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RP-HPLC method for simultaneous estimation of domperidone and pantoprazole in capsule dosage form

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ABSTRACT

A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous determination of Domperidone (DOM) and Pantoprazole(PAN) in capsules. The drugs were separated on an analytical column, HiQ Sil C18V, 4.6mm x 250 mm, 5 µm (Kya Tech Japan). The mobile phase was a mixture of acetonitrile and 0.05M potassium di- hydrogen phosphate buffer (pH 6.5adjusted with ortho phosphoric acid) in ratio of 50:50%v/v plus 0.05%TEA. UV detection was performed at 290 nm. Total chromatographic analysis time per sample was approximately 8 min with DOM, PAN and acetophenone (internal standard) eluting with retention times of 4.57, 5.58, and 7.05 min, respectively. Calibration plots were linear over the concentration ranges 1–10 µg mL^{-1} and 2–20 µg mL^{-1} for DOM and PAN respectively. The limit of detection (LODs) were 0.15 and 0.03 µg/ml and limit of quantification (LOQs) were 0.51 and 0.10 µg/ml for DOM and PAN respectively. The method was validated for accuracy, precision, specificity, linearity, robustness and sensitivity. The developed method was successfully used for quantitative analysis of DOM and PAN in Pantop-D capsules.

Key Words: RP-HPLC, Domperidone, Pantoprazole, Validation, Pantop-D

INTRODUCTION

Domperidone is chemically 5-chloro-1-[1-[3-(2-oxo-1,3- dihydrobenzoimidazol-1-yl)propyl]-4-piperidyl] -1,3-dihydrobenzoimidazol-2-one and it is used as gastrointestinal emptying (delayed) adjunct, a peristaltic stimulant, and also as an antiemetic and dopaminergic blocking agent[1,2].The drug comes in many different trade names such as Costi,Motinorm, and Motillium[3] The structure of domperidone is given in Figure 1.

Pantoprazole is proton-pump inhibitor that inhibits gastric acid by blocking the H+/K+-adenosine triphosphatase enzyme system of the gastric parietal cell [4]. Its application is in the short-term treatment of erosion and ulceration of the esophagus [5]. Pantoprazole is 5- (Difluoromethoxy) – [[(3,4- dimethoxy-2-Pyridiynyl) Methyl] sulphinyl] -1*H*– benzimidazole. Its sodium form that is used in pharmaceuticals is known as Pantoprazole sodium has the structure given in figure 2.

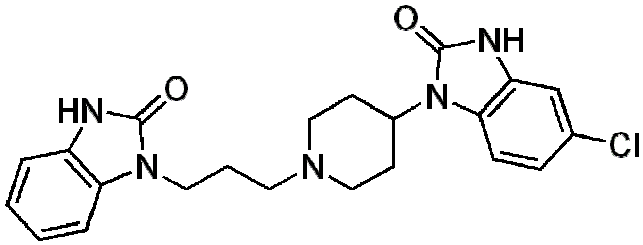

Fig .1 Structure of Domperidone

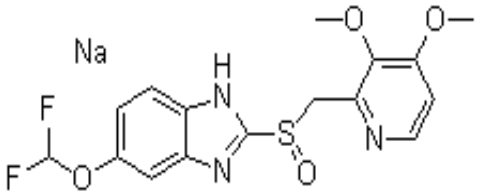

Fig.2 Structure of Pantoprazole Sodium

Combinations of DOM and PAN are many in market under different trade names such as Pantocid-D, Jipan-D, Impan-D, and Panpot Dsr, just to mention but a few. The combinations are formulated to give symptomatic relief from dyspepsia /heartburn/acid pepsin disorder associated with nausea, vomiting and epigastric pain/chronic gastritis[6]. The presence of combinations of DOM and PAN in market requires analytical method for the simultaneous determination of these drugs. Literature search showed some many analytical methods for determination of these drugs individually [7-17] and very few methods for their simultaneous determination [18-21].

The combinational use of DOM and PAN is continuously increasing in every country. However, the simultaneous analysis of these two drugs in their pharmaceutical preparation is not official in Pharmacopoeias. The few methods in the literature for the estimation of these drugs also suffer from one drawback or the other. These include relatively long runtime and low sensitivity. Thus, it became imperative to develop analytical methods for the simultaneous analysis of DOM and PAN in pharmaceutical dosage forms that will be more sensitive and accurate than the existing ones. We describe here in a simple, sensitive and validated RP- HPLC method using C18 column with short retention time for the simultaneous determination of DOM and PAN in pharmaceutical formulations. The developed method can be successfully applied to quality control and other analytical purposes.

MATERIALS AND METHODS

Instrumentation

A Jasco (Japan) PU-2080 pump was used to deliver the mobile phase to the analytical column, HiQ Sil C18V, 4.6mm x 250 mm, 5 µm (Kya Tech Japan). Sample injection was performed via a Rheodyne 7725 injection valve (Rheodyne,USA) with a 20-µl loop. Detection was achieved by an UV-2075 A UV-Visible detector (Jasco, Japan). Jasco borwin software was used for quantitative determination of eluted peaks. Degassing of solvents was achieved by helium purging before use. Dissolution of compound was enhanced by sonication on Bandelin sonerex (Bandelin, Berlin). The pH of the solution was measured using Digital pH Meter, Model DI 707 (Digisun electronics, Hyderabad, India). A UV spectrum of all drugs for selecting the working wavelength of detection was taken using a V-550 UV-Visible spectrophotometer (Jasco, Japan).

Standards and chemicals

Domperidone, Pantoprazole and Omeprazole (used as an internal standard) were gifts obtained from Aurobindo Pharma Ltd. Hyderabad, India. Pantop-D capsules (containing 10mg DOM and 20mg PAN) were purchased from local market in Hyderabad. Purified water was prepared using a Millipore Milli-Q (Bedford, M.A., USA) water purification system. Acetonitrile of HPLC grade, potassium dihydrogen phosphate, ortho Phaosphoric acid and triethyl amine of A.R. grade were purchased from Merck Ltd. (Mumbai, India).

A 0.05 M solution of potassium di hydrogen phosphate was prepared by dissolving 6.804g of potassium dihydrogenphosphate in 800 ml water and diluting to 1000 mL with water. The pH was adjusted to 6.5 with ortho phosphoric acid.

Preparation of standard drug solutions

Stock solution of PAN and DOM were prepared by dissolving 25 mg of each in separate 25mL of volumetric flask with small quantity of acetonitrile. The mixture was sonicated for about 15min and then made up to volume with acetonitrile. Daily working standard solutions of DOM and PAN were prepared by suitable dilutions of the stock solution with appropriate mobile phase.

Stock solutions of Omeprazole (I.S) were prepared by dissolving 25mg of Omeprazole in 25mL of standard volumetric flask with small quantity of acetonitrile. After getting a clear solution final volume was made up to the mark with acetonitrile. From the stock solution 20 µg/ml of solution was prepared.

Chromatographic conditions

The mobile phase consists of acetonitrile and 0.05M potassium dihydrogen phosphate buffer (pH 6.5adjusted with ortho phosphoric acid) in ratio of 50:50%v/v plus 0.05%TEA. The mobile phase was filtered before use through a 0.45µ membrane and degassed for 15 min. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 0.8 mL/min and the injection volume was 20µl. The column temperature was maintained at 23 ± 1^0C.The eluents were monitored at 290nm and a sensitivity setting of 0.005 AUFS (range 0.3). The identification of the separated drugs and I.S. were confirmed by running the chromatograms of the individual compounds under identical conditions. The optimized conditions of DOM and PAN are shown in Tables 1 and 2.

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Table:1 Optimized chromatographic conditions of the proposed method for the simultaneous analysis of pantoprazole and domperidone

S.NO	PARAMETERS	CONDITION
1	Mobile phase	acetonitrile:0.05M Potassium di-hydrogen Phosphate buffer, pH:6.5 (50:50 %, v/v) + 0.05% TEA.
2	Stationary phase	HiQ Sil C_{18}V, 4.6mm x 250 mm, 5µm.
3	Flow rate (ml/min)	0.8
4	Runtime (min.)	10
5	Column temperature ^oC	23
6	Volume of injection (µl)	20
7	Detection wavelength (nm)	290
8	Internal standard	Omeprazole
9	Retention times(min) Domperidone Pantoprazole Omeprazole	 4.6 5.5 7.5

Calibration of standards

The separate standard calibration curves were constructed for each of the drugs. Different volumes of stock solutions were separately and accurately transferred into different 25mL volumetric flasks and diluted to mark to yield concentration range 2-20µg/ml for PAN and 1-10 µg /ml DOM. To the above solutions 20 µg/mL of Omeprazole (IS) was added and the final volume was made up to the mark. The calibration curve was obtained by plotting the analyte to I.S peak area ratio against concentration of drug (Figures 4 and 5).

A 20-µL aliquot was injected into the analytical column. Quantitative analysis was based on peak area measurements as ratios to the peak area of internal standard

Method validation

Validation of the developed method was performed in accordance with International Conference on Harmonization (ICH) guidelines [22-24]. Validation parameters examined were specificity, linearity, sensitivity, LOQ, LOD, precision, accuracy, robustness and system suitability.

Specificity and selectivity

Selectivity of the developed method was tested by checking for the interference in the analysis of a blank solution (without any sample) and then a mixture of pure drug solution of 20µg/ml was injected into the column, under optimized chromatographic conditions, (Figure 3) to demonstrate the separation of both DOM and PAN from any of

the impurities, if present. Furthermore, four different samples of the drug matrix solution (drug standard and excipients) were injected to examine the interference of the excipients.

Linearity

The linearity of the HPLC method was determined at six concentration levels ranging from 1-10 µg ml^{-1} for DOM and 2-20 µg ml^{-1} for PAN**.** Each of these drug solutions (20µl) was injected into the chromatographic system (n=3). The peak area and retention time were recorded, and the mean values of peak area ratio were plotted against concentrations to obtain the calibration curves (Figures 4 and 5).

Limit of detection & Limit of quantification

The LOD is defined as the smallest level of analyte that gives a measurable response. Limit of quantification LOQ is defined as the lowest concentration at which the precision expressed by relative standard deviation (RSD) is less than 2% and accuracy expressed by relative difference in the measured and true value is also less than 2%. In other words, the analyte response is 10 times greater than the noise response.

The LOD and LOQ for both DOM and PAN were determined according to ICH guideline Q2B1. The LOD and LOQ were estimated from the standard calibration curve. The residual standard deviation of regression line or standard deviation of y intercepts of regression lines used to calculate LOD and LOQ. Here, LOD=3.3* D/S and LOQ=10*D/S. Where, D is the standard deviation of y intercept of regression line and S is the slope of calibration curves. The analytical characteristics of the proposed method derived from the calibration curves are as shown in Table 3.

Precision

The intra - day precision (repeatability) was studied by analyzing each of the samples repeatedly (n=6) on the same day, at three different concentration levels (3, 5, 10 and 5, 15, 20 µg ml^{-1} for DOM and PAN respectively). The results are shown in tables 4and 5 respectively. The inter - day precision (reproducibility) was studied by analyzing the three different concentration levels of drugs six times (n=6) per day for three consecutive days. Results were expressed as RSD% (Table 6). The intra- and inter-assay RSD% values were satisfactory. Both intra-day and inter-day sample concentrations were estimated from standard curve concurrently prepared on the day of analysis.

Accuracy

The accuracy of the method was tested based on % Recovery by the assay of known and added amount of analyte. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the tablets (DOM 7.5 µg/ml and PAN 12.5 µg/ml) with three different concentrations of standards(DOM 3, 5, 10 µg/ml and PAN 5, 10, 20µg/ml) Accuracy was presented as percent error (relative error), [(measured concentration-added concentration)/added concentration] x 100 (%), (Table 6).

Robustness

Robustness of the method was determined by small deliberate changes in flow rate, mobile phase pH and mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low ratio value of relative standard deviation indicating that the method was robust.

System suitability

It is defined as tests to measure the method that can generate result of acceptable accuracy and precision. The system suitability was carried out after the method development and validation have been completed. For this, six replicates of the working standard sample were injected and parameters like plate number (N), tailing factor (k), resolution (R), relative retention time (α), HETP, capacity factor (k^{l}), plates per meter and peak symmetry of samples were measured. The results are listed in Table 7.

Recovery of domperidone and pantoprazole in Capsules

The contents of ten capsules were weighed and sample of powder equivalent to 25 mg of domperidone and pantoprazole was extracted with acetonitrile in a 25mL volumetric flask using ultra sonicator. This solution was filtered through whatmann No 1 filter paper. The solution obtained was diluted with the mobile phase so as to obtain a concentration in the range of linearity previously determined. An aliquot of the internal standard was added to the sample solution prior to the dilution so as to give a final concentration of internal standard 20µg/mL. All determinations were carried out in triplicate, (Table 8).

RESULTS

A non-polar C-18 analytical chromatographic column was chosen as the stationary phase for the separation and determination. The mobile phase system used for this method was arrived at after trial of a number of eluting systems. Preliminary trials using solvent systems consisting of methanol, water and acetonitrile in different ratios for the separation of DOM and PAN gave prolonged retention time and tailing. However the use of mobile phase system with composition of 50:50 % v/v acetonitrile and 0.05M potassium dihydrogen phosphate buffer, pH 6.5 and 0.05% TEA used as modifier, provided an efficient separation of DOM and PAN with optimum retention time, Table 2. A flow rate of 0.8 ml/min was found to be optimum for the studied range 0.5-1.5 ml/min. The rate chosen (0.8 ml/min) gave an optimum retention time, baseline stability and reduced noise.

Table: 2. Optimization of mobile phase for the determination of domperidone and pantoprazole

S. No	Composition of mobile phase	Retention time (min)		Total runtime (min)
		DOM	PAN	
1	45:55	4.2	5	10
2	50:50	4.6	5.5	10
3	55:45	5	6	10

Figure 3. HPLC chromatogram of blank spiked with Domperidone (10µg/mL) and Pantoprazole (20 µg /mL).

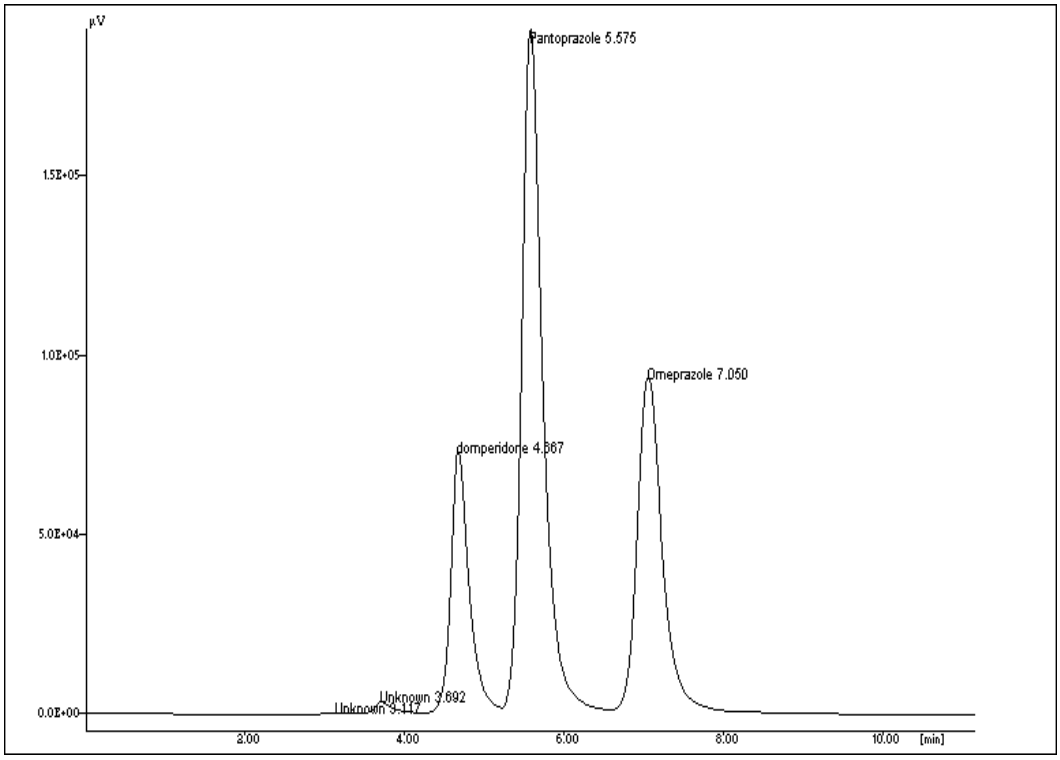

Table-3: Analytical Characteristics of the proposed method derived from the standard calibration curve

Parameters	DOM	PAN
Slope	0.0665	0.2669
Intercept	0.0029	0.296
equation of regression	y=0.0665x+0.0029	y=0.2669x+0.296
Correlation coefficient	0.9973	0.9991
LOD (µg/mL)	0.15	0.03
LOQ (µg/mL)	0.51	0.10

Figure 4. Calibration curve of Domperidone

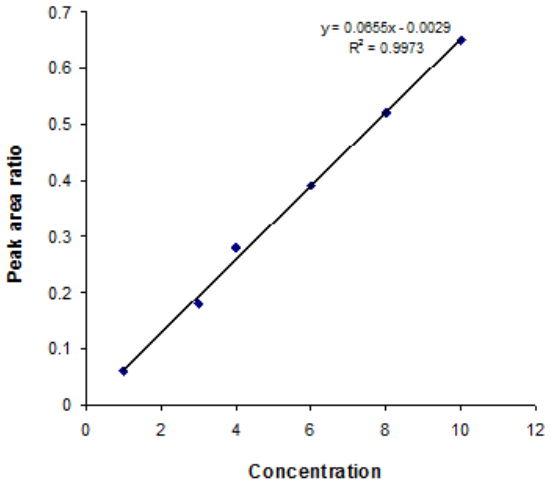

Figure 5: Calibration curve of Pantoprazole

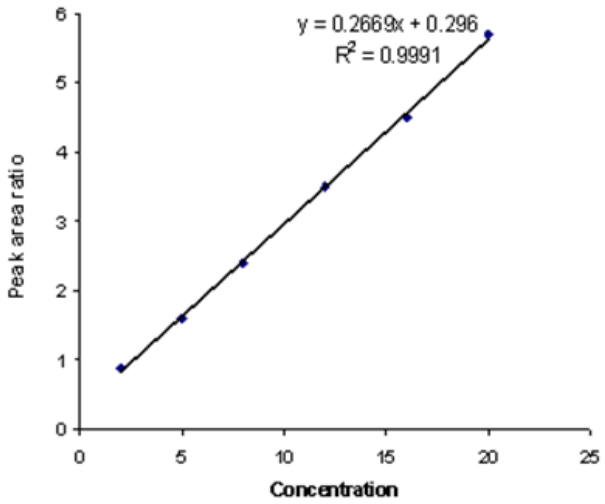

Table: 4. Precision of proposed HPLC method for DOM

S.NO	Concentration taken (µg/mL)	Intra-day		Inter-day	
		Measured Concentration (µg/mL)±S.D	% C.V	Measured Concentration (µg/mL)	%C.V
1	3	3.13 ± 0.05	1.6	2.98 ± 0.03	1.3
2	5	4.95 ± 0.07	1.4	5.05 ± 0.02	0.39
3	10	9.89 ± 0.1	1.01	10.07 ± 0.06	0.50

Table: 5. Precision of proposed HPLC method for PAN

S. NO	Concentration taken (µg/mL)	Intra-day		Inter-day	
		Measured Concentration (µg/mL)±S.D	% C.V	Measured Concentration (µg/mL)	% C.V
1	5	4.95 ± 0.06	1.2	5.04± 0.04	0.9
2	15	15.25 ± 0.07	0.5	14.95 ± 0.12	0.7
3	20	20.13 ± 0.15	0.8	19.93 ± 0.11	0.6

Table-6: Results of accuracy studies

Analyte	Amount of drug added to the analyte (µg/mL)	Measured con. (n=5)(µg/mL)±SD	% Recovery	±%RSD
DOM	3	2.98±0.16	99.63	1.32
	5	5.05±0.12	101.00	0.45
	10	9.97±0.21	99.70	0.21
PAN	5	4.95±0.12	99.02	1.32
	15	15.20±0.22	101.30	0.45
	20	20.15±0.21	100.70	0.21

Table-7. System suitability parameters of DOM and PAN

S.No	Parameter	Value	
		Domperidone	Pantoprazole
1	**Relative retention time**	1.26	1.19
2	**Resolution**	3	2
3	**Capacity factor**	2.3	2.9
4	**Theoretical plates**	2752	2785
5	**Peak Symmetry**	1	1
6	**HETP**	0.01	0.009

Table-8. Amount of DOM and PAN in Pantop-D capsule by proposed HPLC method

Formulation	Labeled amount (mg)	Amount (mg) Found. Mean ±S.D., n=3	% Found Mean ±S.D
Domperidone	10	9.95 ± 0.15	99.5 ± 0.4
Pantoprazole	20	20.25±0.20	101.25±0.3

The internal standard mode of quantification was applied in order to minimize the contribution of sample preparation, injection variation and column deterioration to the final results. Among several drugs that were tested, Omeprazole was found to be good internal standard for this method, because it fulfilled the requirements for good internal standard. Its physicochemical properties are similar to that of DOM and PAN and elute close to analyte.

The HPLC representative chromatograms recorded for the mixture of domperidone and pantoprazole drugs and internal standard is shown in Figure 3.It can be seen from Figure 3 that the two drugs were well separated from each other and they were clearly separated from the internal standard. Thus, the HPLC method presented in this study is very selective for determination of DOM and PAN.

The linearity of calibration curves for the drugs in pure forms was established over the concentration ranges of 2-20 µg/mL and 1-10 µg/mL for PAN and DOM respectively. Separate calibration plots for DOM and PAN were constructed by plotting DOM/IS or PAN/IS peak-area ratios against respective concentrations. This linearity is represented by a linear regression equation as follows.

Y_{DOM} =0.0655 x – 0.0029 (r^2 = 0.9973) and Y_{PAN} = 0.2669 x + 0.296 (r^2 = 0.9991)

The mean ± standard deviation (SD) for the slope, intercept and correlation coefficient of standard curves (n=6) were calculated. Limit of detection was found to be 0.15µg/mL for domperidone and 0.03 µg/ml for pantoprazole (signal to noise ratio 3) and Limit of quantification was found to be 0.5 µg/mL for DOM and 0.1 µg/mL for PAN, Table 3.

Intra-day precision and accuracy were studied by six replicate measurements at three concentration levels. Inter-day precision and accuracy were conducted during routine operation of the system over a period of three consecutive days. Statistical evaluation revealed that relative standard deviation of two drugs at different concentration for six injections was less than 2.0. Precision and accuracy data are shown in Tables 4-6.

For system suitability, six replicates of standard sample were injected and studied the parameters like plate number (N), resolution (R) and relative retention time (α), HETP, capacity factor (k^I), plates per meter and peak symmetry of samples. The data is as shown in Table 7.

The percent recoveries of DOM and PAN was good under most conditions and didn't show any significant change when the critical parameters were modified, Table 8. The tailing factor was always less than 2.0 and the components were well separated under all the changes carried out. Considering the modifications in the system suitability parameters and the specificity of the method, as well as the carrying out of the experiment at room temperature one may conclude that the method conditions were robust.

DISCUSSION

The chromatographic method was optimized by changing various parameters, such as pH of the mobile phase, organic modifier and buffer used in the mobile phase. Retention of pantoprazole and domperidone had more dependence on pH of the mobile phase when compared to omeprazole. The separation of peaks was also dependent on pH of the buffer and the percentage of acetonitrile.

Under the presently prescribed conditions, the recoveries of two drugs were found to be from 99.5 to 101.25 % respectively,(Table 8). Medium concentration of buffer (0.05Mpotassiumdihydrogen phosphate, pH adjusted to 6.5 with orthophsphoric acid) was used to reduce the tailing. A HiQ sil C18V column was used and the buffer pH in the mobile was 6.5, which is within the limits (pH 2-8) specified by the manufacturers (Kya tech).

The differences of less than 2.0 % for both intra- and inter-day data reflect the accuracy of the method. The observation of C.V. less than 2.0 for both intra- and inter-day measurements also indicates high degree of precision. In the present method, we have established a linearity range of 2-20 µg /mL; this linearity range covers all the strengths of DOM and PAN. Hence this method can be applied for quantifying the low levels of the drugs in pharmaceutical dosage forms and other pharmacokinetic studies.

CONCLUSION

A simple reversed-phase high-performance liquid chromatographic method was developed and validated for the simultaneous determination of Domperidone and Pantoprazole in capsules . The method has significant advantage over other techniques used for simultaneous determination of the two compounds. The major advantages of this method are the simple preparation, the rapidity of separation, the efficiency of analyzing the two compounds simultaneously. However, further studies should be performed in order to use the method to evaluate the stability of pharmaceutical formulations that contain these compounds.

REFERENCES

[1] Sethi PD. HPLC-Quantitative Analysis of Pharmaceutical Formulations. CBS Publications and Distributors, New Delhi,**1993**.
[2] British Pharmacopoeia **1999**; Vol-I: 545-546.
[3] www.wikipedia.com
[4] Ritter J. M., Lewis L.D., Mant T.G.K., "A Textbook of Clinical Pharmacology", 4th ed., Arnold LTD London, **1999**, 365.
[5] Ewin K.J., "Goodman & Gilman's. The Pharmacological Basis of Therapeutics", 10th ed., McGraw-Hill Inc., London, **2001**, 1007.
[6] A text book of "Essentials of Medical Pharmacology" By K.D.Tripath, Jaypee Publications, 6th edition.
[7] SS. Zarapkar, BB. Salunke, *Indian Drugs* **1990**, 27, 537-540.
[8] M. Varalakshmi, J. Vijaya Ratna, K. Krishna Chaitamya, and D. Samson Israel. *IJPRD*, **2011**, 3(4) 61-64.
[9] MJ Smit, FCW Sutherland, HKT Humdt, KJ Swart, AF Humdt, J Els. *Journal of Chromatography* A **2002**, 949, 65-70.
[10] Y Rama Mohan, AB Avadhanulu. *Indian drugs* **1998**, 35, 754-755.
[11] M. Tanaka, H. Yamazaki, *Anal. Chem*., **1996**, 68 (9), 1513-1516.
[12]ABN Nageswara Rao, Ojeyemi M. Olabemiwo, V.J. Rao, JVLNS. Rao, *Der Pharmacia Lettre*, **2011**, 3(5), 318-325.
[13]AKM. Pawar, ABN Nageswara Rao, D. Gowri Sankar, *Der Pharmacia Lettre*, **2011**, 3(6):58-67
[14] Rajnish Kumar, Harinder Singh and Pinderjit Singh, *J. Chem. Pharm. Res*., **2011**, 3(2):113-117.
[15] QB. Cass , ALG. Degani, NM. Cassiano, JJ. Pedrazolli, *J. Chromatogr. B*, **2001**, 766,153-160.
[16] K. Basavaiah, UR. Anil Kumar, *Indian Journal of Chemical Technology*, **2007**, 14, 611-615.

[17] A. Tivesten,S. Folestad, V. Schonbacher, K. Svensson, *FABAD J. Pharm. Sci*., **2003**, 28, 85-92.
[18] RB. Kakde, SM. Gedam ,NK. Chaudhary, AG. Barsagade, DL. Kale DL, AV. Kasture, *International Journal of Pharm Tech Research*, **2009**, 1(2), 386-389.
[19] Deeptijain and sahu R., Preeti, *Indian Journal of Pharmaceutical Science*, **2005**, 503-505
[20] T. Sivakumar , R. Manavalan, K. Valliapan, *Acta Chromatographica*, **2007**, 18, 130-142.
[21] N. Ramakriskna, KN. Vishwottam, and Kotaeshwara, *Journal of Chromatography* B, **2005**,326-329
[22] ICH, Q2B- Validation of Analytical Procedures: Methodology, International Conference on Harmonization, Nov, **1996**.
[23] International Conference on Harmonization, ICH Q1 A (R2); Stability Testing of New Drug Substances and Products **2003**.
[24] ICH Steering Committee, International conference on harmonization (ICH) of technical requirements for registration of pharmaceuticals for human use, validation of analytical procedure methodology, Geneva, **1996**.