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Validated RP-HPLC Method for Simultaneous Quantitation of Domperidone Maleate and Naproxen Sodium in Bulk Drug and Formulation

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ABSTRACT

This paper describes a new, simple, precise, and accurate HPLC method for simultaneous quantitation of Domperidone Maleate (DOM) and Naproxen Sodium (NAP) as the bulk drug and in tablet dosage form. Chromatographic separation of the drugs was achieved on a Eurosphere C_{18} column (5 µm, 250 mm × 4.6 mm, i.d.) with the isocratic mobile phase methanol: sodium acetate buffer (60:40 v/v, pH 3.6 adjusted with acetic acid) at a flow rate of 1 mL/min. The UV detection wavelength was 266 nm. The two drugs were satisfactorily resolved with retention times 3.21 min and 7.45 min for DOM and NAP, respectively. Linearity was found to be in the range of 10-60 µg/mL for DOM and 25-150 µg/mL for NAP with significantly high value of correlation coefficient (0.9974 for DOM and 0.9992 for NAP). The percentage recovery obtained was 98.86 % for DOM and 99.55 % for NAP.

Keywords: RP-HPLC, Domperidone Maleate, Naproxen Sodium, Method validation

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INTRODUCTION

Domperidone (DOM) (Fig. 1a) is chemically 5-Chloro-1-[1-[3-(2-oxo-1, 3-dihydrobenzoimidazol-1-yl)propyl]-4-piperidyl]-1,3-dihydrobenzimidazol-2-one maleate. Its gastroprokinetic properties are related to its peripheral dopamine receptor blocking properties. It facilitates gastric emptying and decreases small bowel transit time. Antiemetic property is related to its dopamine receptor blocking activity at both the chemoreceptor trigger zone and at the gastric level. It is used for the symptomatic management of upper GI motility disorders associated with chronic and subacute gastritis and diabetic gastroparesis; prevention of GI symptoms associated with use of dopamine-agonist anti-Parkinson agents [1-2]. Naproxen

(NAP) (Fig. 1b) is chemically 2-(6-methoxynaphthalen-2-yl)propanoic acid. It inhibits prostaglandin synthesis by decreasing the activity of the enzyme, cyclooxygenase, which results in decreased formation of prostaglandin precursors, like that of other NSAIDs. It is used for the management of ankylosing spondylitis, osteoarthritis, and rheumatoid disorders (including juvenile rheumatoid arthritis); acute gout; mild to moderate pain; tendonitis, bursitis; dysmenorrhea; fever, migraine headache [3].

Literature review reveals that methods have been reported for analysis of DOM by Spectrophotometry [4-6], Spectrofluorimetry [7]. Capillary Electrophoresis [8], High-Performance Liquid Chromatography (HPLC) [9-11] and High-Performance Thin Layer Chromatography (HPTLC) [12-14] and for NAP methods reported are Spectrophotometry [15-17], Spectrofluorimetry [18-19], Gas Chromatography (GC) [20], Capillary zone Electrophoresis [21], HPLC [22-24] and HPTLC [25-26] either alone or in combination with other drugs.

To date, there have been no published reports about the simultaneous quantitation of DOM and NAP by HPLC in bulk drug and in pharmaceutical dosage forms. This present study reports for the first time simultaneous quantitation of DOM and NAP by RP-HPLC in bulk drug and in pharmaceutical dosage forms. The proposed method is validated as per ICH guidelines [27].

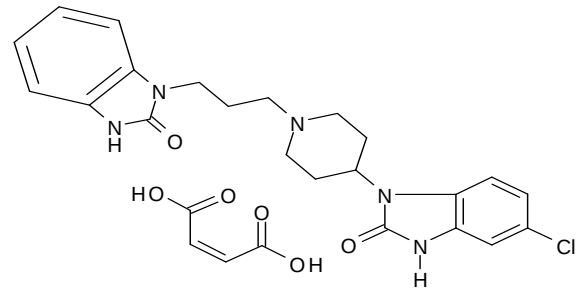

Fig. 1a Domperidone maleate

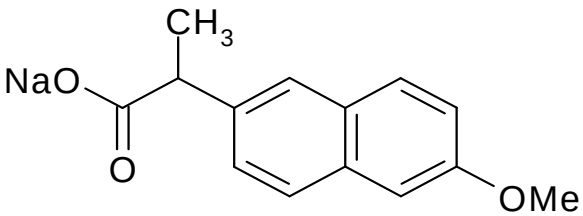

Fig. 1b Naproxen sodium

MATERIALS AND METHODS

2.1. Materials

Working standards of pharmaceutical grade DOM and NAP were obtained as generous gifts from T. M. Thakore Pharmaceutical Labs. Pvt. Ltd. Mumbai (Maharashtra, India). It was used without further purification and certified to contain 99.25 % and 99.48 % (w/w) on dry weight basis DOM and NAP, respectively. Fixed dose combination tablet (Napra-D 250) containing 10

mg DOM and 250 mg NAP was purchased from Local pharmacy. Acetic acid was of analytical grade, HPLC grade methanol and water were obtained from Merck Chemicals, Mumbai, India.

2.2. Instrumentation

The High Performance Liquid Chromatographic experimentation were performed on Systronic LC 6600 system equipped with LC 6600 pump and UV-VIS detector. Data acquisition and processing were performed using Chemittochrom software. A Eurosphere C_{18} (KNAVER, Berlin, Germany) column (250 × 4.6 mm) with particle size 5µm forms the stationary phase.

2.3. Preparation of Standard Stock Solutions

Reference standard of DOM 10 mg and NAP 10 mg was transferred to 10 mL volumetric flask separately and dissolved in methanol. The flask was shaken for 30 min and the volume was made up to the mark with mobile phase to obtain standard stock solution of DOM and NAP, 1000 µg/mL each. Stock solution was filtered through a 0.2 µm membrane filter.

2.4. Working Standard Solution:

From standard stock solution, the combined working standard solution was prepared using the methanol to contain 10µg/mL of DOM and 250µg/mL of NAP.

2.5. Optimization of the HPLC Method

The RP-HPLC procedure was optimized with a view to develop a simultaneous assay method for DOM and NAP. The mixed standard stock solution of drugs (DOM and NAP) were injected and run in different solvent systems. Initially methanol and water in different ratios were tried, and then methanol and acetate buffers of different pH were also tried. It was found that methanol: sodium acetate buffer in ratio 60:40, v/v pH 3.6 adjusted with glacial acetic acid at flow rate 1 mL/min gives acceptable retention time and resolved peaks with minimum tailing as compared to other mobile phases. The scanning wavelength selected was 266 nm where good response was observed for both DOM and NAP. (Fig. 2)

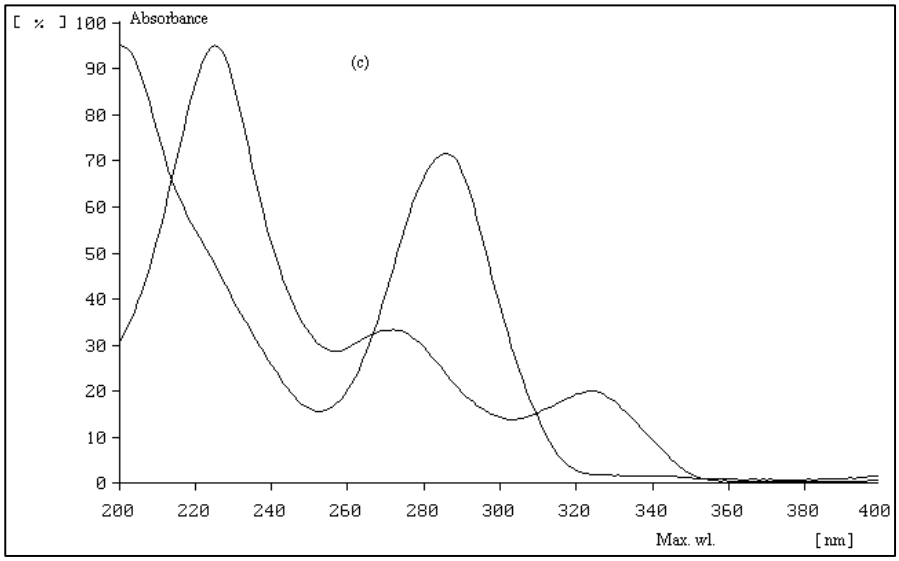

Fig. 2 In-situ overlain spectrum of DOM and NAP

2.5. Validation of the method

Validation of the optimized RP-HPLC method was carried out with respect to the following parameters.

2.5.1. Linearity

From DOM standard stock solution 1, 2, 3, 4, 5, 6 mL was transferred to 100 mL volumetric flask and volume was made up to the mark with mobile phase to obtain concentration of DOM 10–60 µg/mL. In the same way 2.5, 5, 7.5, 10, 12.5, 15 mL of NAP was transferred to 100 mL volumetric flask from the NAP standard stock solution and volume was made up to the mark with mobile phase to obtain concentration 25–150 µg/mL of NAP. The solutions (20 µL) were injected into column with the help of Hamilton syringe. All measurements were repeated six times for each concentration. The calibration curves of the area under curve Vs concentration were recorded for both drugs.

2.5.2. Precision

Precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by hexaplicate injections of three different concentrations 10, 30, 60 µg/mL of DOM and 25, 75, 150 µg/mL of NAP on the same day. The studies were also repeated on different days to determine intermediate precision.

2.5.3. Limit of detection and Limit of quantitation

The limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. It may be expressed as a concentration that gives a signal to noise ratio of 2:1 or 3:1. Limit of quantitation (LOQ) is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. A signal to noise ratio of 10:1 can be taken as LOQ of the method.

2.5.4. Robustness of the method

To evaluate robustness of HPLC method, a Eurosphere C_{18} column, 250 mm × 4.6 mm i.d., 5 µm particle size, (KNAVER, Berlin, Germany) was considered. Operating in the above conditions with minimal variation of flow rate, percentage of methanol in the mobile phase, pH of mobile phase. Robustness of the method was done at three different concentration levels 10, 30, 60 µg/mL and 20, 75, 150 µg/mL for DOM and NAP, respectively.

2.5.5. Accuracy

Accuracy of the method was carried out by applying the method to drug sample (DOM and NAP in combination tablet) 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), mixed and the powder was extracted and analyzed by running chromatogram in optimized mobile phase.

2.6. Analysis of a marketed formulation

To determine the content of DOM and NAP in conventional tablet (Brand name: NAPRA-D 250, Label claim: 10 mg Domperidone Maleate and 250 mg Naproxen Sodium), average weight of tablet equivalent to 10 mg DOM and 250 mg NAP was transferred into a 50 mL volumetric flask

containing 25 mL methanol, sonicated for 10 min and diluted to 50 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min. The above stock solution was further diluted to get sample solution containing (10µg/mL) DOM and (250µg/mL) NAP. A 20 µL volume of sample solution was injected into HPLC. The analysis was repeated in triplicate. The possibility of excipient interference with the analysis was examined.

RESULTS AND DISCUSSION

The results of validation studies on simultaneous estimation method developed for DOM and NAP in the current study involving methanol: sodium acetate in ratio 60:40, v/v pH 3.6 adjusted with glacial acetic acid as the mobile phase for RP-HPLC are given below.

3.1. Validation

3.1.1. Linearity

The drug response was linear (r^2 = 0.9974 for DOM and 0.9992 for NAP) over the concentration range between 10-60 µg/mL for DOM and 25-150 µg/mLfor NAP. The mean (± % RSD) values of the slope, intercept and correlation coefficient for DOM and NAP were 44.37, 31.06, 0.9974 and 209.3, 66.93, 0.9992, respectively.

3.1.2. Precision

The results of the repeatability and intermediate precision experiments are shown in Table 1. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were < 2 %, respectively as recommended by ICH guidelines.

Table 1. Precision studies

Conc. µg/mL	Repeatability (n= 6)		Intermediate precision (n= 6)	
	Found conc. ± SD	RSD (%)	Found conc. ± SD	RSD (%)
For NAP				
25	24.89 ± 0.129	0.518	24.77 ± 0.386	1.55
75	74.63 ± 1.23	1.64	74.72 ± 1.47	1.96
150	148.25 ± 1.56	1.05	147.36 ± 1.76	1.19
For DOM				
10	9.84 ± 0.052	0.528	9.72 ± 0.12	1.23
30	28.57 ± 0.321	1.12	29.13 ± 0.221	0.758
60	57.14 ± 0.261	0.456	58.42 ± 0.579	1.05

3.1.3. LOD and LOQ

The LOD and LOQ were found to be 0.185µg/mL, 0.563µg/mL for DOM and 0.023µg/mL, 0.071µg/mL, for NAP, respectively.

3.1.4. Robustness of the method

Each factor selected was changed at three levels (-1, 0 and 1). One factor at the time was changed to estimate the effect. Thus, replicate injections (n = 6) of mixed standard solution at three concentration levels were performed under small changes of three chromatographic parameters (factors). Insignificant differences in peak areas and less variability in retention time were observed.

Table 2. Robustness testing

Factor	Level	Retention time		Assymmetry	
Flow Rate (mL/min)		**NAP**	**DOM**	**NAP**	**DOM**
0.9	-1	7.63	3.33	1.45	1.26
1.0	0	7.54	3.25	1.41	1.22
1.1	+1	7.43	3.17	1.37	1.16
Mean ± S.D (n = 6)		7.53 ± 0.1	3.25 ± 0.08	1.41 ± 0.04	1.21 ± 0.04
% of methanol in the mobile phase (v/v)					
59	-1	7.69	3.41	1.48	1.31
60	0	7.54	3.25	1.41	1.22
61	+1	7.37	3.12	1.28	1.13
Mean ± S.D.(n = 6)		7.53 ± 0.16	3.26 ± 0.145	1.39 ± 0.1	1.22 ± 0.09
pH of mobile phase					
3.5	-1	7.52	3.11	1.35	1.12
3.6	0	7.56	3.27	1.41	1.24
3.7	+1	7.50	3.24	1.14	1.32
Mean ± S.D. (n = 6)		7.52 ± 0.03	3.20 ±0.085	1.3 ± 0.14	1.22 ± 0.1

Table 3. Recovery studies (n = 6)

Drug	Label claim (mg/tablet)	Amount added mg (%)	Total amount (mg)	Amount recovered (mg) ± S.D.	Recovery (%)
NAP	250	125 (50 %)	375	374.3 ± 0.29	99.81
		250 (100 %)	500	494.4 ± 0.64	98.88
		375 (150 %)	625	618.6 ± 0.84	98.97
DOM	10	5 (50 %)	15	14.87 ± 0.25	99.13
		10 (100 %)	20	19.85 ± 0.17	99.25
		15 (150 %)	25	24.55 ± 0.38	98.2

3.1.5. Recovery Studies

As shown from the data in Table 3 good recoveries of the DOM and NAP in the range from 99.25 to 99.81 % were obtained at various added concentrations. The average recovery for DOM and NAP were 98.86 % and 99.55 % respectively.

3.2. Analysis of a formulation

Experimental results of the amount of DOM and NAP in tablet, expressed as a percentage of label claims were in good agreement with the label claims, showing no interference of excipients in analysis (Fig. 3). The drug content was found to be 99.82 ± 0.06 for DOM and 99.48 ± 0.16 for NAP (Table 4).

Table 4. Analysis of Commercial Formulation

Drug	Labelled amount[a] (mg/tab)	Amount Present (mg/tab)	% of Drug Found*
NAP	250	248.7	99.48 ± 0.16
DOM	10	9.98	99.82 ± 0.06

**Average of six determinations*

[a]*NAPRA-D 250 Tablets, Sun Pharmaceuticals Ltd.*

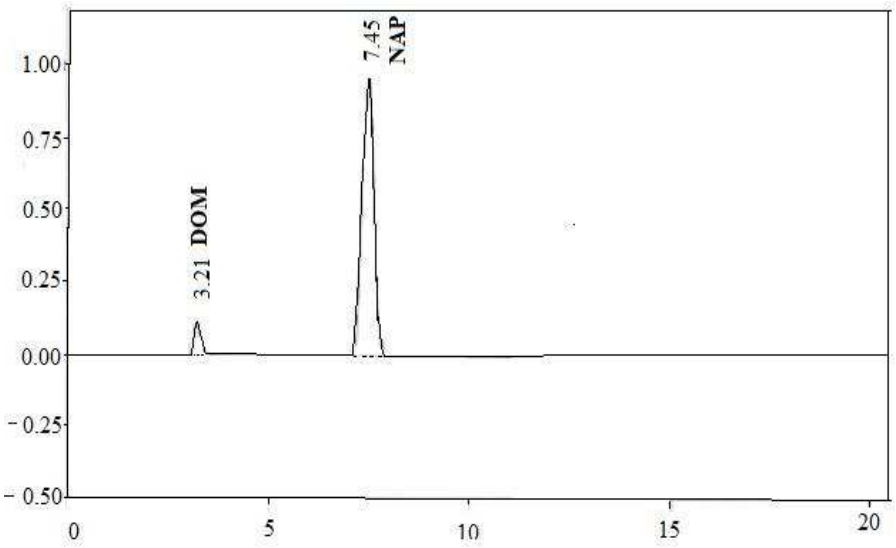

Fig. 3 Chromatogram of DOM (RT 3.21) and NAP (RT 7.45) of Marketed Formulation (NAPRA-D 250)

CONCLUSION

A simple RP-HPLC method using a C18 column was developed for the analysis of DOM and NAP in bulk drug and in pharmaceutical formulation. The developed method is simple, accurate, precise and linear within the desired range. Therefore this method can be useful in routine quality control analysis of DOM and NAP.

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