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Validated HPTLC Method for Simultaneous Quantitation of Domperidone Maleate and Naproxen Sodium in Bulk Drug and Formulation

Shubhangi M. Pawar[a], Bharat S. Patil[b], Ravindra Y. Patil[a*]

[a] Department of Pharmaceutical Chemistry, Hon. L.M.C., College of Pharmacy, Faizpur,Jalgaon, India
[b] Bharati Vidyapeeth's Institute of Pharmacy, Erandwane, Pune, Maharashtra, India.

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Abstract

This paper describes a new, simple, precise, and accurate HPTLC 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 performed on aluminum plates precoated with silica gel 60 F_{254} as the stationary phase and the solvent system consisted of toluene: methanol: acetone (8: 2: 2, v/v/v). Densitometric evaluation of the separated zones was performed at 266 nm. The two drugs were satisfactorily resolved with R_F values 0.44 ± 0.02 and 0.56 ± 0.02 for DOM and NAP, respectively. The accuracy and reliability of the method was assessed by evaluation of linearity (20–140 ng.spot^{-1} for DOM and 500-3500 ng.spot^{-1} for NAP), precision (intra-day RSD 0.4–1.01 % and inter-day RSD 0.316–0.876 % for DOM, and intra-day RSD 0.488–1.329 % and inter-day RSD 0.450–1.026 % for NAP), accuracy (98.38 ± 0.55 % for DOM and 98.64 ± 0.49 % for NAP), and specificity, in accordance with ICH guidelines.

Keywords:

High performance thin layer chromatography; densitometry**;** validation; quantification; domperidone maleate; naproxen sodium

1. Introduction

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]. 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

* **Corresponding Author**
E-mail: rypatil1971@yahoo.co.in
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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 HPTLC in bulk drug and in pharmaceutical dosage forms. This present study reports for the first time simultaneous quantitation of DOM and NAP by HPTLC in bulk drug and in pharmaceutical dosage forms. The proposed method is validated as per ICH guidelines [27].

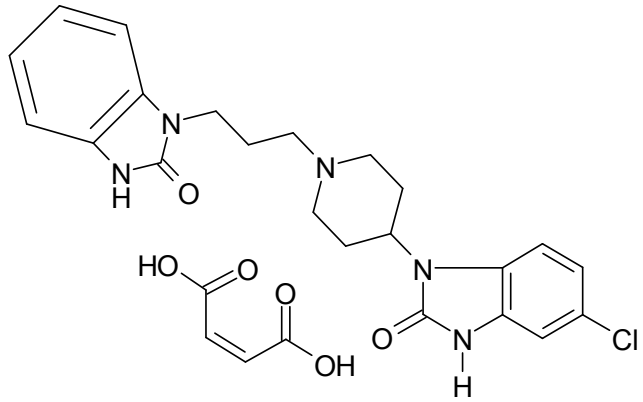

Fig. 1a Domperidone maleate

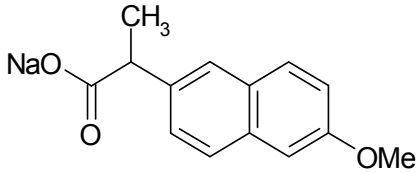

Fig. 1b Naproxen sodium

2. Experimental

2.1. Materials

Working standards of pharmaceutical grade Domperidone maleate (DOM) and Naproxen sodium (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 medical, Pune, India. All chemicals and reagents of analytical grade were purchased from Merck Chemicals, Mumbai, India.

2.2. Instrumentation

The samples were spotted in the form of bands of width 6 mm with a Camag 100 µL sample (Hamilton, Bonaduz, Switzerland) syringe on silica gel precoated aluminum 60 F_{254} plates, [10 cm × 10 cm with 250 µm thickness; E. Merck, Darmstadt, Germany] using a Camag Linomat V (Switzerland) sample applicator. The plates were prewashed with methanol and activated at 110 ^{o}C for 5 min prior to chromatography. A constant application rate of 0.1 µL s^{-1} was used and the space between two bands was 6 mm. The slit dimension was kept at 5 mm × 0.45 mm and the scanning speed was 10 mm s^{-1}. The monochromator bandwidth was set at 20 nm, each track was scanned three times and baseline correction was used. The mobile phase consisted of toluene: methanol: acetone (8: 2: 2, v/v/v*)* and 12 mL of mobile phase was used per chromatography run. Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25 ^{o}C ± 2) at relative humidity of 60 % ± 5. The length of each

chromatogram run was 8 cm. Following the development the TLC plates were dried in a current of air with the help of an air dryer in a wooden chamber with adequate ventilation. The air flow in laboratory was maintained unidirectional (laminar flow, towards the exhaust). Densitometric scanning was performed using a Camag TLC scanner III in the reflectance-absorbance mode at 266 nm and operated by CATS software (V 3.15, Camag). The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. Concentrations of the compound chromatographed were determined from the intensity of the diffused light. Evaluation was used by peak areas with linear regression.

2.3. Preparation of Standard Stock Solutions

Standard stock solutions of concentration 0.2 $mg.mL^{-1}$ of DOM and 5 $mg.mL^{-1}$ of NAP were prepared separately using methanol. From the standard stock solution, the mixed standard solution was prepared using methanol to contain 0.02 $mg.mL^{-1}$ of DOM and 0.5 $mg.mL^{-1}$ of NAP, respectively. The stock solution was stored at 2-8 °C, protected from light.

2.4. Optimization of the HPTLC method

The HPTLC procedure was optimized with a view to develop a simultaneous assay method for DOM and NAP respectively. The mixed standard stock solution (0.02 $mg.mL^{-1}$ of DOM and 0.5 $mg.mL^{-1}$ of NAP) was spotted on to HPTLC plates and run in different solvent systems. Initially, toluene, methanol & acetone were tried in different ratios. Toluene was used to impart the necessary non-polarity to mobile phase to obtain a suitable R_F value**.** Initially toluene, methanol & acetone in the ratio of 5: 2: 2.5, v/v/v was tried but R_F was found more towards the right side (0.61 for DOM and 0.71 for NAP). Then the volume of toluene in mobile phase was increased from 5 mL to 8 mL & that of acetone was decreased from 2.5 mL to 2 mL to decrease R_F and improve separation. Finally, the mobile phase consisting of toluene: methanol: acetone in the ratio of 8: 2: 2, v/v/v was found optimum. In order to reduce the neckless effect, HPTLC chamber was saturated for 20 min using saturation pads. The mobile phase was run up to a distance of 8 cm; which takes approximately 20 min for complete development of the HPTLC plate. The scanning wavelength selected was 266 nm where good response was observed for both DOM and NAP. (Fig. 2)

2.5. Validation of the method

Validation of the optimized HPTLC method was carried out with respect to the following parameters.

2.5.1. Linearity and range

From the mixed standard stock solution 0.02 $mg.mL^{-1}$ of DOM and 0.5 $mg.mL^{-1}$ of NAP, 1 to 7 µL solutions were spotted on HPTLC plate to obtain final concentration 20-140 $ng.spot^{-1}$ for DOM and 500-3500 $ng.spot^{-1}$ for NAP. The plate was then developed using the previously described mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves.

2.5.2. Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentration (20, 80, 140 $ng.spot^{-1}$ for DOM and 500, 2000, 3500 $ng.spot^{-1}$ for NAP) of the drug six times on the same day. The intermediate precision of the method was checked by repeating studies on three different days.

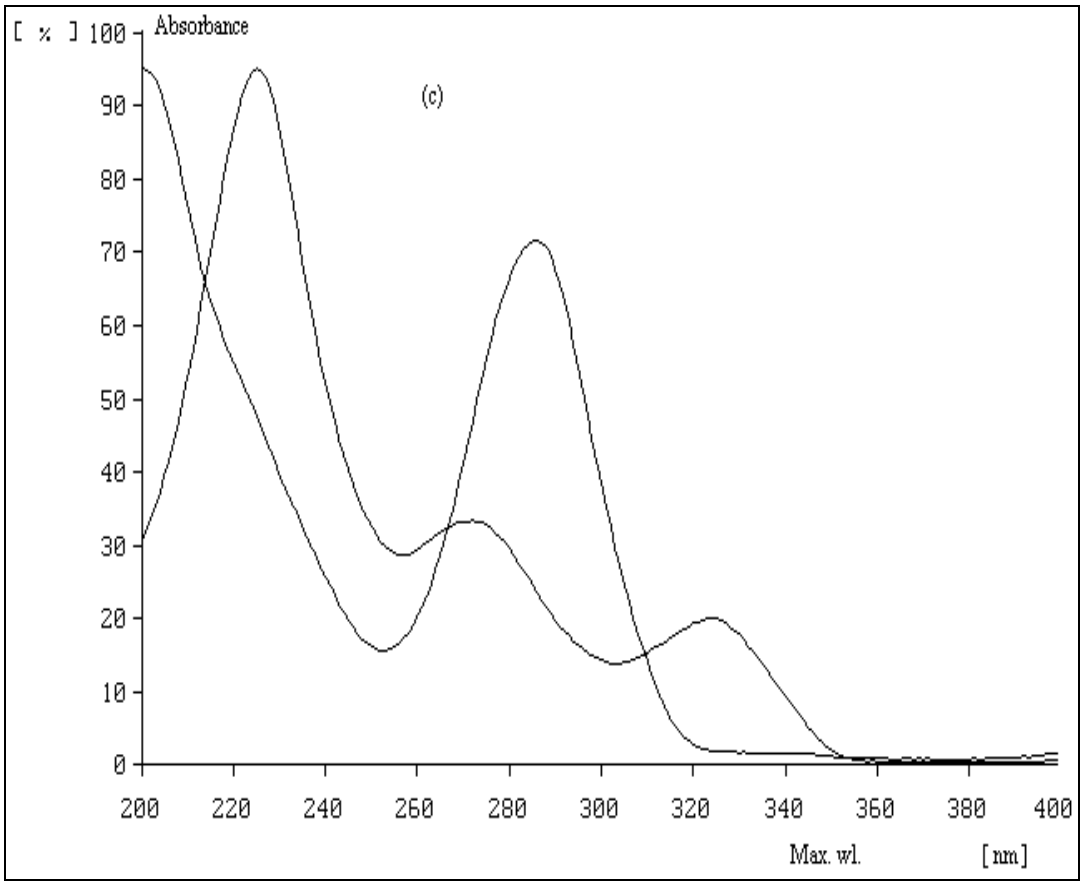

Fig. 2. UV absorbance spectra overlain of Domperidone (DOM) and Naproxxen (NAP).

2.5.3. Limit of detection and Limit of quantitation

Limits of detection (LOD) and quantification (LOQ) represent the concentration of the analyte that would yield signal-to-noise ratio 3 for LOD and 10 for LOQ, respectively. LOD and LOQ were determined by measuring the magnitude of analytical background by spotting a blank and calculating the signal-to-noise ratio for DOM and NAP by spotting a series of solutions until the signal-to-noise ratio 3 for LOD and 10 for LOQ. The samples were applied to HPTLC plate and the chromatograms were run and measured signal from the samples was compared with those of blank samples. Limit of detection (LOD) and Limit of quantitation (LOQ) were calculated as 3.3 σ/S and 10 σ/S, respectively as per ICH guidelines, where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

2.5.4. Robustness of the method

Following the introduction of small changes in the mobile phase composition (± 0.1 mL for each component), the effects on the results was examined. Mobile phases having different compositions, e.g. toluene: methanol: acetone (8.1:2:2, v/v/v), (7.9:2:2, v/v/v), (8:2.1:2.5, v/v/v), (8:1.9:2, v/v/v) were tried and chromatograms were run. The amount of mobile phase was varied over the range of ± 5 %. The plates were prewashed with methanol and activated at 110 °C for 2, 5, and 7 min respectively prior to chromatography. The time from spotting to chromatography and from chromatography to scanning was varied by ± 10 min. The robustness of the method was determined at three different concentration levels 20, 80 and 140 ng.spot^{-1} and 500, 2000 and 3500 ng.spot^{-1} for DOM and NAP, respectively.

2.5.5. Specificity

The specificity of the method was determined by analyzing standard and drug samples. The spot for DOM and NAP in the samples was confirmed by comparing the R_F and spectrum of the spot with that of a standard. The peak purity of DOM and NAP was determined by comparing the spectrum at three different regions of the spot i.e. peak start (S), peak apex (M) and peak end (E).

2.5.6. 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 & of 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. 1 mL of above solution diluted upto 10 mL with methanol and the drug content was determined (0.02 and 0.5 $mg.mL^{-1}$ for DOM and NAP, respectively). Then 4 µL of above filtered solution was applied to a HPTLC plate to produce a concentration of 80 and 2000 $ng.spot^{-1}$ for DOM and NAP, respectively and was developed in optimized mobile phase. The analysis was repeated in triplicate. The possibility of excipient interference with the analysis was examined.

3. Results and discussion

The results of validation studies on simultaneous estimation method developed for DOM and NAP in the current study involving toluene: methanol: acetone (8:2:2, v/v/v) as the mobile phase for HPTLC are given below.

3.1. Linearity and range

The drug response was linear (r^2 = 0.9993 for DOM and 0.9953 for NAP) over the concentration range between 20-140 $ng.spot^{-1}$ for DOM and 500-3500 $ng.spot^{-1}$ for NAP.

3.2. 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.

3.3. LOD and LOQ

Signal-to-noise ratios of 3:1 and 10:1 were obtained for the LOD and LOQ respectively. The LOD and LOQ were found to be 0.79 $ng.spot^{-1}$and 2.39 $ng.spot^{-1}$ for DOM and 4.66 $ng.spot^{-1}$ and 14.14 $ng.spot^{-1}$, for NAP, respectively.

Table 1. Precision studies

Concentration precision ($ng.spot^{-1}$) (n= 6)	Repeatability (n= 6)		Intermediate	
	Measured concentration ± SD	RSD (%)	Measured concentration ± SD	RSD (%)
For DOM				
20	19.14 ± 0.1	0.701	19.36 ± 0.1	0.876
80	77.67 ± 0.3	0.400	78.22 ± 0.6	0.831
140	135.95 ± 1.3	1.01	136.10 ± 0.4	0.316
For NAP				
500	486.77 ± 6.4	1.329	488.9 ± 4.6	0.954
2000	1945.49 ± 18.5	0.954	1938.26 ± 19.89	1.026
3500	3400.64.3 ± 16.6	0.488	3409.5 ± 15.35	0.450

3.4. Robustness of the method

The standard deviation of the peak areas was calculated for each parameter and the % RSD was found to be less than 2 %. The low values of the RSD, as shown in Table 2 indicated robustness of the method.

Table 2. Robustness testing (n = 6)

Parameters	SD of peak area for DOM	RSD,%	SD of peak area for NAP	RSD, %
Mobile phase composition (± 0.1 mL)	2.15	1.23	2.76	1.08
Amount of mobile phase (± 5%)	4.12	1 .45	1.33	1.84
Time from spotting to chromatography (± 10 min.)	3.29	1.31	2.63	1.15
Time from chromatography to scanning (± 10 min.)	2.76	1.82	3.63	1.52

3.5. Specificity

The peak purity of DOM and NAP was assessed by comparing their respective spectra at the peak start, apex and peak end positions of the spot i.e., r (S, M) = 0.9992 and r (M, E) = 0.9988. A good correlation (r = 0.9973) was also obtained between the standard and sample spectra of DOM and NAP, respectively.

3.6. Recovery studies

As shown from the data in Table 3 good recoveries of the DOM and NAP in the range from 98.06 to 99.20 % were obtained at various added concentrations. The average recovery of three levels (nine determinations) for DOM and NAP were 98.38 % (0.55) and 98.64 % (0.49) respectively.

3.7. 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 thereby suggesting that there is no interference from any of the excipients, which are normally present in tablet (Fig. 3). The drug content was found to be 97.20 % ± 0.23 for DOM and 97.60 % ±12 for NAP. Two different lots of DOM and NAP combination tablet were analyzed using the proposed procedures.

Table 3. Recovery studies (n = 6)

Drug	Label claim, mg mL^{-1}	Amount added, %	Total amount, mg	Amount recovered, mg ± SD	RSD, %	Recovery, %
DOM	10	50	15	14.71 ± 0.6	1.622	98.07
		100	20	19.61 ± 0.2	1.010	98.06
		150	25	24.75 ± 0.4	1.617	99.03
NAP	250	50	375	372.03 ± 1.7	0.175	99.20
		100	500	492.33 ± 2.5	0.126	98.4
		150	625	614.16 ± 3.2	0.111	98.26

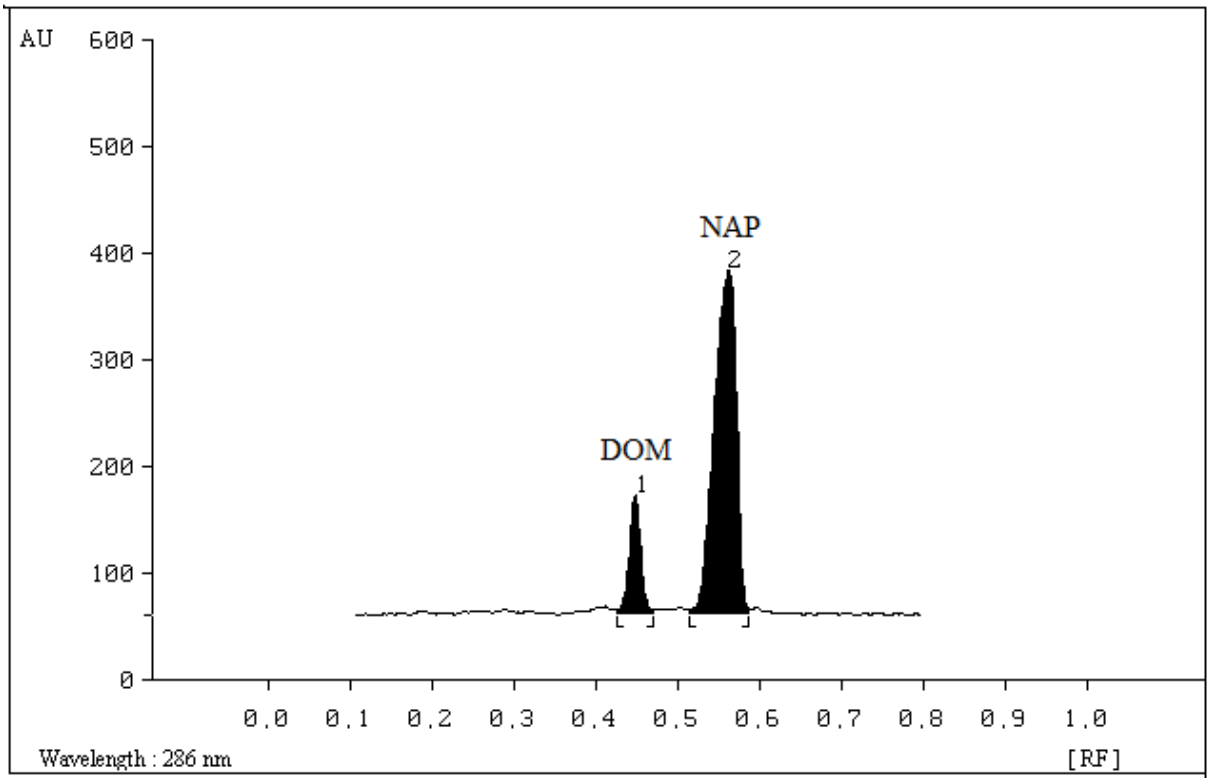

Fig. 3. Densitogram of DOM (R_F 0.44) and NAP (R_F 0.56) of marketed formulation (NAPRA-D 250) showing no interference of excipients in analysis.

4. Conclusion

Introducing HPTLC into pharmaceutical analysis represents a major step in terms of quality assurance. Today HPTLC is rapidly becoming a routine analytical technique due to its advantages of low operating costs, high sample throughput and the need for minimum sample preparation. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase-unlike HPLC - thus reducing the analysis time and cost per analysis.

The developed HPTLC technique is precise, specific and accurate. Statistical analysis proves that the method is suitable for the analysis of DOM and NAP as bulk drug and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of DOM and NAP and also for its estimation in plasma and

other biological fluids. The proposed HPTLC method is less expensive, simpler, rapid, and more flexible than HPLC.

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