Research Article
www.ijrap.net

METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ESOMEPRAZOLE AND DOMPERIDONE AS BULK DRUGS AND IN TABLET DOSAGE FORM BY HPTLC

Shaikh N. C.[1]*, Bandgar S. A.[1], Kadam A. M.[1], Dhaneshwar S. R.[2]
[1]Ashokrao Mane College of Pharmacy, Pethvadgaon, Maharashtra, India
[2]Bharati Vidyapeeth College of Pharmacy, Pune, Maharashtra, India

Received on: 15/03/12 Revised on: 14/04/12 Accepted on: 04/05/12

***Corresponding author**
Shaikh N. C., Assistant Professor, Ashokrao Mane College of Pharmacy, Pethvadgaon, Kolhapur, Maharashtra, India
Email:shaikh.nissar@gmail.com

ABSTRACT
A simple, selective, precise, specific and reproducible indicating high-performance thin layer chromatographic method for routine analysis of esomeperazole (ESO) and domperidone (DOM) as bulk drugs and in tablet formulation has been developed and validated. Aluminium foil TLC plates precoated with silica gel 60F 254 were used as stationary phase and toluene: ethyl acetate: methanol (2:7:0.5 v/v/v) as mobile phase. A compact band (R_f values 0.54±0.01 and 0.33±0.01) was obtained for esomeprazole and domperidone respectively. Linear regression analysis revealed a good linear relationship (r^2 =0.9972±0.01) between peak area and concentration in the range 200-600ng/spot for esomeperazole and (r^2 =0.9985±0.01) in the range 400-1200ng/spot for domperidone. The mean values ± SD of the slope and intercept were 12.967±0.033 and 1513.4±1.20 respectively for esomeperazole and 8.192±0.035 and 1664±0.62 respectively for domperidone. Densitometric analysis was performed in absorbance mode at 286 nm selected as isobestic point. Review of literature reveals that few HPLC, LC-MS and HPTLC methods have been reported for estimation of esomeprazole and domperidone in single and in combination with other drugs, but no HPTLC method is reported so far for simultaneous estimation of this combination. The limits of detection and quantitation were 50 and 80ng/spot respectively for esomeperazole and 80 and 100ng/spot respectively for domperidone. The method was validated for precision, recovery, and robustness. Statistical analysis proved the method enables repeatable, selective, and accurate analysis of the drug. It can be used for identification and quantitative analysis of esomeperazole and domperidone in the bulk drugs and in tablet formulations.

Keywords: Esomeperazole, Domperidone, HPTLC, Validation.

INTRODUCTION

Esomeperazole (ESO) S-bis (5-methoxy-2-[(S)-[(4-methoxy-3,5-dimethyl-2 pyridinyl) methyl] sulfinyl-1]-1H-benzimidazole-1-yl) magnesium trihydrate is a proton pump inhibitor that suppresses gastric acid secretion by specific inhibition of the H+/K+-ATPase in the gastric parietal cell. The S- and R- isomers of omeprazole are protonated and converted in the acidic compartment of the parietal cell forming the active inhibitor, the achiral sulphenamidey[1,2]. Domperidone (DOM) 5-chloro-1-[-1-[3-(2,3-dihydro-2-oxo-1H-benzimadozol-1-yl)propyl]-4-piperidinyl]-1,3-dihydro-2H-benzimidazol-2-one is a synthetic benzimidazole compound that acts as dopamine antagonist with antiemetic properties. It is also used as prokinetic agent for treatment of upper gastrointestinal motility disorders[3-5]. Review of literature reveals that few HPLC, LC-MS and HPTLC methods have been reported for estimation of esomeprazole and domperidone in single and in combination with other drugs, but no HPTLC method is reported so far for simultaneous estimation of this combination[6-12]. The objective of present work was to develop a simple, accurate and reproducible procedure for determination of esomeprazole and domperidone by HPTLC as bulk and in tablet dosage form.

MATERIALS AND METHODS

Chemicals and Reagents

Esomeperazole was supplied as gift sample by Torrent pharmaceutical ltd. Ahemedabad and Domperidone was gift sample by Vamsi labs ltd. Solapur. All chemicals and reagents used were of analytical grade and were purchased from Merk Chemicals, Mumbai, India. Tablets containing 20mg of ESO and 30mg of DOM (ESOZ-D-20) was purchased from local market.

HPTLC Instrumentation and Conditions

Chromatography was performed on 20 cm × 20 cm on aluminium foil plates precoated with 0.2-mm layers of silica gel 60F254 (E. Merck, Germany). Before use the plates were prewashed by development with methanol then dried in the current of dry air and activated at 110 °C for 5 min. Samples were applied as bands 6 mm wide, 6 mm apart. Camag Linomat V semiautomatic sample applicator, Hamilton syringe (100 µl), Camag TLC Scanner-3 with CATS 4 software, Camag twin- trough chamber (20×10 cm) and Remi centrifuge (Model C30) were used for the present study. The mobile phase was toluene: ethyl acetate: methanol (2:7:0.5). Linear ascending development was performed in a twin-trough glass chamber previously saturated with mobile phase vapour for 30 min at room temperature and relative humidity 60±5%. Ten µl of standard solutions of esomeprazole (200 µg/ml) and domperidone (600 µg/ml) were applied on pre-washed and activated plate under nitrogen stream using semiautomatic spotter. They were developed at constant temperature in a Camag twin trough chamber previously saturated for 20 min with mixture of toluene ethyl acetate methanol (2:7:0.5) as mobile phase. The plates were removed from the chamber and dried in air. Densitometric measurements were performed at 286 nm in reflectance mode with Camag TLC Scanner 3 using CATS 4 software incorporating track optimization position using deuterium lamp as source of radiation. The slit dimensions were 6 mm × 0.45 mm were shown in figure 1.[13]

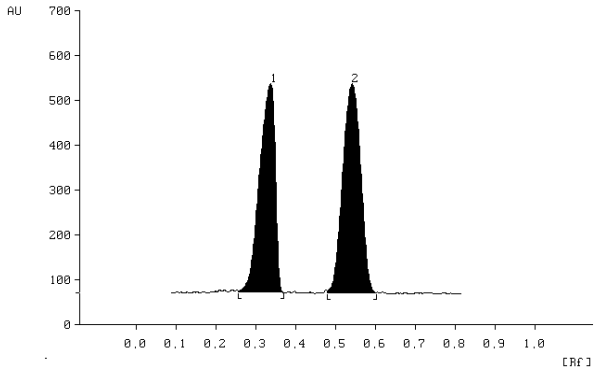

Figure 1: Densitogram of standard esomeprazole (4000 ng/spot) and domperidone. (6000 ng/spot); (Rf, 0.54±0.01 for esomeprazole and Rf, 0.33±0.01 for domperidone); Mobile phase; toluene ethyl acetate methanol (2:7:0.5)

Calibration

A stock solution containing 100 µg/ml ESO and 200 µg/ml DOM was prepared in methanol. Different volumes of this solution were applied to the plate resulting in application of 200- 600 ng/spot for ESO and 400-1200ng/spot for DOM to the plate. Each concentration was applied seven times to the plate and the plate was developed as described above. Peak areas were plotted against corresponding concentrations to furnish the calibration plot. The overlain UV spectrum which were shown in figure 2.

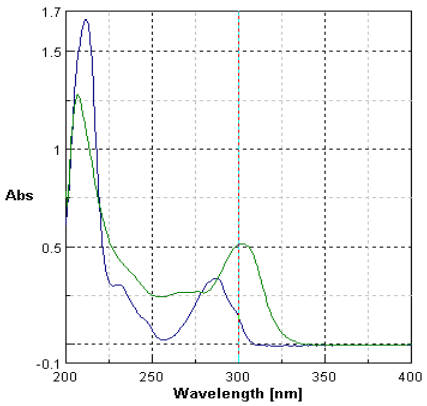

Figure 2: Overlain UV spectrum for esomeprazole and domperidone

Method Validation

Precision

Repeatability of sample application and measurement of peak area were assessed by chromatography of six replicates of the same concentration (600 ng /spot ESO and 1200ng/spot DOM).Intra-day and inter-day variation for determination of ESO (200, 400, 600 ng/spot).and DOM was measured at concentrations (400, 800, 1200 ng/spot). [13-15]

Robustness

Small changes in the chromatographic conditions were introduced and the effects on the results were examined. Small variations in mobile phase composition (±0.1 %), amount of mobile phase (±5 %), time from spotting to chromatography (±20 min) and scanning time (±20 min). The % RSD of peak area for each parameter was calculated.

Limits of Detection and Quantitation

To determine the limits of detection and quantitation, concentrations in the lower part of the linear range of the calibration plot were used. Stock solution of ESO (100 µg/ml) and DOM (200 µg/ml) was prepared and applied in triplicates in different volumes in the range of 10 to 200ng. The time from spotting to chromatography and time from chromatography to scan was varied at 0 and 20 min.

Specificity

The specificity of the method was determined by analysis of drug standards and samples. The band for ESO and DOM in the sample was identified by comparing the R value and F spectrum of the band with those of the band from a standard. The peak purity of ESO and DOM was assessed by comparing spectra acquired at three different positions on the peak.

Recovery

Recovery studies were carried out by applying the method to drug sample to which known amount of esomeprazole and domperidone corresponding to 80, 100 and 120 % of label claim had been added (standard addition method). At each level of the amount, six determinations were performed and the results obtained were compared with expected results.

Analysis of the Marketed Formulation

To determine the ESO & DOM contents of conventional tablets, twenty tablets were weighed and powdered in a glass mortar. An amount of powder equivalent to the average weight of the one tablet ESO & DOM was transferred to a 50 ml volumetric flask, extracted with methanol. The solution was centrifuged for 15minutes at 600 RPM. The resulting solution was filtered through whatman filter paper no.41 and residue was washed with methanol and volume was adjusted 250ml with the same solvent to obtained final concentration of 200µg/ml of ESO & 600µg/ml of DOM. The two drugs were satisfactorily resolved with Rf values 0.55±0.01 and 0.34±0.01 for esomeprazole and domperidone respectively.

RESULTS AND DISCUSSION

HPTLC Method Optimisation and Validation

A solvent system that would give dense and compact spots with appropriate and significantly different Rf values was desired for quantification of esomeprazole and domperidone in pharmaceutical formulations. Various solvent systems like chloroform methanol, chloroform toluene acetic acid, benzene methanol toluene were tried to separate and resolve spots of esomeprazole and domperidone from their impurities and other excipients of formulations. The mixture of toluene ethyl acetate methanol (2:7:0.5) could resolve esomeprazole and domperidone with better peak shape. The two drugs were satisfactorily resolved with Rf values 0.54±0.01 and 0.33±0.01 for esomeprazole and domperidone respectively.

Validation

Linearity and range

The linear regression data for calibration curves (n = 6) showed good linear relationship over a concentration range of 200-600 ng/spot for esomeprazole and 400-1200 ng/spot for domperidone shown in Table 1-4.

Table 1: Linearity and range of esomeprazole

Sr. No.	Concentration (ng/spot)	Area (AU)*
1	200	4182
2	300	5407
3	400	6604
4	500	7878
5	600	9430

*n=6

Table 2: Linear regression data for calibration curve of esomeprazole

Parameters	Data*
Linearity range	200-600 ng/spot
r^2	0.9972
Slope ± SD	12.967±0.033
Intercept ± SD	1513.4 ± 1.20

*n=6

Table 3: Calibration linearity and range of domperidone

Sr. No.	Concentration (ng/spot)	Area (AU)*
1	400	4934
2	600	6686
3	800	8119
4	1000	9760
5	1200	11589

*n=6

Table 4: Linear regression data for calibration curve of domperidone

Parameters	Data*
Linearity range	400-1200 ng/spot
r^2	0.9985
Slope ± SD	8.192 ± 0.035
Intercept ± SD	1664 ± 0.62

*n = 6

Precision

Tables 5-8 shown that intraday and interday relative standard deviations are found in the range 0.21-0.54 % and 0.18-0.29% for esomeprazole and 0.36-0.53 % and 0.33-0.58 % for domperidone. The smaller values of intraday and interday variation in the analysis indicate that the method is precise.

Table 5: Intraday precision of esomeprazole

Concentration (ng)	Average Area	SD	% RSD*
200	4190	22.91	0.54
400	6608	14.50	0.21
600	9443	24.54	0.25

*n = 3

Table 6: Inter day precision of esomeprazole

Concentration (ng)	Average Area	SD	% RSD*
200	4181	12.34	0.29
400	6606	12.05	0.18
600	9439	19.67	0.20

*n = 3

Table 7: Intra day precision of domperidone

Concentration (ng)	Average Area	SD	% RSD*
400	4918	23.02	0.46
800	8133	43.46	0.53
1200	11584	42.15	0.36

*n = 3

Table 8: Inter day precision of domperidone

Concentration (ng)	Average Area	SD	% RSD*
400	4948	17.61	0.35
800	8176	48.04	0.58
1200	11605	39.06	0.33

*n = 3

Robustness

When the standard deviation of peak area was calculated for each change of conditions RSD was found to be less than 2%. These low RSD values given in Table 9 & 10 indicated the method is robust.

Table 9: Robustness of esomeprazole

Parameters	% RSD
Mobile phase composition (± 0.1 ml)	1.47
Amount of mobile phase (± 0.5 %)	1.32
Time from spotting to chromatography (± 20 min)	0.54
Time from chromatography to scanning (± 20 min)	1.01

*n = 3

Table 10: Robustness of domperidone

Parameters	% RSD
Mobile phase composition (± 0.1 ml)	1.14
Amount of mobile phase (± 0.5 %)	1.22
Time from spotting to chromatography (± 20 min)	0.36
Time from chromatography to scanning (± 20 min)	0.92

*n = 3

Recovery

When the method was used for extraction and subsequent analysis of ESO & DOM from pharmaceutical dosage forms after spiking with 80, 100, and 120% of additional drug, recovery was 98.51±0.23% for ESO and 99.01±0.15 % for DOM were shown in Table 11.

Table 11: Recovery of esomeprazole and domperidone

Label claim (mg/tablet)	Amount added (%)	Total amount added (mg)	Amount recovered (mg)	% Recovery ± SD*	% RSD
Esomeprazole					
20	80	36	35.7	98.58±0.27	0.28
	100	40	39.6	98.25±0.20	0.21
	120	44	43.7	98.70±0.22	0.23
Domperidone					
30	80	54	53.8	99.16±0.25	0.25
	100	60	59.8	99.00±0.21	0.21
	120	66	65.7	98.87±0.23	0.23

*n=6

Limits of Detection and Quantitation

The limit of detection was found to be 50 ng/spot for esomeprazole and 80 ng/spot for domperidone and limit of quantitation was found to be 80 ng/spot for esomeprazole and 100 ng/spot for domperidone. This indicates the sensitivity of the method is adequate were shown in Table 12.

Table 12: LOD and LOQ for esomeprazole and domperidone

Drug	LOD	LOQ
Esomeprazole	50 ng/spot	80 ng/spot
Domperidone	80 ng/spot	100 ng/spot

Specificity

It was observed that excipients present in formulation did not interfere with peaks of esomeprazole (R_f, 0.55±0.01) and domperidone (R_f, 0.34±0.01) shown in figure 3.

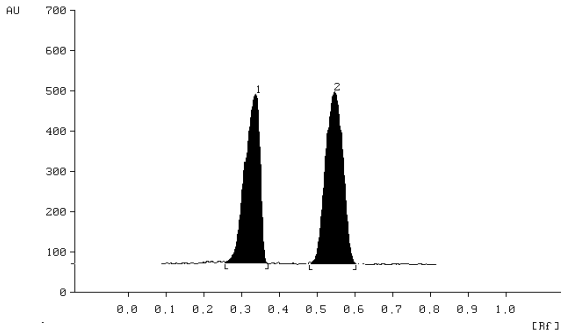

Figure 3: Densitogram of marketed formulation ESOZ-D-20 containing 20 mg of esomeprazole and 30 mg ofdomperidone; (Rf, 0.55±0.01 for esomeprazole and Rf, 0.34±0.01 for domperidone)

Assay of marketed formulation

The spot was resolved into two peaks in the chromatogram of drug samples, extracted from the marketed formulation of R_f, 0.55±0.015 for ESO and R_f, 0.34±0.01 for DOM. The content of drug was calculated from the peak areas recorded. There was no interference from the excipients commonly present in the tablets. The drug content was found to be 98.62 ± 0.50% and 99.05± 0.30% for ESO and DOM respectively. The low value of RSD indicates the method is suitable for routine analysis of ESO and DOM in pharmaceutical dosage forms were shown in Table 13.

Table 13: Assay of esomeprazole and domperidone

Sr. No.	Label claim (mg)		Amount found (mg)		% Drug content*	
	ESO	DOM	ESO	DOM	ESO	DOM
1	20	30	19.82	29.90	98.22	99.03
2			19.74	29.87	97.47	98.77
3			19.91	29.93	99.18	99.36

*n=3

CONCLUSION

The developed HPTLC proposed method is rapid, accurate, precise, specific and reproducible which could be used as effective quality control tool for routine analysis of esomeprazole and domperidone as bulk drugs and in tablet formulations without any interference from the excipients.

ACKNOWLEDGEMENT

The authors are grateful to Mr. V. K. Gupta and Mr. Vaidya, Torrent pharmaceutical Ahemedabad for providing samples of Esomeperazole as gift and Vamsi labs ltd. Solapur for providing sample of Domperidone. The authors are also grateful to Bharati Vidyapeeth University, Poona College of Pharmacy, Pune, India, for providing excellent facilities for carrying out this research work.

REFERENCES

1. Esomeprazole Safety Sheet 2007 [cited 2007 Apr 22] Available from: http // www. Seq chem. Com / safety sheet. Php.
2. Beale JM, Block JH Editors. Wilson and Gisvold's Text Book of Organic Medicinal and Pharmaceutical Chemistry. 11th ed. Philadelphia :Lippincott Williams & Wilkins;2004.
3. O'Neil, Maryadele J Editors. The Merck index : an encyclopedia of chemicals, drugs, and biological. 14th ed. New Jersey: Merck Research Lab., Division of Merck and Con.,Inc. Whitehouse Station;2006.pp. 578,3418.
4. Parfitt K Ed. The Complete Drug Reference. 32nd ed. London, UK: Pharmaceutical Press; 1999.pp.1190-1191.
5. GENERIC NAME: DOMPERIDONE - ORAL [cited 2007 March 14] Available from: http //www. medcinenet. com/ script/ main/ art.asp
6. Joseph A, Kumar R . Simultaneous estimation of esomeprazole and domperidone by UV spectrophotometric method. Journal of Pharmaceutical Sciences 2008; 70(1):128-131.
7. Hultman Ia, Stenhoft H. Determination of esomeprazole and its two main metabolites in human, rat and dog plasma by liquid chromatography with tandem mass spectrometry. J. chromatography2007; 848(2): 317-322.
8. Oin Y, Zhi L, Yan Z. RP-HPLC determination of plasma esomeprazole concentration. Huaxi Medical 2005; 21(1): 93-95.
9. Bhavesh HP, Suhagia B, Patel J. Simultaneous estimation of pantoprazole and domperidone in pure drug and a pharmaceutical formulation by HPLC method. Journal of AOAC International 2007; 90(1):142-146.
10. Lakshmi S, Anilkumar V. Simultaneous estimation of omeprazole and domperidone from tablet by HPLC method. Indian Journal of Pharmaceutical Sciences 2007; 69(5): 674-676.
11. Kartik A, Udupa N. Simultaneous estimation of paracetamol and domperidone in tablet by RP-HPLC method. Indian Journal of Pharmaceutical Sciences 2007; 69(1): 142-144.
12. Sivakumar T, Kannappam V. Stability indicating HPLC method for simultaneous estimation of pantoprazole and domperidone from their combination drug product. J. chromatography 2008; 67(2): 41-47.
13. W. Bertsch, Hara S, Kaiser RE, Zlatkis A, Editors .Instrumental HPTLC. Huthig, Heidelberg: 1980.
14. ICH Guidance on Analytical Method Validation, in: Proceedings of the International Convention on Quality for the Pharmaceutical Industry, Toronto, Canada, and September, 2002.
15. ICH, Q2A, Harmonized Tripartite Guideline, Test On Validation of Analytical Procedures, IFPMA, in: Proceedings of the International Conference on Harmonization, Geneva, March, 1994.

Source of support: Nil, Conflict of interest: None Declared