International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

RP-HPLC METHOD FOR THE DETERMINATION OF DOMPERIDONE AND ESOMEPRAZOLE IN COMBINED DOSAGE FORM

*Kukalakunta Kavya, Gajja Vijaykumar and Kudipudi Harinadhbabu

NOVA College of Pharmacy, Vegavaram, Jangareddigudem, West Godavari, A.P, India

***Corresponding author e-mail:** kukalakunta.kavya@gmail.com

ABSTRACT

A simple, specific and sensitive reverse phase high performance liquid chromatographic method was developed and validated for simultaneous determination of esomeprazole and domperidone from pharmaceutical dosage forms. The method uses ODS C_{18} column and isocratic elution. The mobile phase composed of methanol: phosphate buffer (pH 4.0) in the ratio of 65:35 v/v was used at a flow rate of 1.0 ml /min. DAD detector was programmed at 230 nm for run time 7 min. All the validation parameters were in acceptable range. The developed method was effectively applied to quantitate amount of esomeprazole and domperidone from tablets. The method was also applied suitably for determining the degradation products of esomeprazole and domperidone.

Keywords: Esomeprazole, Reverse Phase High Performance Liquid Chromatography, Domperidone

INTRODUCTION

Domperidone (DOM), 5-chloro-1-[1-[3-(2,3-dihydro-2-oxo-1Hbenzimidazole-1-yl)propyl]-4-piperidinyl]-1-3-dihydro-2Hbenzimidazole-2-one is a dopamine antagonist and exerts its effect at peripheral D2 receptors in the GI tract; the CTZ, which is outside the blood-brain barrier; and the pituitary. [1, 2] It has antiemetic property similar to metoclopramide and neuroleptic drugs. Unlike these drugs, however, domperidone does not readily cross the blood brain barrier and seldom causes extra pyramidal side effects. [3, 4] Esomeprazole (ESO), S-isomer of omeprazole inhibits gastric acid secretion and is cost effective in the treatment of gastric oesophageal reflux diseases. It is the first single optical isomer proton pump inhibitor. It provides better acid control than current racemic proton pump inhibitors and has a favorable pharmacokinetic profile relative to omeprazole. It is chemically bis (5-methoxy-2-[(S)-[(4-methoxy-3,5-dimethyl-2-pyridinyl) methyl] sulfinyl] -1H-benzimidazole). [5, 6] Estimation of DOM included spectrophotometric methods [7, 8], HPLC [9-12] and HPTLC [13] in dosage forms. A detailed literature revealed that several analytical methods available for determination of ESO includes UV spectrophotometric method [14], Reversed Phase Liquid Chromatography when present with other drugs [15], HPLC Method [16]. The present work involves the efficient RP-HPLC method forthe estimation of DOM and ESO in combined dosage form.

MATERIALS AND METHODS

Materials: Domperidone was donated by Sun Pharmaceuticals Ltd., Mumbai, India. Esomeprazole was generously gifted by Lupin Pharmaceutical Ltd., Pune, India. Methanol (HPLC grade) was purchased

from Qualigens fine chemicals, Mumbai, India. Distilled, 0.45 µm filtered water used for HPLC analysis and preparation of buffer. Buffers and all other chemicals were analytical grade.

Instrumentation: A Waters HPLC system consisting of a Water 2695 binary gradient pump, an inbuilt auto sampler, a column oven and Water 2487 dual wavelength absorbance detector (DAD) was employed throughout the analysis. The data was acquired using Empower 2 software.

The column used was ODS C_{18} (150×4.6mm, 3.5µm). A Bandline sonerex sonicator was used for enhancing dissolution of the compounds. A Digisum DI 707 digital pH meter was used for pH adjustment. The mixture of methanol and phosphate buffer (pH 4.0) in the proportion of 65:35 v/v was used as mobile phase at 1.0 mL/min. The column was maintained at ambient temperature.

Stock solution and calibration standard sample: A stock solution was prepared by dissolving accurately weighed 25 mg of DOM and ESO in a 25 ml volumetric flask to obtain 1 mg/ml solution using HPLC grade methanol. The stock solution was diluted suitably with methanol to obtain concentrations in the range of 50 to 100 µg/ml and calibration curves were plotted.

System suitability: The system suitability was assessed using five replicate analyses of drugs at concentration of 60 µg/mL. The acceptance criterion was ±2 % of coefficient of variation (% CV) for retention times and peak areas for both drugs.

Detection and quantitation limits: Limit of detection (LOD) and limit of quantitation (LOQ) were obtained from signal to noise ratio. The detection limit was defined as the lowest concentration level resulting in a peak area of three times the baseline noise. The quantitation limit was defined as the lowest concentration level that provided a peak area with a signal-to-noise ratio higher than 10.

Accuracy and precision: Accuracy of the assay method was determined for both intra-day and inter-day variations using the triplicate analysis of drugs at three levels viz. 50%, 100% and 150%. These samples are denoted as QC samples. Precision of the assay was determined by repeatability (intra-day) and intermediate precision (interday) in triplicate. Repeatability refers to the use of the analytical procedure over a short period of time that was evaluated by assaying the QC samples during the same day. Intermediate precision was assessed by comparing the assays on different days (3 days).

Application of method to dosage form: The developed and validated HPLC method was applied for determination of DOM and ESO from dosage forms. Contents of 20 capsules having ESO and DOMPE were emptied and weighed accurately. A quantity of the powder equivalent to about 20 mg of ESO and 30 mg of DOM was taken in to 100 mL volumetric flask, completely dissolved and filtered through Whatmann filter paper No. 41. The residue was washed thoroughly with methanol. The filtrate and washings were combined and diluted to the mark with methanol. One mL of extract was transferred into 10 mL volumetric flask and diluted to the mark with methanol to get an approximate concentration of 60 µg/mL of ESO and 90 µg/mL of DOM. The solutions were injected into HPLC and analyzed for drug content.

RESULTS AND DISCUSSION

Both DOM and ESO have limited aqueous solubilities hence methanol was used for the extraction of drugs from the formulations and for preparation of stock solutions. The HPLC analysis was done at 230 nm for both drugs. Mobile phase optimization was initiated using methanol and phosphate buffer (pH 3.0-5.5) at the flow rate of 1.0 mL/min. The flow rate was decreased from 1.5 ml/min to 1.0 ml/min to resolve the degradation product from main drug peaks. The peak shape and separation was found to be good when a mobile phase composition of 65:35 (v/v, methanol: phosphate buffer pH 4.0) was used at the flow rate of 1.0 mL/min. The retention times of DOM and ESO were found to be 2.309 min and 4.307 min, respectively.

The resolution (Rs) between DOM and ESO was found to be 2.57, indicating good separation of both analytes from each other. The theoretical plate number for DOM and ESO were found to be 2982 and 2904, respectively, thus indicating good column efficiency. A typical chromatogram was recorded at 230 nm, shown in Figure 1. The calibration curve constructed was evaluated by its correlation coefficient. The peak area was linear in the range of 60-100 µg/mL for DOM and 50-90 µg/mL for ESO (Table 1).

The correlation coefficients for both the calibration plots of drugs were more than 0.999. The percent CV of retention time for both the drugs was less than 0.3 % indicating high stability of the system. The percent

CV of peak area was within the range of 2 % limit signifying suitability of the system. The USP tailing factor was 1.205±0.4 (mean±% CV) for ESO and 1.049±0.3 (mean±% CV) for DOM. The recovery of DOM and ESO from placebo was determined at three different concentrations. Mean recovery was 99.66-100.02% for DOM and 99.29-100.09% for ESO (Table 2).

The precision of the test method was demonstrated by intra-day and inter-day variation studies. The intra-day (repeatability) studies were carried out by injecting six repeated injections of standard solution on the same day, by one analyst under the same experimental conditions. The RSD values for peak areas were found to be 0.30% for DOM and 0.39% for ESO (Table 2). The results obtained from intermediate precision (inter-day) also indicated a good method precision. All the data were in the acceptable range of criteria of 2%. Robustness was done by small deliberate changes in the chromatographic conditions. There were no significant changes in the peak areas and retention times of DOM and ESO when the pH and flow rate of the mobile phase were changed. The results were indicating that the proposed method is robust.

The assay results show that the proposed method was selective for the simultaneous determination of DOM and ESO without interference from the excipients used in the tablet dosage form. Assay results for three samples of tablets expressed as the percentage of the label claim, were found 98.24 for DOM and 99.16% for ESO. Results showed (Table 3) that the content of DOM and ESO in tablet formulation were to the counter requirements (98-102 % of the label claim).

CONCLUSION

A rapid, simple and specific reverse phase HPLC method has been developed for simultaneous determination of ESO and DOM from tablet dosage form. The method was validated for accuracy, precision, linearity. Application of this method for simultaneous determination of DOM and ESO from tablet dosage form showed that neither the degradation product nor the excipients interfere with the determination.

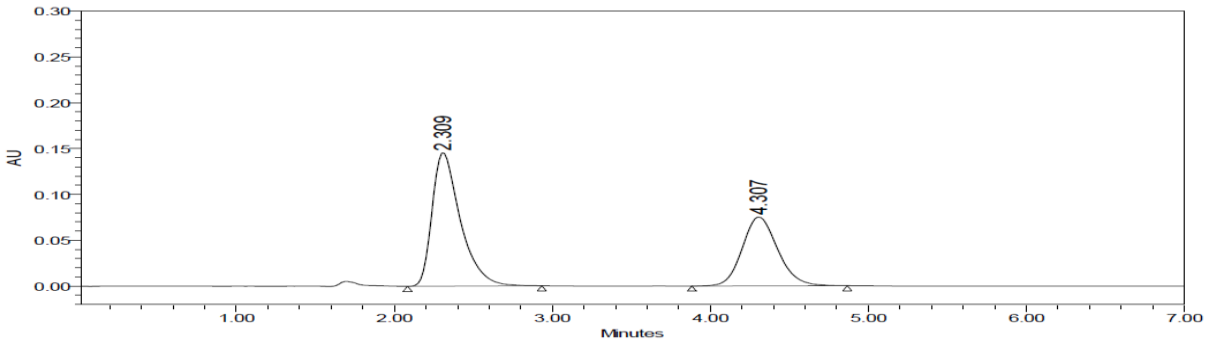

Figure 1: A typical Chromatograph of DMO (2.309) and ESO (4.307)

Table 1: Linearity data of DOM and ESO

Analyte	Conc. (µg/mL)	Mean peak area ± SD (n=3)	RSD (%)	Linear regression
DOM	60	1810101±9161	0.5061	R^2=0.9995
	70	2074287±20715	0.9987	
	80	2360133±26597	1.1269	
	90	2602279±24089	0.9257	
	100	2869778±35465	1.2358	
ESO	50	1164173±5196	0.4463	R^2=0.9993
	60	1342535±6636	0.4943	
	70	1555931±19964	1.2831	
	80	1737973±29079	1.6732	
	90	1942319±29912	1.54	

Table 2: Validation parameters and data for proposed method

Validation parameter	Results	
	DOM	ESO
Limit of detection (µg/mL)	0.08	0.28
Limit of quantitation (µg/mL)	0.126	0.455
Accuracy (% recovery)*	99.66-100.02	99.29-100.09
Precision		
Intra-day precision (%RSD)	0.30	0.39
Inter-day precision (%RSD)	0.11	0.16
System suitability parameter		
Peak area (%RSD)	0.133	0.196
Retention time (%RSD)	0.219	0.146
Tailing factor	1.38	1.12
Number of theoretical plates	2982	2904
Resolution	2.57	

** Replicates of three concentration levels (in three determinations)*

Table 3: Estimation of amount present in tablet dosage form

Tablet Formulation	Label Claim per Tablet (mg)	% Drug found ± SD (n=3)	RSD (%)
DOM	30	98.35±0.9401	0.9558
ESO	20	99.27±0.9576	0.9647

REFERENCES

1. Domperidone. Cited on January 25 2009. Available from: http://www.rxmed.com/ motilium-drug.htm.
2. Barone JA. Hosp Pharm, 1998; 33(2):191-7.
3. Reyntjens AJ, Niemegeers CE, Van Nueten JM, Laduro P, Heykants J 1978 Arzneim Forsch/Drug Res 28:1194.
4. Brogden RN, Carmine AA, Heel RC. Drugs, 1982; 24:360.
5. Andersson T, Hassan-Alin M, Hasselgren G, Rohss K, Weidolf L. Clinical Pharmacokinetics, 2002; 40: 411-26.
6. Scott LJ, Dunn CJ, Mallarkey G, Sharpe M. Drugs, 2002; 62: 1503-38.
7. Al Khamis KL, Hagga ME, Al-Khamis HA. Anal Lett, 1990; 23: 451-60.
8. Ramamohan Y, Avadhanulu AB. Indian Drugs, 1998; 35:754-6.
9. Yamamoto K, Hagino M, Kotaki H, Iga I. J Chromatogr B, 1998; 720:252-5.
10. Argekar AP, Shah SJ. J Pharm Biomed Anal, 1999; 19: 813-7.
11. Zarapkar SS, Kanyawar NS. Indian Drugs, 2002; 39: 217-21.
12. Kobylinska M, Kobylinska K. J Chromatogr B, 2000; 744: 207-12.
13. Zarapkar SS, Salunkhe BB. Indian Drugs, 1990; 27: 537-40.
14. Putta Rajesh Kumar, Somashekar Shyale , Mallikarjuna Gouda M and S. M. Shanta Kumar. J Chem Pharm Res, 2010; (2):484-90.
15. BH Patel, BN Suhagia, MM Patel, JR Patel. A Springer Open Journal, 2007; 65: 743-8.
16. ArmaĞAn ÖNal and Aysel ÖZtunÇ. J Food Drug Anal, 2006; 14: 12-8.