From the stock solution, 2 ml was taken into the 10 ml volumetric flask and made upto the mark using the mobile phase to contain 10.17 µg/ml of DOM and 200 µg/ml of NAP. Twenty microlitre of the working standard solution was injected and chromatogram was recorded.

### Sample preparation

Twenty tablets were accurately weighed, and finely powdered. A quantity of powder equivalent to 250 mg of NAP and 12.72 mg of DOM tablet powder was weighed

and transferred into 250 ml volumetric flask and dissolved with mobile phase, sonicated for 30 min. The solution was then filtered through 0.2 micron membrane filter paper and made up the volume with mobile phase. From that solution, 2 ml was taken into 10 ml volumetric flask and made up the volume with mobile phase. Twenty microlitre of the test solution was injected and chromatogram was recorded for the same, and amounts of the drugs were calculated.

### Method validation

The chromatographic conditions were validated by evaluating specificity, linearity, accuracy, method and system precision, limit of detection (LOD), limit of quantification (LOQ), ruggedness, and robustness in accordance with ICH guideline Q2(R1)<sup>28</sup>.

### Linearity

From the standard stock solution 0.5, 1, 1.5, 2, 2.5, and 3 ml was transferred to six 10 ml flasks and made up the volume with mobile phase. The concentrations of DOM and NAP were found to be 2.54-15.264 µg/ml and 50-300 µg/ml respectively. Twenty microlitre of the each standard solution was injected and chromatograms were recorded.

### Method and system precision

Precision of the method was verified by repeatability (system precision) and intermediate precision (method precision) studies. Repeatability studies were performed by six replicate injections of 10.17 µg/ml of DOM and 200 µg/ml of NAP on the same day. The studies were replicated on different days to determine intermediate precision.

### Accuracy

Accuracy of the method was carried out by applying the method to drug sample to which known amount of DOM and NAP standard powder corresponding to 50%, 100% and 150% of label claim had been added (standard addition method), the solutions are analyzed by optimized method.

### Limit of detection (LOD) and Limit of quantification (LOQ)

The limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected, but not necessarily quantified, under the stated experimental conditions. LOD & LOQ was calculated by using standard deviation and slope values obtained from calibration curve.

$$\text{LOD} = 3.3 \sigma / S$$

$$\text{LOQ} = 10 \sigma / S$$

Where,  $\sigma$  is standard deviation (intercept of calibration line); S is slope.

### Robustness of the method

To evaluate robustness of the HPLC method, a sunfire C<sub>18</sub> column, 250 mm x 4.6 mm, i.d., 5  $\mu$ m parcel size was considered. Slight variations were made in flow rate and percentage of acetonitrile in the mobile phase. Robustness of the method was carried out by 10.17  $\mu$ g/ml of DOM and 200  $\mu$ g/ml of NAP.

## RESULTS AND DISCUSSION

### Chromatographic condition

The RP-HPLC procedure was optimized with a view to develop a simultaneous estimation method for DOM and NAP. The mixed standard solution of drugs (DOM and NAP) was run in different solvent systems. Initially methanol, water (80:20), acetonitrile, water (70:30) and potassium phosphate buffer, acetonitrile with different ratio, different pH were tried. It was found that phosphate buffer and acetonitrile (50:50 pH 6.5 is adjusted with the orthophosphoric acid) and at flow rate 1ml/min gives acceptable retention time and resolved peaks without tailing. The scanning wavelength selected was 274 nm as isobestic point of DOM and NAP.

### Validation of Developed Method

#### Specificity

Specificity of the HPLC method is illustrated in Fig. 3 where complete separation of DOM and NAP was noticed in presence of table excipients. In addition there was no interference at the retention time of DOM and NAP in the chromatogram of placebo solution.

### Linearity

The correlation coefficient ( $r^2$ ) values of both drugs were found to be 0.999. The equation of regression line for DOM was found to be  $y = 76684x - 3531.2$ . The equation of regression line for NAP was found to be  $y = 56016x + 87257$ .

### Precision

The precision of an analytical method is the degree of agreement among individual test results obtained when the method is applied to multiple sampling of a homogenous sample. Precision is a measure of the reproducibility of the whole analytical method (including sampling, sample preparation and analysis) under normal operating circumstances. Precision is determined by using the method to assay a sample for a sufficient number of times to obtain statistically valid results (i.e. in between 5-10). The precision is then expressed as the relative standard deviation. Acceptance criteria for the precision of the method, the %RSD should not be more than 2%. In the present study, for the system precision % RSD for NAP and DOM was found to be 0.17%, 0.81% respectively and for the method precision % RSD for NAP and DOM was found to be 0.39%, 1.19% respectively. The % RSD value indicates a good degree of precision within the specified range (Table 1,2,3).

### Accuracy

Accuracy is a measure of the closeness of test results obtained by a method to the true value. It indicates the deviation between the mean value found and the true value. In our study, the percentage recovery of NAP was found to be 98.43%, 100.52%, and 98.08% from 50%, 100% and 150% sample solutions respectively. For DOM it was found to be 100.02%, 98.12% and 99.93% from 50%, 100% and 150% sample solutions respectively. The obtained percentage recovery of both drugs was found to be within the range. This indicates the proposed method was more accurate than the existing methods. The results were displayed in table 4.

**LOD and LOQ**

LOD and LOQ were calculated by from standard deviation and slope values obtained from calibration curve using non instrumental method. The LOD and LOQ value for DOM were 0.15 µg/ml, 0.50 µg/ml respectively and for NAP were 5.14 µg/ml, 15.42 µg/ml, respectively.

**Robustness**

Robustness of analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. In present study it was observed that there was no significant change in the peak area with change in flow rate and mobile phase. The results of robustness study were shown in Table 5.

**Analysis of pharmaceutical dosage form**

Experimental results of the amount of DOM and NAP in pharmaceutical formulation were in good agreement with the label claims. The drug content was

found to be 99.6% for DOM 99.8% for NAP, the chromatograms were shown in Fig 4 and 5 and results were shown in Table 6.

**CONCLUSION**

A simple RP-HPLC method using a C<sub>18</sub> column was developed for analysis of DOM and NAP in pharmaceutical tablet dosage formulation. The developed method is simple, accurate, precise, reproducible and retention time was less than the existing methods. Therefore this method can be useful for the routine quality control analysis of DOM and NAP.

**Conflict of interest statement**

We (the authors of this manuscript) have no financial and personal relationships with other people or organizations that could influence our work.

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**Table 1: System Precision data results for the NAP and DOM**

Drug	concentration µg/ml	Average(n=6)		SD		RSD%	
		RT	Peak area	RT	Peak area	RT	Peak area
NAP	200	4.2783	11405062	0.001506	44787.11	<b>0.04</b>	<b>0.39</b>
DOM	10.17	2.6371	780789.3	0.001329	9308.342	<b>0.05</b>	<b>1.19</b>

**Table 2: Method Precision data results for the NAP and DOM**

Drug	concentration µg/ml	Average(n=6)		SD		RSD%	
		RT	Peak area	RT	Peak area	RT	Peak area
NAP	200	4.2781	11446018	0.002137	19613.19	<b>0.05</b>	<b>0.17</b>
DOM	10.17	2.6363	784344.8	0.001033	6383.431	<b>0.04</b>	<b>0.81</b>

**Table 3: System suitability parameters for the NAP and DOM**

Parameters	NAP	DOM
Theoretical plates	6968	4074
Resolution	8.98	-
Symmetric factor	1.09	1.10

**Table 4: Accuracy data results for the NAP and DOM at the level of 50%, 100% and 150%.**

Drug	Spiked sample (µg/ml)	Total amount (µg/ml)	Amount recovered %	Recovery %
NAP	100	300	49.21	98.43
	200	400	100.52	100.52
	300	500	147.12	98.08
DOM	5.085	15.185	50.01	100.02
	10.17	20.34	98.12	98.12
	15.835	26.005	149.9	99.93

**Table 5: Robustness parameters for the NAP and DOM  
Flow rate change**

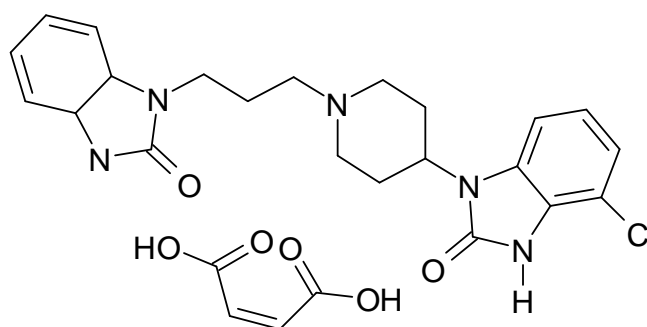
Factor	Level	Retention time		Asymmetry	
		DOM	NAP	DOM	NAP
Flow rate (ml/min)					
0.9	-0.1	2.765	4.483	1.07	1.09
1	0	2.635	4.269	1.09	1.10
1.1	+0.1	2.514	4.274	1.05	1.08
Mean ±S.D		2.638±0.125	4.274±0.205	1.07±0.02	1.09±0.11

**Mobile phase ratio change**

Factor	Level	Retention time		Asymmetry	
		DOM	NAP	DOM	NAP
ACN : Buffer					
55:45	-5	2.567	3.714	1.12	1.09
50:50	0	2.636	4.270	1.19	1.11
45:55	+5	2.723	5.159	1.09	1.08
Mean ±S.D		2.642±0.078	4.381±0.728	1.133±0.05	1.093±0.075

**Table 6: Assay data results for the NAP and DOM**

Drug	Labeled amount	Amount present	% of drug found
NAP	250	249.62	99.8
DOM	12.7	12.6	99.6

**Fig. 1: Domperidone maleate**

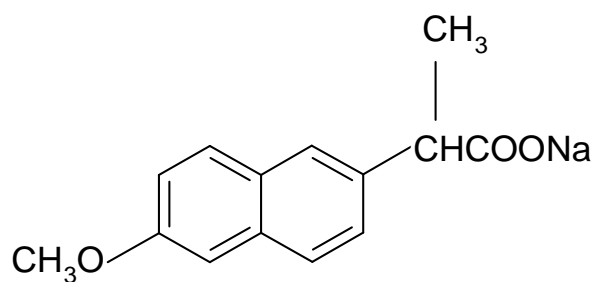


Fig. 2: Naproxen sodium

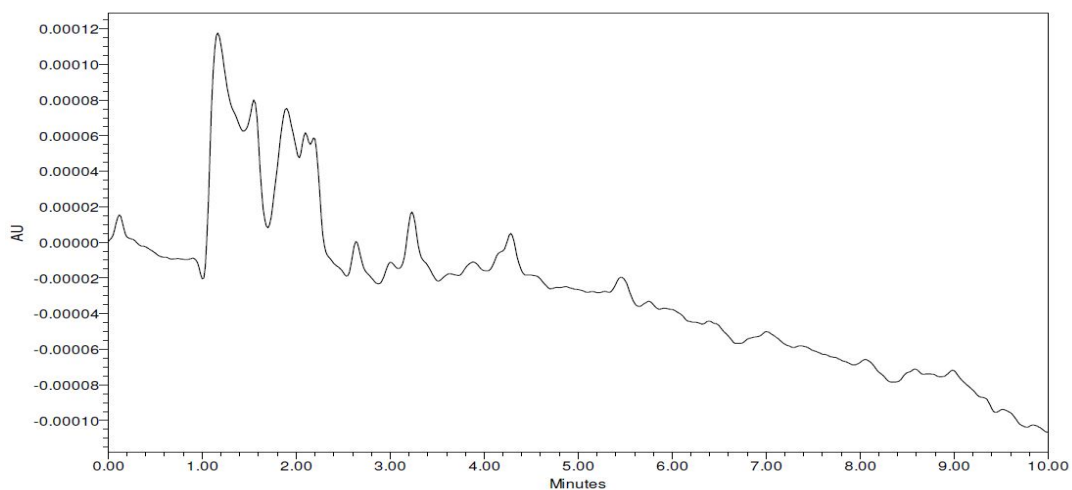


Fig. 3: Chromatogram of placebo solution

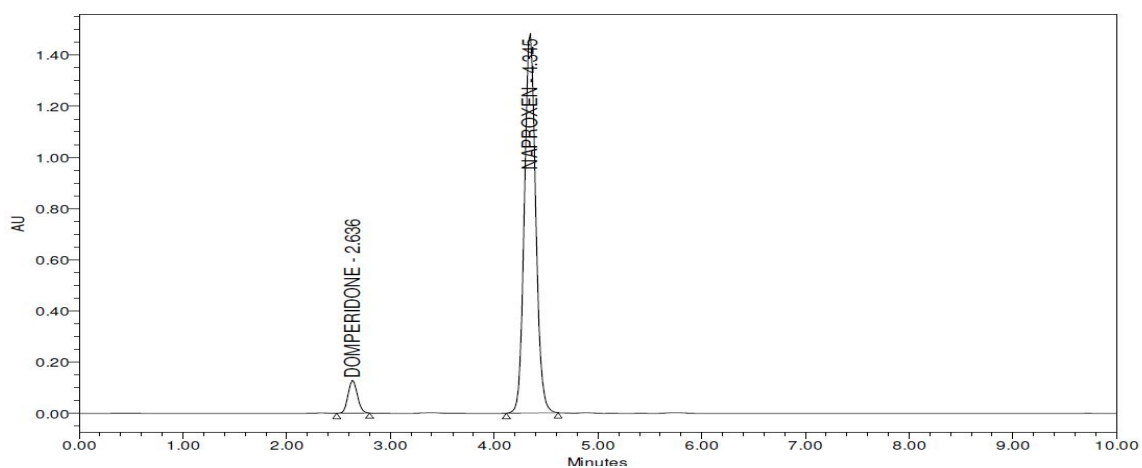


Fig. 4: Chromatogram of DOM and NAP tablet formulation

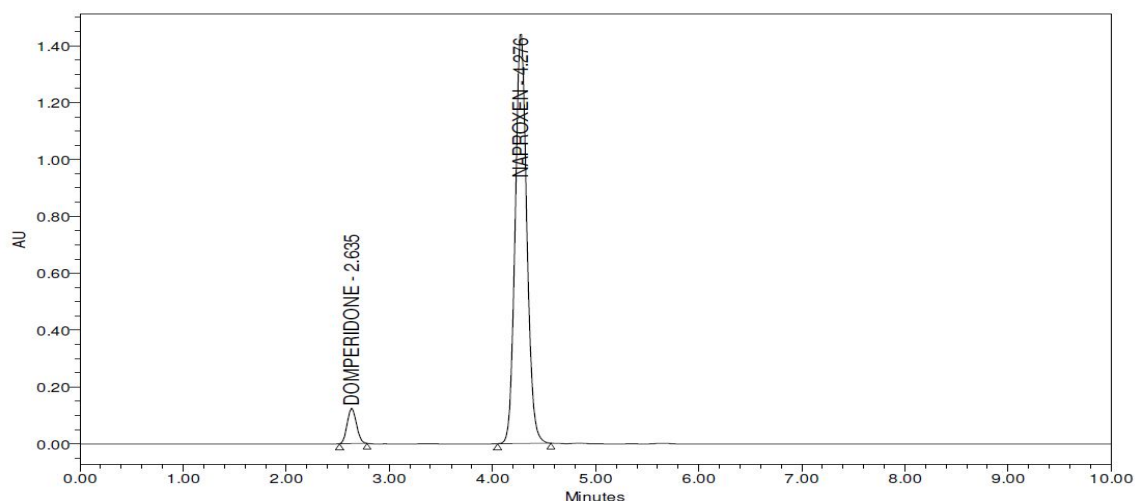


Fig. 5: Chromatogram of DOM and NAP standard solution

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