DRUG FORMULATIONS AND CLINICAL METHODS

Simultaneous Estimation of Pantoprazole and Domperidone in Pure Powder and a Pharmaceutical Formulation by High-Perfomance Liquid Chromatography and High-Performance Thin-Layer Chromatography Methods

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This paper describes validated high-performance liquid chromatography (HPLC) and high-performance thin-layer chromatography (HPTLC) methods for the simultaneous estimation of pantoprazole (PANT) and domperidone (DOM) in pure powder and capsule formulations. The HPLC separation was achieved on a Phenomenex C18 column (250 mm id, 4.6 mm, 5 μm) using 0.01 M, 6.5 pH ammonium acetate buffer–methanol–acetonitrile (30 + 40 + 30, v/v/v, pH 7.20) as the mobile phase at a flow rate of 1.0 mL/min at ambient temperature. The HPTLC separation was achieved on an aluminum-backed layer of silica gel 60F_{254} using ethyl acetate–methanol (60 + 40, v/v) as the mobile phase. Quantification was achieved with ultraviolet (UV) detection at 287 nm over the concentration range 400–4000 and 300–3000 ng/mL with mean recovery of 99.35 ± 0.80 and 99.08 ± 0.57% for PANT and DOM, respectively (HPLC method). Quantification was achieved with UV detection at 287 nm over the concentration range 80–240 and 60–180 ng/spot with mean recovery of 98.40 ± 0.67 and 98.75 ± 0.71% for PANT and DOM, respectively (HPTLC method). These methods are simple, precise, and sensitive, and they are applicable for the simultaneous determination of PANT and DOM in pure powder and capsule formulations.

Received February 8, 2006. Accepted by SW May14, 2006.
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Pantoprazole (PANT), 5-(difluoromethoxy)-2-[(3,4-dimethoxy-2-pyridyl)methyl-sulfinyl]1H-benzimidazole, is a selective and irreversible proton pump inhibitor (PPI) used in medicine as an antiulcerative agent (1, 2). PANT is characterized by its favorable pharmacokinetic properties and low potential to interact with other drugs in humans. The latter is probably due to its unique metabolism as compared with other PPIs (e.g., omeprazole, lansoprazole, rabeprazole, and esomeprazole; 3–5). PANT is metabolized by a combination of Phase I and Phase II metabolisms (6). PANT accumulates in the acidic compartment of the parietal cell, where it is protonated and chemically rearranged to the active inhibitor that then covalently binds to the H^+/K^+-ATPase. This results in a long duration of action. The chemical name of domperidone (DOM) is 5-chloro-1-[1-[3-(2,3-dihydro-2-oxo-1H-benzimidazole-1-yl)propyl]-4-piperidinyl]-1-3-dihydr- 2H-benzimidazole-2-one (7). It is a peripheral dopamine–2-receptor antagonist and a unique gastrokinetic and antiemetic drug.

A literature survey revealed that different analytical methods involving high-performance column liquid chromatography (HPLC) for determination of PANT in biological fluids (8, 9), spectrophotometry (10–13), HPLC detection (14), and thin-layer chromatography (TLC; 15) have been developed. PANT is a nonofficial drug substance (16); a review of its pharmacology, clinical efficacy, and tolerability (17), as well as the similarities and differences between PANT and other PPIs (omeprazole, lansoprazole, and rabeprazole; 18) have been published recently. Several literature reports concerning HPLC determination of PANT in serum and plasma (19) and tablet dosage forms (20), as well as chiral resolution of PANT sodium and related sulfoxides by capillary zone electrophoresis using bovine serum albumin as the chiral selector (21) and enantiomeric determination of PANT by multidimensional HPLC (22), have been published.

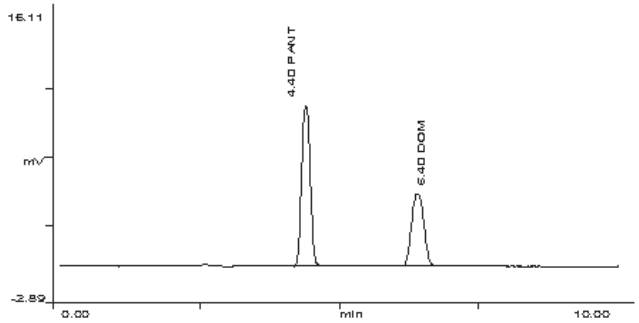

Figure 1. High-performance liquid chromatogram of PANT and DOM and corresponding retention times with detection at 287 nm.

Recently, PANT and lansoprazole have been determined by spectrophotometric procedures: 2 methods were based on charge transfer complexation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone and iodine, and a third method on ternary complex formation with eosin and cupric ions (23).

Many reports are available for estimation of DOM in pure powder and formulations using HPLC, spectrophotometry, and HPTLC in combination with omeprazole, cinnarazine, and ranitidine (24–27). The present report describes precise, accurate, specific, and sensitive HPLC and HPTLC methods for simultaneous estimation of PANT and DOM in capsules.

Experimental

Apparatus

A Shimadzu (Columbia, MD) HPLC instrument (LC-10AT vp) equipped with an ultraviolet-visible (UV-Vis) detector, manual injector with 20 μL loop, and Phenomenex (Torrance, CA) C_{18} column (250 mm × 4.6 mm id, 5 μm particle size) was used. For HPTLC, a Linomat V autosprayer, Scanner-III, flat bottom and twin trough developing chambers and viewing cabinet with dual wavelength UV lamps (Camag, Muttenz, Switzerland), used. HPTLC plates used were silica gel with fluorescent indicator 254 nm, layer thickness 0.2 mm, 20 × 10 cm, aluminum backing (E. Merck KGaA, Darmstadt, Germany).

Reagents and Materials

PANT and DOM pure powder were kindly donated by Torrent Pharmaceutical (Ahmedabad, India) with 99.94 and 99.92% purity, respectively. HPLC grade methanol was purchased from SDfine Chemical (Ahmedabad, India). The water for HPLC was prepared by triple glass distillation and filtered through a nylon 0.45 μm–47 mm membrane filter (Gelman Laboratory, Mumbai, India). Ethyl acetate, ammonium acetate, acetic acid, and ammonia were procured from SDfine Chemical and were of analytical grade.

Chromatographic Conditions

(**a**) *HPLC method*.—A Phenomenex C_{18} (2) column was used at ambient temperature. The mobile phase consisted of 0.01 M, 6.5 pH ammonium acetate buffer–methanol–acetonitrile (30 + 40 + 30, v/v/v) final pH adjusted to 7.20 ± 0.02 with acetic acid–ammonia and was pumped at a flow rate of 1 mL/min. The mobile phase was filtered through a nylon 0.45 μm–47 mm membrane filter and degassed before use. The elution was monitored at 287 nm, and the injection volume was 20 μL.

(**b**) *HPTLC method*.—Solutions of the PANT and DOM were applied to silica gel $60F_{254}$ HPTLC plates (20 × 10 cm) by means of a Linomat V automatic spotter equipped with a 100 μL syringe and operated with settings of band length, 6 mm; distance between bands, 8 mm; distance from the plate edge, 10 mm; and distance from the bottom of the plate, 10 mm. The plate was developed in a twin trough chamber previously saturated for 30 min with the mobile phase, ethyl acetate–methanol (60 + 40, v/v), for a distance of 8 cm. The spots on the air-dried plate were scanned with a Scanner III at 287 nm using the deuterium source.

Preparation of PANT and DOM Standard Stock Solutions

(**a**) *HPLC method*.—Accurately weighed PANT (20 mg) and DOM (15 mg) were transferred to a 50 mL volumetric flask and dissolved in and diluted to the mark with methanol to obtain a standard solution of PANT (400 μg/mL) and DOM (300 μg/mL). Of this solution, 1 mL was further diluted to 100 mL with mobile phase to obtain a working standard solution with PANT (4 μg/mL) and DOM (3 μg/mL) for the HPLC method.

(**b**) *HPTLC method*.—Accurately weighed PANT (20 mg) and DOM (15 mg) were transferred to a 50 mL volumetric flask and dissolved in and diluted to the mark with methanol to obtain a standard solution of PANT (400 μg/mL) and DOM (300 μg/mL). This solution (1.0 mL) was further diluted to 50 mL with methanol to obtain a working standard solution of PANT (80 μg/mL) and DOM (60 μg/mL) for the HPTLC method.

Preparation of Sample Solutions

Powders (pellets) of each of 10 capsules (2 brands) were weighed and analyzed as follows: A mass of pellets equivalent to the powder of 1 capsule was weighed and transferred in a 100 mL volumetric flask, and methanol (80 mL) was added. The solution was sonicated for 15 min, and the final volume

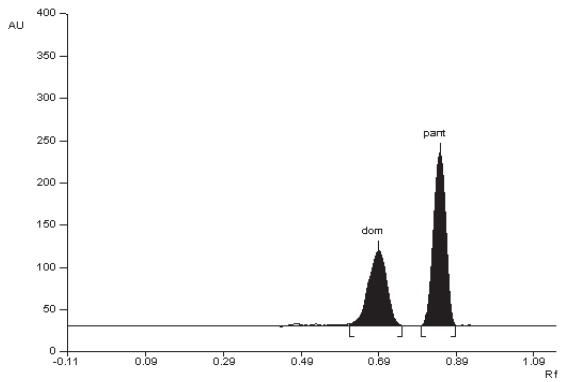

Figure 2. HPTLC densitogram of PANT and DOM with scanning at 287 nm.

Table 1. Regression analysis of the calibration curves for PANT and DOM for the proposed HPLC and HPTLC methods

	HPLC method		HPTLC method	
Parameter	PANT	DOM	PANT	DOM
Concentration range	400–4000 ng/mL	300–3000 ng/mL	80–240 ng/spot	60–180 ng/spot
Slope	334.34	282.72	17.97	14.993
Standard deviation of the slope	6.508	0.701	0.032	0.047
Intercept	–16474	3938.4	–178.2	22.2
Standard deviation of the intercept	132.21	31.37	0.72	0.52
Correlation coefficient	0.997	0.996	0.999	0.994

was diluted to the mark with methanol to obtain solution of PANT (400 μg/mL) and DOM (300 μg/mL). The mixture was then filtered through a nylon 0.45 μm–47 mm membrane filter.

Method Validation

(**a**) *Calibration curve (linearity of the HPLC method)*.—Calibration curves were constructed by plotting peak areas vs concentrations of PANT and DOM, and the regression equations were calculated. The calibration curves were plotted over the concentration range 400–4000 and 300–3000 ng/mL for PANT and DOM, respectively. Accurately measured standard working solutions of PANT and DOM (1.0, 2.0, 4.0, 6.0, 8.0, and 10.0 mL) were transferred to a series of 10 mL volumetric flasks and diluted to the mark with mobile phase. Aliquots (20 μL) of each solution were injected under the operating chromatographic conditions described above.

(**b**) *Calibration curve (linearity) of the HPTLC method*.—Calibration curves were plotted over a concentration range of 80–240 and 60–180 ng/spot for PANT and DOM, respectively. Accurately prepared standard solutions of PANT and DOM (10.0, 15.0, 20.0, 25.0, and 30.0 μL) were applied to the plate. The calibration curves were constructed by plotting peak areas vs concentrations with the help of win-CATS software. Each reading was the average of 3 determinations.

Accuracy (% Recovery)

The accuracy of the methods was determined by calculating recoveries of PANT and DOM by the standard addition method. Known amounts of standard solutions of PANT (400, 800, and 1600 ng/mL) and DOM (300, 600, and 1200 ng/mL) for the HPLC method and PANT (160, 200, and 240 ng/spot) and DOM (120, 150, and 180 ng/spot) for the HPTLC method were added to prequantified sample solutions of capsule dosage forms for the HPLC and HPTLC methods, respectively. The amounts of PANT and DOM were estimated by applying these values to the regression equation of the calibration curve.

Method Precision (Repeatability)

The precision of the instruments was checked by repeatedly injecting (n = 6) standard solutions of PANT (1600 ng/mL) and DOM (1200 ng/mL) for the HPLC method and by repeated scanning of the same spot (n = 6) of PANT

Table 2. Summary of validation parameters for the proposed HPLC and HPTLC methods

	HPLC method		HPTLC method	
Parameter	PANT	DOM	PANT	DOM
LOD[a]	147.51 ng/mL	85.82 ng/mL	29.42 ng/spot	19.03 ng/spot
LOQ[b]	400.63 ng/mL	260.08 ng/mL	89.16 ng/spot	57.69 ng/spot
Accuracy, %	98.97–99.15	99.00–99.62	97.35–99.35	98.39–98.90
Repeatabilty (RSD[c], %, n = 6)	0.538	0.167	0.194	0.302
Precision (RSD, %)				
Interday (n = 3)	0.639796–1.719493	0.198987–0.804209	0.185981–0.512181	0.281866–1.177167
Intraday (n = 3)	0.745621–1.732184	0.199954–0.826354	0.188541–0.529541	0.295411–1.179562

[a] LOD = Limit of detection.
[b] LOQ = Limit of quantification.
[c] RSD = Relative standard deviation.

Table 3. System suitability test parameters for PANT and DOM for the proposed HPLC method

Parameter	PANT ± % RSD[a]	DOM ± % RSD
Retention time, min	4.40 ± 0.01	6.40 ± 0.02
Tailing factor	1.03 ± 0.05	1.06 ± 0.02
Asymmetry	1.08 ± 0.04	1.13 ± 0.03
Theoretical plates	3400 ± 0.08	3164 ± 0.09

[a] RSD = Relative standard deviation.

(200 ng/spot) and DOM (150 ng/spot) without changing the position of plate for the HPTLC method.

Intermediate Precision (Reproducibility)

The intraday and interday precisions of the proposed methods were determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of PANT (400, 1600, and 4000 ng/mL) and DOM (300, 1200, and 3000 ng/mL) for the HPLC method and PANT (80, 160, and 240 ng/spot) and DOM (60, 120, and 180 ng/spot) for the HPTLC method. The results are reported in terms of relative standard deviation (RSD).

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines (28).

$$LOD = 3.3 \times \sigma/S$$
$$LOQ = 10 \times \sigma/S$$

where σ = the standard deviation of the response and S = the standard deviation of *y*-intercept of regression lines.

Analysis of PANT and DOM in Combined Capsule Dosage Forms

Capsules containing PANT (40 mg) and DOM (30 mg) of the following 2 brands: Alkem Lab Ltd. (Mumbai, India), and Shaimil Laboratory (Baroda, India) were purchased from local market.

The responses of capsule dosage forms were measured at 287 nm for quantification of PANT and DOM, respectively, by using HPLC and HPTLC instruments as described above. The amounts of PANT and DOM present in sample solution were determined by fitting the responses into the regression equation for PANT and DOM.

Results and Discussion

HPLC Method

To optimize the HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for PANT and DOM were obtained with a mobile phase consisting of 0.01 M, 6.5 pH ammonium acetate buffer–methanol–acetonitrile (30 + 40 + 30, v/v/v) with the final pH adjusted to 7.20 ± 0.02 with acetic acid–ammonia to obtain better reproducibility and repeatability. Quantification was achieved with UV detection at 287 nm based on peak area. Complete resolution of the peaks with clear baseline separation was obtained (Figure 1).

HPTLC Methods

Several mobile phases were tried to accomplish good separation of PANT and DOM. Using the mobile phase ethyl acetate–methanol (60 + 40, v/v) and 20 × 10 cm HPTLC silica gel $60F_{254}$ aluminum-backed plates, better separation was attained with R_f values of 0.82 for PANT and 0.61 for DOM. A wavelength of 287 nm was used for the quantification of the drugs. Resolution of the peaks with clear baseline separation was found (Figure 2).

Validation of the Proposed Method

Linearity.—Linear correlation was obtained between peak areas and concentrations of PANT and DOM in the range of 400–4000 and 300–3000 ng/mL, respectively, for HPLC and 80–240 and 60–180 ng/spot, respectively, for HPTLC. The linearity of the calibration curves was validated by the high value of correlation coefficients of regression (Table 1).

Accuracy.—The recovery experiments were carried out by the standard addition method. The recoveries obtained were 99.35 ± 0.80 and 99.08 ± 0.57% for PANT and DOM, respectively, by HPLC and 98.40 ± 0.67 and 98.75 ± 0.71% for PANT and DOM, respectively, by HPTLC (Table 2). The high values indicate that both methods are accurate.

Method precision.—The RSD values for PANT and DOM in the combined formulation were found to be 0.538 and 0.167% respectively, using HPLC and 0.194 and 0.302%, respectively, for HPTLC (Tables 2–4). The low RSD values indicate the proposed methods are repeatable.

Intermediate precision.—The low RSD values of interday (0.693–1.719 and 0.198–0.804%) and intraday (0.745–1.732 and 0.199–0.826%) variations for PANT and DOM, respectively, reveal that the proposed methods are robust (Table 2).

LOD and LOQ.—LOD for PANT and DOM was found to be 147.51 and 85.82 ng/mL, respectively, for HPLC and 29.42 and 19.03 ng/spot, respectively, for HPTLC. LOQ for PANT and DOM was found to be 400.63 and 260.08 ng/mL, respectively, for HPLC and 89.16 and 57.69 ng/spot, respectively, for HPTLC

Table 4. System suitability test parameters for PANT and DOM for the proposed HPTLC method

Parameter	PANT ± % RSD[a]	DOM ± % RSD
R_f value	0.85 ± 0.07	0.69 ± 0.21
Area (average)	4111.16 ± 0.19	2715.30 ± 0.30

[a] RSD = Relative standard deviation.

Table 5. Assay results for the combined dosage form using the proposed HPLC and HPTLC methods

	PANT ± SD[a] (n^b = 5), %		DOM ± SD (n = 5), %	
Formulation	HPLC	HPTLC	HPLC	HPTLC
A	99.72 ± 0.43	98.40 ± 0.68	99.65 ± 0.36	98.75 ± 1.14
B	100.18 ± 0.48	100.65 ± 0.72	99.12 ± 0.82	99.33 ± 0.97

[a] SD = Standard deviation.
[b] n = Number of determinations.

(Table 2). These data show that both methods are sensitive for the determination of PANT and DOM.

Assay of the Capsule Dosage Form (PANT 40 mg and DOM 30 mg/capsule)

The proposed validated methods were successfully applied to determine PANT and DOM in their combined capsule dosage form (Capsules A and B). The results obtained for PANT and DOM were comparable with the corresponding labeled amounts (Table 5).

Comparison of the Proposed Methods

The assay results for PANT and DOM in their combined dosage form obtained using the HPLC and HPTLC methods were compared by applying the paired *t*-test. The calculated *t*-value of 0.43 for PANT and 0.19 for DOM is less than the tabulated *t*-value (4.60) at the 95% confidence interval. Therefore, there is no significant difference in a determined content of PANT and DOM by the HPLC and HPTLC methods.

Conclusions

The results of the analysis of pharmaceutical dosage forms by the proposed methods are highly reproducible and reliable and are in good agreement with the label claim of the drug. The additives usually present in the pharmaceutical formulations of the assayed samples did not interfere with determination of PANT and DOM. The methods can be used for the routine simultaneous analysis of PANT and DOM in pharmaceutical preparations.

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