BJMHR
British Journal of Medical and Health Research
Journal home page: www.bjmhr.com

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Pantoprazole and Domperidone In Pharmaceutical Dosage Forms

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ABSTRACT

A simple, rapid, precise, accurate and sensitive reverse phase high performance liquid chromatography(RP-HPLC) method for simultaneous estimation of Pantoprazole and Domperidone in pharmaceutical dosage forms have been developed and validated. Drug was resolved on a C18 column (Phenomenex phenyl hexyl column, 250mm*4.6mmi.d,5um). Utilizing mobile phase of water with 0.4%v/v triethyl amine and acetonitrile with diluted orthophosphoric acid pH adjusted to 5.2 in a ratio of 50:50 of water and acetonitrile respectively. Mobile phase was delivered at the flow rate of 1.0ml/min. Ultraviolet detection was carried out at 236nm. Separation was completed within 7.75 minutes. Calibration curve was linear with correlation coefficient (r2) =0.999. Using etoricoxib 10ug/ml as IS. Recovery was between 99.26, 100.2 percentage. The standard deviation was found to be less than 1% for the assay of tablet.. The proposed methods were successfully employed for the estimation of Pantoprazole and Domperidone in combined tablet formulation.

Keywords: Pantoprazole (PAN), Domperidone (DOM) and Etoricoxib, RP-HPLC, CI8 (4.6*250) mm, 5 micron column, Validation.

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Received 19 September 2016, Accepted 22 October 2016

Please cite this article as: Sharma A *et al.*, Development and Validation of RP-HPLC Method for Simultaneous Estimation of Pantoprazole and Domperidone In Pharmaceutical Dosage Forms. British Journal of Medical and Health Research 2016.

INTRODUCTION

Quantitative analysis is of enormous importance in science and pharmaceutical Industry. Modern medicines for human use are required to meet exact standards which relate to their quality, safety and efficacy. The importance of various quantitative analytical methods namely assays are very much essential to find out the percentage purity, the active content of the pharmaceutical product etc. The choice and selection of the assay methods will depend on the type of substance to the estimated to find out the purity of the pharmaceutical product.

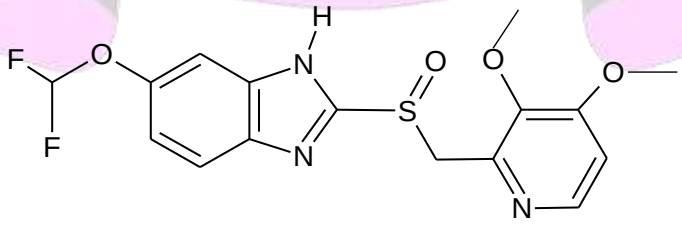
The drug analysis plays an important role in the development of drugs their manufacture and therapeutic use. Pharmaceutical industries rely upon quantitative chemical analysis to ensure that the raw material used and the final product obtained meet the required specifications. The number of drugs and drug formulations introduced into the market has been increasing at an alarming rate. These drugs or formulation of the existing drugs or novel dosage forms or multicomponent dosage forms. With the growth of Pharmaceutical Industry during the last few several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. In the absence of suitable and simple methods of analysis for complex formulation, it would be difficult to control the quality of drug products. Standard analytical procedures for the drugs in formulations are complex mixture may not be available in pharmacopoeia or in other literature. Hence it becomes essential to develop newer analytical methods which are accurate, precise, linear, specific, simple& rapid.

The multicomponent dosage forms proved to be effective due to the combined mode of action on the body. The complexity of dosage forms including the presence of multiple drug entities posses considerable challenge to the analytical chemist during the development of assay procedure. The estimation of individual drugs in these multicomponent dosage forms becomes difficult due to cumbersome extraction or isolation procedure.

PANTOPRAZOLE

Molecular formula : $C_{16}H_{15}N_3F_2O_4S$

Structure



Chemical name : 5-(difluromethoxy)_2{(3,4-dimethoxypyreidin2yl) methyl sulfinyl }-3H-benzoimidazole

Molecular weight : 383.371

Description : A white to off-solid

Solubility : Freely soluble in water; practically insoluble in hexane; very soluble in phosphate buffer

Melting point : 139° to 140°

p Ka : 3.29; 8.19(21 ° to 22°)

Category : proton pump inhibitor

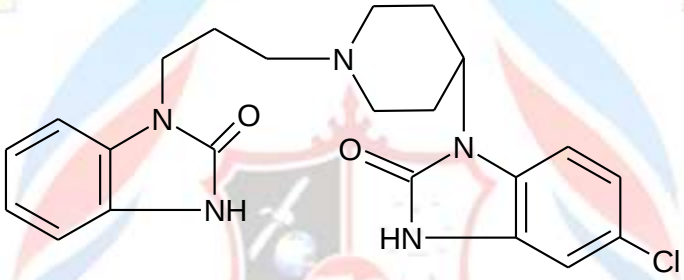
Dose : 20 – 40 mg

Mechanism of Action: Pantoprazole inhibit H^+K^+ATPase pump function there by healing the acid related conditions. pantoprazole is a proton pump inhibitor drug used for short treatment erosion and ulceration of the esophagus caused by gastro esophageal reflux

DOMPERIDONE

Molecular formula : $C_{22}H_{24}ClN_5O_2$

Structure :



C**hemical name** : 5-chloro-1-{1-(3-(2,3-dihydro2-oxo1H-benzimidazol–yl)propyl)-4-piperdinyl)-2H-benzimiazol-2 one

Molecular weight : 425.9

Description : A white to slightly beige coloured.

Melting point : 236 - 239 C

Solubility : In alcohol 1in 400, 1n water 1in 50,000

Category : Dopamine receptor blocker, antiemetic

Mechanism of Action : Abolishes nausea and vomiting due to a wide variety of causes .Increases peristaltic activity of the antrum and duodenum and improves gastric emptying Increase lower oesophageal sphincter pressure and reduces oesophageal and pressure

Dose: 10-20 mg daily

MATERIALS AND METHOD

Pantoprazole and Domperidone standards were obtained from Alidac Laboratories, Ltd. (Ahmedabad, India), methanol, acetonitrile and buffer (HPLC grade) were obtained from Qualigens Fine Chemicals (Mumbai, India). Double distilled water was used throughout the

experiment. Other chemicals used were have analytical or HPLC grade.

Chromatographic Conditions:

A chromatographic system prominence consisting of quaternary solvent delivery pump, a degasser, and column oven and photodiode array detector, LC20-AT series, C18 (4.6*250) mm, 5 micron column was used. The instrumental settings were a flow of 1Ml/min. The injection volume was 10ul.The detection wavelength was 236 nm for all three analytes. The peak purity was checked with the photodiode array detector from LC20-AT.

Mobile phase:

Acetonitrile and buffer (pH 4) with 50:50, 60:40 and 75:25 ratios were tried as mobile phases. At 50:50 ratio, the peaks were eluted at 4.91 min and 2.94 min for pantoprazole and domperidone. At 60:40 ratio, the peaks were eluted at 3.8 min and 2.9 min for pantoprazole and domperidone respectively. At 75:25 ratio, the peaks were eluted at 3.1 min and 2.1 min for pantoprazole and domperidone. So the ratio of 50:50 was selected for further studies.

Standard Stock Solution:

a)10 mg of domperidone was taken in a 10 ml standard flask and diluted with few ml of mobile phase until the sample dissolves completely and make up the volume to 10 ml with mobile phase .

b)10 mg of Pantoprazole was taken in a 10ml standard flask. To this 2ml of mobile phase was added for dissolving the drug. Shake it for 1 minutes, to get a clear solution and make up the volume to 10 ml with mobile phase solution.

The internal standard solution was prepared by taking 10 mg of Etrocoxib in a 10 ml standard flask. It is dissolved by adding 3 ml of mobile phase, shake it for few minutes to get a clear solution and make up the final volume to 10 ml with mobile phase.

The final standard solution was prepared in such a way that each standard flask contains 2,4,6,8,10µg and 4,8,12,16,20µg of domperidone and pantoprazole and 10µg of etrocoxib (IS).

Preparation of Formulation Solutions:

Twenty tablet containing 20mg of pantoprazole and 10 mg domperidone were weighed and finely powdered. From the powdered tablet, a quantity of powder equivalent to 10mg was weighed .Then was then extracted This was then filtered and finally it is diluted to 1000µg/ml with mobile phase. From this final dilution were prepared contain 4 µg/ml of domperidone and 8 µg/ml of pantoprozole& 10µg/ml of internal standard for brand A .For brand B it was 6 µg/ml of domperidone, and 12µg/ml of pantoprozole & 10µg/ml of internal standard.

Method or Recording of chromatogram:

With the optimized chromatographic conditions mentioned above, a steady baseline for about

15 min. was recorded and shown in Figure 1. After the stabilization of the baseline for about 15 min., the standard solution were injected and chromatograms were recorded until the reproducibility of the peak areas was satisfactory and finally 20 µl of the standard solution of the individual samples were injected and the chromatograms were recorded . Successive aliquots of 20µl of mixed standard solutions were injected and the chromatograms were recorded .The typical chromatograms of the sample solutions was also recorded . The chromatograms of sample showing the3D image & peak purity profile and peak purity curves of pantoprazole, domperidone and etrocoxib (IS) were shown This procedure was repeated using the sample solution. The peak areas were noted and the response factors of the standard and sample solution peaks were calculated. The elution order of mixture was found as pantoprazole (retention time 4.986), domperidone (retention time 2.950) and etrocoxib (IS). (retention time 7.541) figure 2-6.

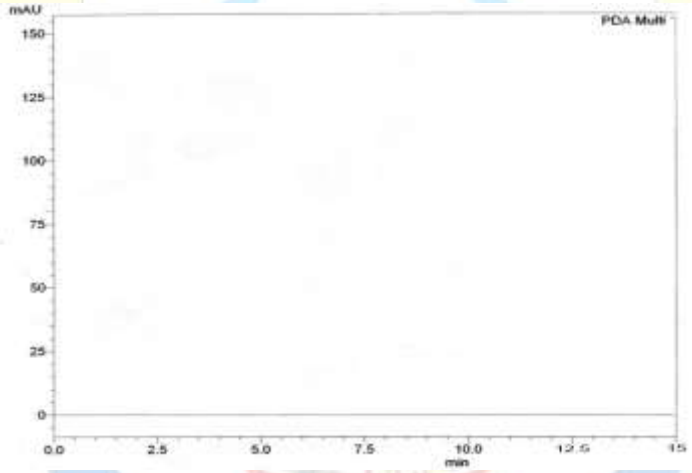


Figure 1: Chromatogram of blank (mobile phase only)

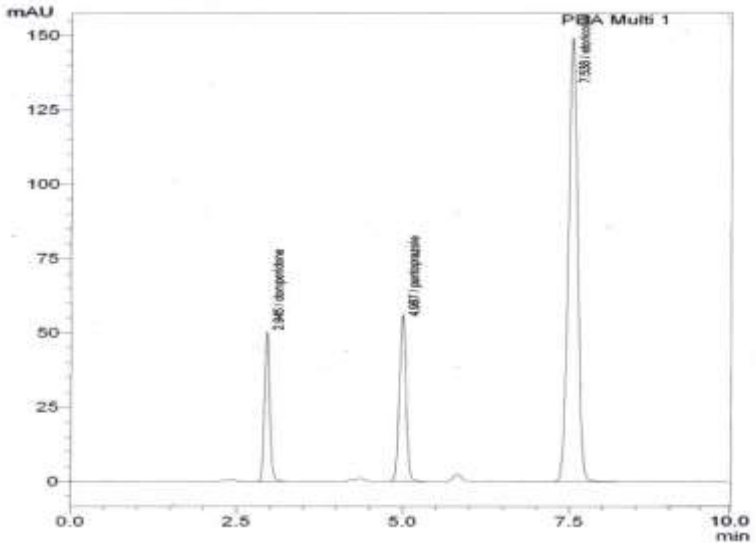


Figure: 2 Typical chromatogram of standard solution of pantoprozole domperidone and Etoricoxib(IS) for brand A

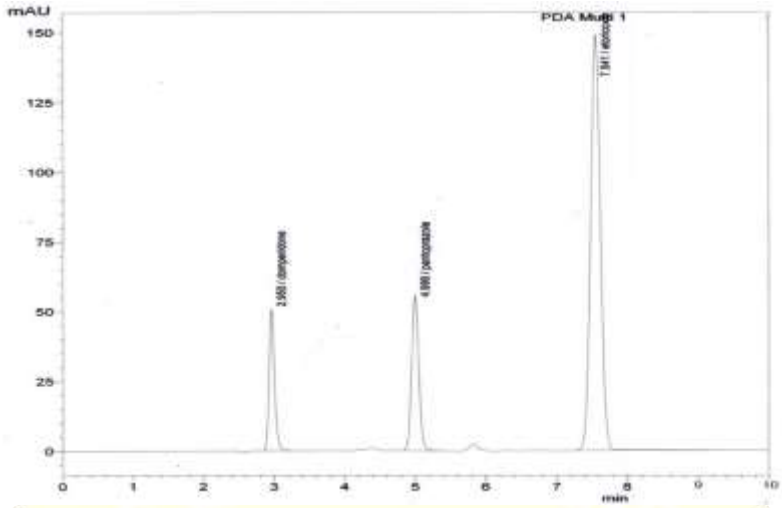


Figure: 3 Typical chromatogram of standard solution of pantoprozole ,domperidone and etoricoxib(IS) for brand B

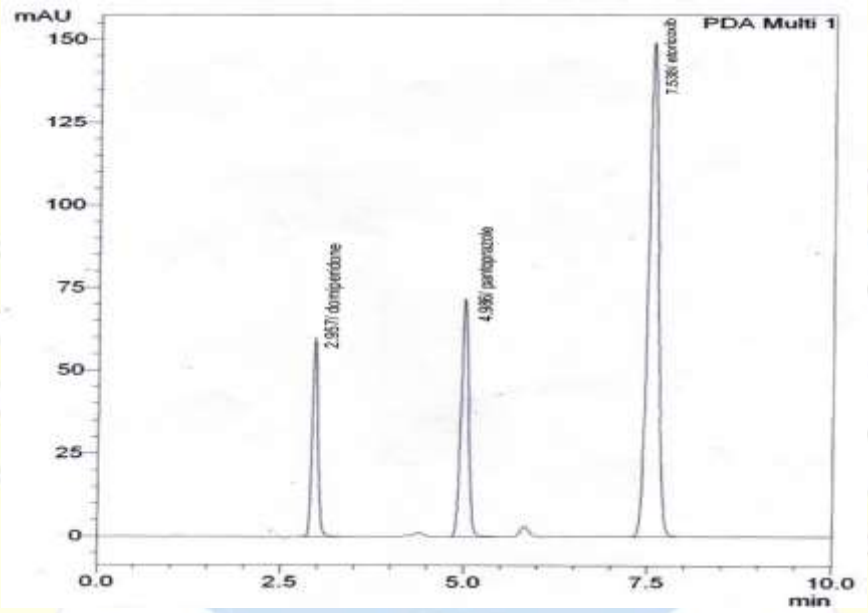


Figure 4: Chromatogram of recovery study

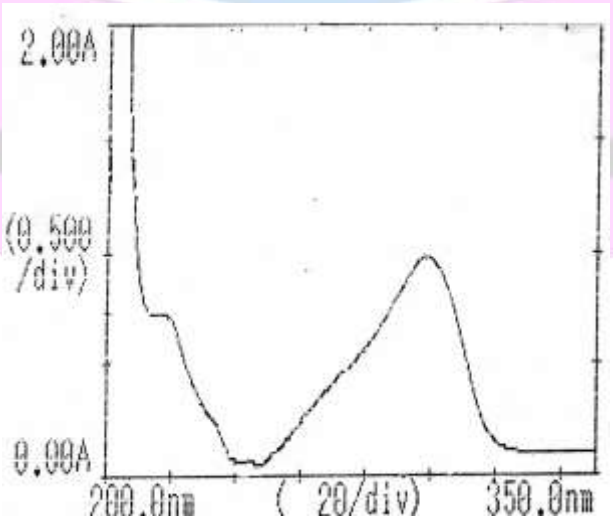


Figure: 5. UV spectrum of pantoprazole

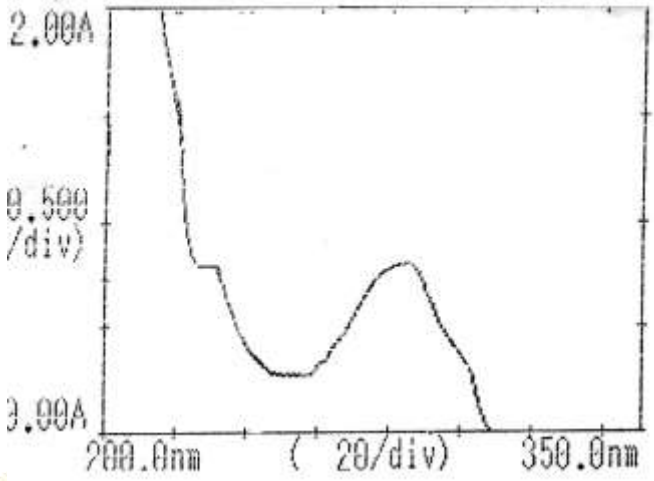


Figure: 6. UV spectrum of domperidone

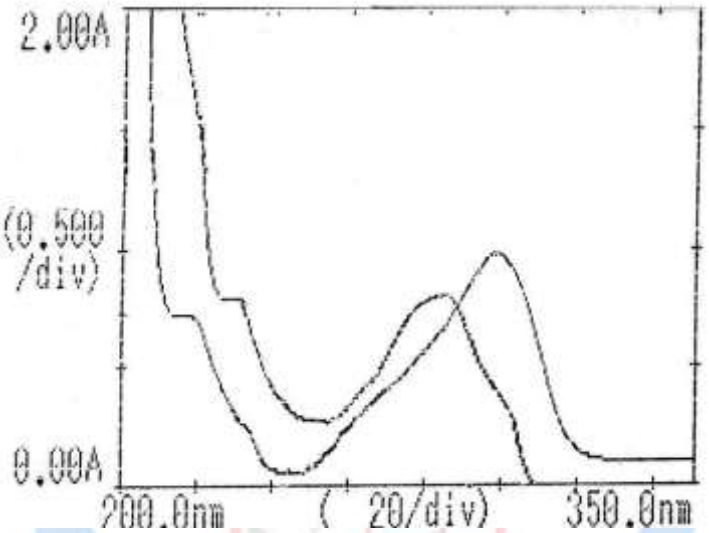


Figure: 7. Overlain UV spectrum of pantoprozole and domperidone

Validation of the HPLC Method

Method validation is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, quality, purity, and potency of the drug substances and drug products. Simply, method validation is the process of proving that an analytical method is acceptable for its intended purpose. A successful validation guarantees that both the technical and regulatory objectives of the analytical methods have been fulfilled. The transfer of a method is best accomplished by a systematic method validation process. The real goal of validation process is to challenge the method and determine limits of allowed variability for the conditions needed to run the method.

Significance of Method Validation:

The quality of analytical data is a key factor in the success of a drug development programme. The process of method development and validation has a direct impact on the quality of these data.

- To trust the method.

- Regulatory requirement.

Analytical validation is a very important feature of any package of information submitted to international regulatory agencies in support of new product marketing or clinical trials applications. A thorough method development can almost rule out all potential problems, at the same time, a thorough validation programme can address the most common ones and provide assurance to the intended purpose (can be used with 100% confidence). In other words, a thorough validation can fulfill all the technical and regulatory objectives. A direct consequence and most significant out come from any method validation exercise is 'the development of meaningful specifications can be predicted upon the use of validated analytical procedures that can assess changes in a drug substance or drug product during its life time.

Analytical characteristics listed below may not be applicable to every test procedure or every particular material. It will mostly depend on the purpose for which the procedure is required, however, these following aspects of validation should be given due importance.

RESULTS AND DISCUSSION

Estimation:

Estimation of pantoprazole and domperidone in dosage forms by High Performance Liquid Chromatography was carried out using optimized chromatographic conditions. The standard and sample solutions were prepared and chromatograms were recorded.

The peak area ratios of standard and sample solutions were calculated. The assay procedure was repeated for 6 times and mean peak area, mean peak area ratio, mean weight of standard drugs, mean weight of sample taken for assay were calculated. The percentages of individual drugs found in formulations, mean, and standard deviation in formulations were calculated and presented in Table 1. The results of analysis shows that the amount of drugs was in good agreement with the label claim of the formulation.

Table 1: Analysis of Formulation

Brand*	Drug	Label Claim Mg/Tablet	Estimated* Amount Mg/Tablet	% Label claim*	% RSD*
A	Domperidone	10mg	9.98	99.82	0.22
	Pantoprazole	20mg	20.09	100.45	0.27
B	Domperidone	10mg	9.98	99.98	0.17
	Pantoprazole	20mg	20.05	100.25	0.14

* Mean of Three Replicates A-Pentab-D, B-Dompan

Validation of the method:

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out 6 times and the percentage recovery and percentage relative standard deviation of the percentage recovery were calculated and presented in Table 2. The

chromatogram of the recovery studies was recorded and shown in Figure. From the data obtained, recoveries of standard drugs were found to be accurate and are within the specified limits.

Table 2: Accuracy (Recovery Studies)

Drug	% Recovery*		% RSD*	
	50% level	100% level	50% level	100% level
pantoprazole	99.94	100.11	0.04	0.05
Domperidone	99.80	100.2.	0.05	0.02

* Mean of three Replicates

The precision of the method was determined by studying repeatability and reproducibility. The response factor of drug peaks and percentage relative standard deviation were calculated and presented in Table. 3 & 4. The results revealed that the method developed is reproducible.

The standard drug solutions in varying concentrations ranging from 50 to 150 % of the targeted level of the assay concentration containing internal standard were examined by the assay procedure. The linearity and range for drugs was found to be from 2 to 10 µg/ml. for domperidone and 4to 20 µg/ml for pantoprazole

Table 3: Intraday Studies:

Level	Concentration (µg/ml)		Peak area		% RSD*	
	Dom *	Pan *	Dom	Pan	Dom	Pan
I	2	4	108150	148726	0.25	0.54
			108700	147926		
			109300	149546		
II	4	8	219000	291792	0.16	0.27
			219860	291142		
			220400	292692		
III	6	12	328500	444510	0.15	0.24
			327900	443173		
			328900	442373		

Mean of three replicate, Dom=Domperidone; Pan*= Pantoprazole

Table 4: Interday Studies:

Day	Concentration (µg/ml)		Peak area		% RSD*	
	Dom *	Pan*	Dom	Pan	Dom	Pan
1	2	4	108650	148726	0.15	0.38
2			108600	147926		
3			108350	147625		
1	4	8	220400	292692	0.37	0.31
2			219000	291142		
3			218992	291736		
1	6	12	328900	442373	0.15	0.03
2			328500	442200		
3			327900	442100		

* Mean of Three replicates, Dom*=Domperidone, Pan*=Pantoprazole.

Repeatability of injection:

A standard solution of mixture of drugs was injected 6 times and its % RSD was calculated, table 6.

The response factor, slope, intercept and correlation coefficient values were calculated. The correlation coefficient of domperidone and pantoprazole were found to be 0.9999 and 0.9999 respectively. The calibration curves were plotted using response factor Vs concentration of the standard solutions (Figure 8 and 9). The calibration graph shows that linear response was obtained over the range of concentrations used in the assay procedure. These data demonstrates that the methods have adequate sensitivity to the concentration of the analytes. The range demonstrates that the method is linear outside the limits of expected use.

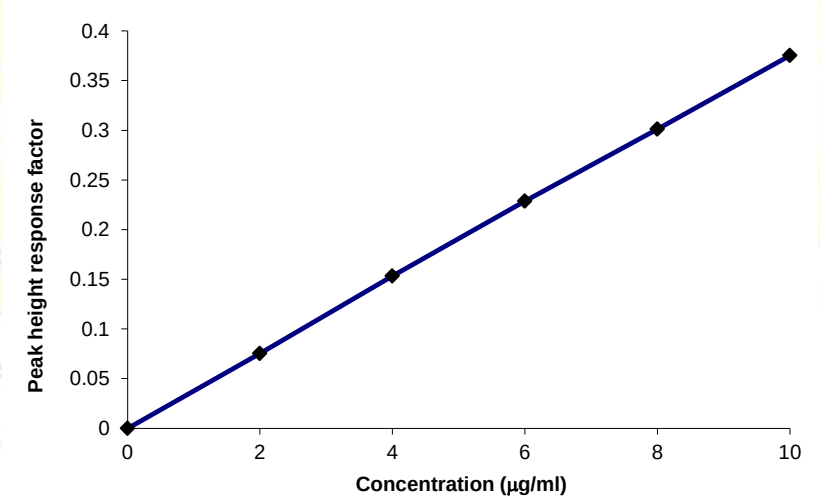


Figure: 8. Calibration graph of Domperidone

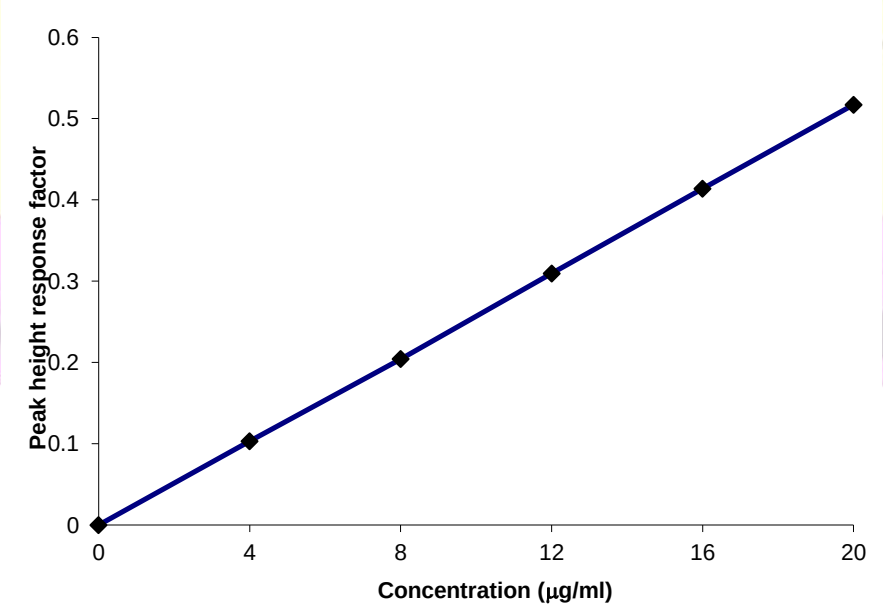


Figure: 9 Calibration graph of Pantoprazole

The LOD and LOQ of the developed method were determined by analyzing progressively low concentration of the standard solutions using the developed methods. The LOD is the

smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). LOD of domperidone and pantoprazole were found to be 175, and 315 ng/ml. the LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ of domperidone, pantoprozole were found to be 530 and 950 ng/ml.

The resolution, capacity factor, theoretical plates/meter, peak symmetry was calculated for the standard solutions and is presented in Table 7. The values obtained demonstrated the suitability of the system for the analysis of the above drug combination.

Table 5: Linearity and Range in HPLC:

Internal Standard	Domperidone			Pantoprozole		
Peak area(10µg/ml)	Concentration (µg/ml)	Peak area	Response factor	Concentration (µg/ml)	Peak area	Response factor
1441144	2	10306	0.07512	4	148726	0.1032
1429653	4	219022	0.1532	8	291792	0.2041
1435756	6	328500	0.2288	12	444510	0.3096
1503337	8	452805	0.3012	16	622080	0.4138
1488863	10	558621	0.3751	20	769742	0.5170

Table 6: Repeatability of injection:

A standard solution of mixture of drugs was injected 6 times and its % RSD was calculated.

Concentration (µg/ml)	Injection	Peak area		% RSD*	
		Dom*	Pan*	Dom	Pan
Domperidone (2 µg/ml) Pantoprazole (2 µg/ml)	1	108650	148726	0.39	0.51
	2	109300	149226		
	3	108150	148726		
	4	108700	147926		
	5	108150	147550		
	6	108700	147330		

Mean of Three Replicate. Dom=Domperidone, Pan*=Pantoprazole

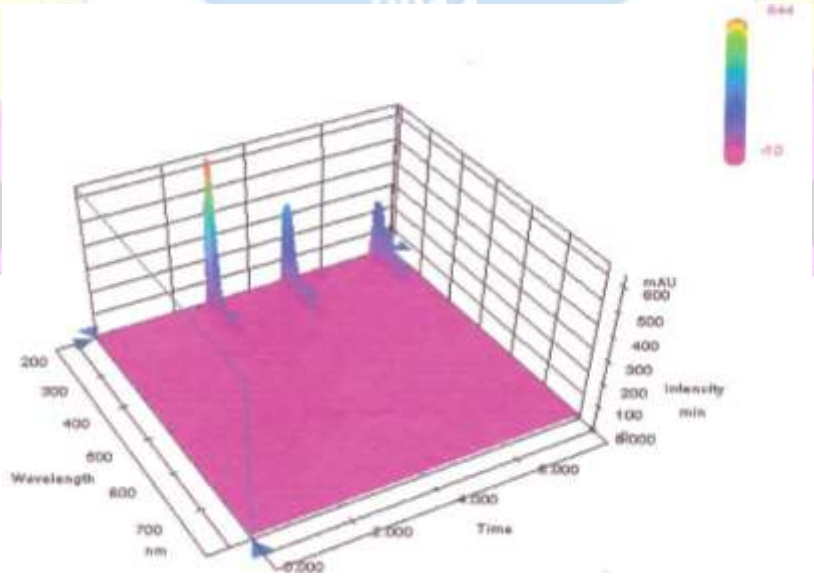


Figure 10: PDA Chromatogram showing 3D view of pantoprazole, domperidone and etoricoxib (IS)

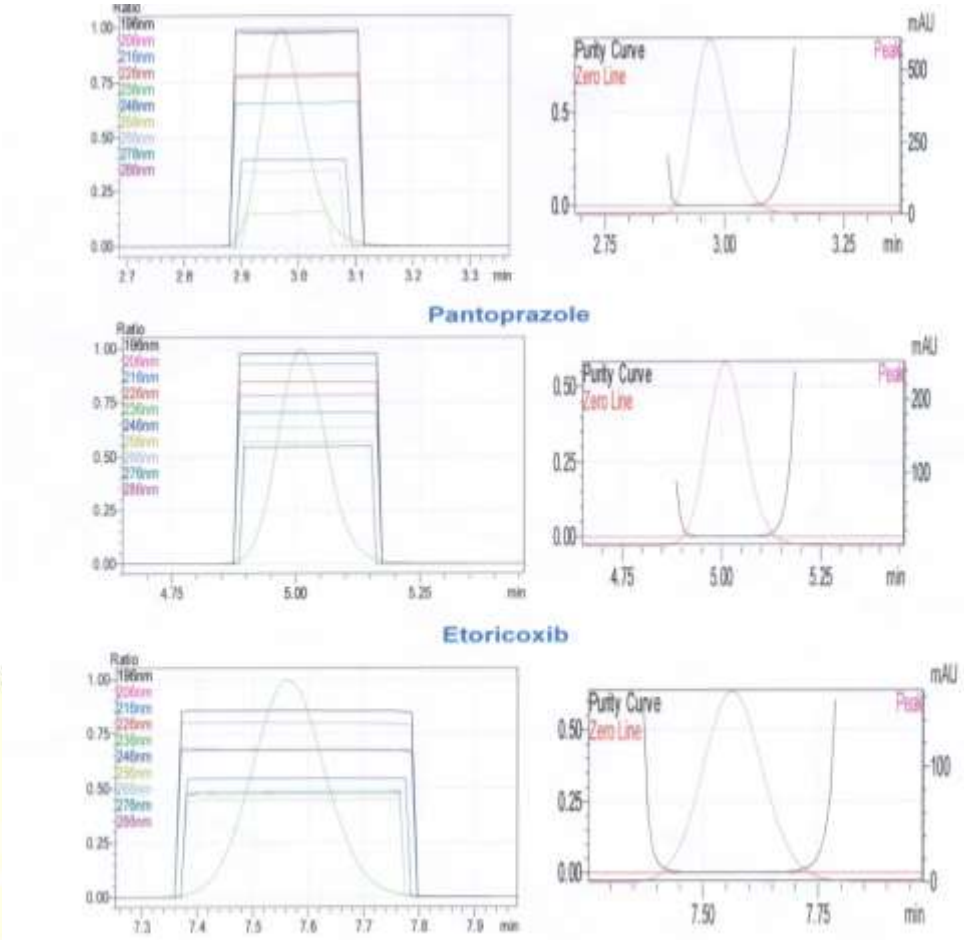


Figure: 11: Peak purify profile and curve for the analytes Domperidone, Pantoprazole and Etoricoxib Domperidone

Table 7: System suitability studies

Drug	Rs*	N*	K'*	Tailing factor	HETP	LOD ng/ml	LOQ ng/ml
Domperidone	10.476	4889.109	0.000	1.1295	28	175	530
Pantoprazole	10.854	9570.065	0.684	1.124	25	315	950

Rs*- resolution , N* –theoretical plates , k*- capacity factor

CONCLUSION

The scope and objective of the present work is to optimize the chromatographic conditions, to develop HPLC method for the estimation of drugs in selected multi-component dosage form and the same is validated. The developed HPLC method requires less time, no tedious extraction procedure were involved, run time were less than10 min, suitable for the analysis of raw material. Hence, the chromatographic method developed for Pantoprazole and Domperidone is said to be simple, precise and accurate that can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutics and bio-equivalence studies and in clinical pharmacokinetic studies.

ACKNOWLEDGMENT

First and fore most I extremely beholden to our group director, Prof.(Dr.) R.D Jaiman, & Prof.(Dr.) Bigala Rajkamal for his constant insight, personal advice, countless serenity and pain taking effort in all stages of study. I submit my sincere thanks and respectful regard to our beloved Chairman, Girish Mittal for all the facilities that were provided to me. My sincere thanks to all other staffs of Siddhi Vinayak College of Sciences & Hr Education (Department of Pharmacy), Alwar-Rajasthan.

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