RESEARCH ARTICLE

Analytical Method Validation for Simultaneous Estimation of Domperidone and Omeprazole by RP-HPLC Method

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ABSTRACT

The developed RP-HPLC technique lets in speedy and particular omeprazole and domperidone determinations, the goal of the current painting is to improve and extend the chromatographic circumstances, to broaden RP-HPLC method. a variety of mobility phases were tested for the duration of technique improvement, some of the numerous cell phases methanol, acetonitrile and phosphate buffer changed into discovered to be a perfect cell phase, because it provided optimal peak forms and high resolution. The go with the flow rate changed into optimized at 0.90 mL/min. The development of the isocratic elution method for omeprazole and domperidone made it perfect for quick and routine analysis. Omeprazole and domperidone linearity and correlation coefficient had been located to be 10 to 50 ug/mL 0.997, and 0.998, respectively. The restriction of detection for omeprazole and domperidone turned into discovered to be 1.78 and 3.15 and the limit of quantification changed into observed to be 544 and 956. With the assay procedure, the methodology has been recognized as accurate. The %assay become determined to be 98 and 73. The evolved technique changed into confirmed to an excellent accuracy and precision. The isocratic elution approach evolved for the dedication of omeprazole and domperidone perfectly suited for rapid and ordinary evaluation. This technique shows that right reproducibility of the effects. moreover this approach become easy, touchy, and accurate. Degradation studies had been accomplished, right here the drug stability outcomes have been within the variety of acceptance standards 85 to 115%. Moreover, this approach was easy, touchy, and correct.

Keywords: Domperidone, Method validation, Omeprazole, Optimization, RP-HPLC.

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INTRODUCTION

Domperidone (molecular weight- 425.9) increases gut bowel movements by lowering esophageal sphincter tone.[1-4] By allowing the movement of acid contents deeper inside the colon and avoiding reflux esophagitis, this increased gastrointestinal motility helps reduce vomiting and nausea.[5-7]

It is an official compound of B. P. omeprazole. The substance is utilised as a proton pump inhibitor in pharmaceutical formulations to treat gastric ulcers.[8-12]

There had been several courses describing various techniques for quantifying such substances in my opinion or in mixture with other tablets. The components of omeprazole have been successfully measured through excessive overall performance liquid chromatography with coulometric detection.[13-18]

HPLC is used for evaluation of omeprazole in bioanalytical sample like human plasma. (RP)-ion couple domperidone and cinnarazine were successfully separated using the HPLC method in pharmaceutical formulations.[19-21]

The prevailing paper describes the improvement of RP-HPLC method the use of isocratic cell section that offers sure blessings as a result of its efficiency and speed.

Drug samples of omeprazole and domperidone have been procured from Swapnaroop drugs Pvt. Ltd. Aurangabad.

MATERIALS AND METHODS

Instruments

Shimadzu HPLC with UV detector is used.

Software Used

Lab Solutions

Reagents Used

HPLC Grade methanol was used as the solvent as well as the Mobile phase. It was procured fromMolychem India. From

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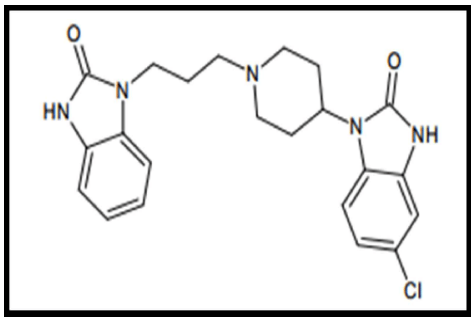

Figure 1: Domperidone Structure

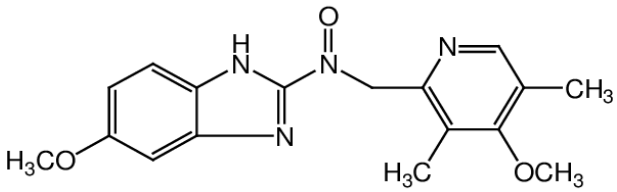

Figure 2: Omeprazole Structure

Qualigens in India, ortho phosphoric acid was bought.

HPLC grade water for the analysis was purchased from Molychem.

Chemicals Used

Methanol, acetonitrile (HPLC Grade), monobasic potassium phosphate (AR Grade), orthophosphoric acid.

Method Development

Method development of omeprazole and domperidone depends upon parameter like pH mobile phase wavelength of drug composition of sample and its physical and chemical properties.

Selection of Chromatographic Method

The reverse phase chromatographic method is selected as the drug is non polar in nature.

Selection of Diluent

Based upon physiochemical nature of drug the solvent is selected. Omeprazole and domperidone are dispersible in methanol as well as in acetonitrile. So combination of both solvents is used as diluent.

Wavelength Selection for Detection

Wavelength selection is selected based upon the UV spectra of omeprazole and domperidone 304 nm is selected as a detection wavelength for both the drugs.

Selection of Column

C18 column selected for the detection of domperidone and omeprazole having dimensions 250 × 4.6 mm.

Selection of Mobile Phase

Mobile phase is selected based on eluation and selected mobile phase is Methanol:Acetonitrate:Buffer (40:45:15).

Preparation of Stock Solution

The 100 mg of omeprazole and domperidone is taken in 100 mL volumetric flask and solution in prepared using diluent.

Trialand Error Method

Out of various trials conducted the results of the optimized method was finalised.

Optimized Method

Preparation of Buffer

Prepared 0.68% monobasic potassium phosphate by dissolving in 100 mL of water. pH of this solution was adjusted to 4.50 with ortho phosphoric acid. Degassed after being filtered *via* 0.45 µ filter paper.

Preparation of Mobile Phase

Mobile phase is prepared in proportion of Methanol: Acetonitrate:Buffer (40:45:15).

Diluent

Mixture (50:50) of HPLC grade methanol and acetonitrile was used as diluent.

Optimized method forHPLCchromatographic conditions

Chromatograms are shown in Figures 12-14.

Instrument HPLC Shimadzu Separation Module

Column C18

Auto sampler Temperature Ambient

Flow rate 0.90 mL/min

Wavelength 304 nm

Run time 8 minutes

Injection volume 10 µL

Analytical method validation Validated as per quality guidelines (ICH).

System Suitability Parameters

Before performing method validation and to check effectiveness of chromatographic system, the system suitability parameter is studied. The 40 µg/mL solution of domperidone and 20 µg/mL solution of omeprazole is injected and results are verified against acceptance criteria.

Table 1: System suitability parameters of Omeprazoleand Domperidone

Name	*Area*	*Retention Time (mins)*	*Theoretical plates*	*Asymmetry*	*Resolution*
Domperidone	185264	3.442	4056	1.292	------
Omeprazole	731039	4.182	5827	1.244	3.397
Limit	-------	----	NLT 1500	NMT 2.0	NLT 2.0

Table 2: Accuracy results

	Area obtained for Domperidone	*Area obtained for Omeprazole*	
Inj. No.	*Area*	*Inj. No.*	*Area*
1	92521	1	1266833
2	91867	2	1267310
AVG	92194	AVG	1267071

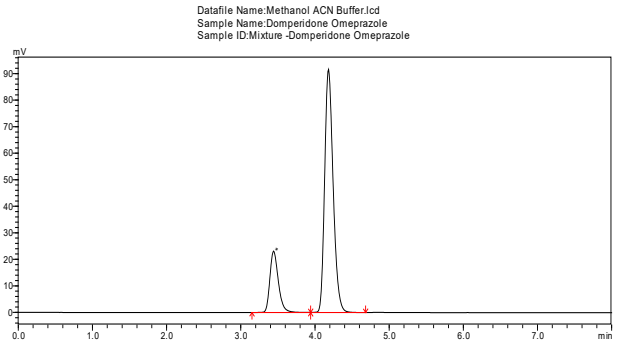

Figure 3: Chromatogram for optimized method.

Linearity

Preparation of Standard Stock Solution

- Have taken 100 mg omeprazole, dissolved and diluted to 100 mL using diluent. (1000 ppm) (Stock solution 1).
- Have taken 100 mg domperidone, dissolved and diluted to 100 mL using diluent. (1000 ppm) (Stock solution 2).
- Take 2 mL of solution from stock 1 & 1-mL of stock 2 were taken to produce 20 ppm domperidone and 10 ppm of omeprazole - Level I
- Take 1.5 mL of solution from stock 1 & 3 mL of stock 2 were taken to produce 30 ppm domperidone and 15 ppm of omeprazole - Level II
- Take 2 mL of solution from stock 1 & 4 mL of stock 2 were taken to produce 40 ppm domperidone and 20 ppm of omeprazole) - Level III
- Take 2.5 mL of solution from stock 1 & 5 mL of stock 2 were taken to produce 50 ppm domperidone and 25 ppm of omeprazole) - Level IV
- Take 3 mL of solution from stock 1 & 6 mL of stock 2 were taken to produce 60 ppm domperidone and 30 ppm of omeprazole) - Level V
- Take 4 mL of solution from stock 1 & 8 mL of stock 2 were taken to produce 80 ppm domperidone and 40 ppm of omeprazole) - Level VI

Accuracy

Results obtained for both drugs were determined to be within the permissible range of pharmacopeial criteria. (95–105% w/w). Domperidone and omeprazole drugs were purchased from Swapnroop Drugs Pvt. Ltd. Aurangabad. %Assay of marketed formulation – Brand name- Omee -D, Mfd. By- Alkem Lab Ltd. Contents of capsules were weighed. Avg. Wt. = 0.2612 g

Total of 10 mg of domperidone and 20 mg of omeprazole, respectively, of the finely powdered contents of capsules, were taken, crushed to fine powder and dilute to prepare 100 µg per mLsolution of omeprazole and domperidone. The 1-mL of solution was diluted to 100 mL and 10 µL solution injected into chromatographic system.

Precision

Solution preparation for inter-day and intra-day precision

a) Have taken 100 mg omeprazole, dissolved and diluted to 100 mL using diluent. (1000 ppm) (Stock solution 1).

b) Have taken 100 mg domperidone, dissolved and diluted to 100 mL using diluent. (1000 ppm) (Stock solution 2).

Level I, II and III solutions

- Take 3.5 mL of solution from stock 1 & 1.7 mL of stock 2 were taken to produce domperidone- 35 ppm and omeprazole- 17 ppm
- Take 5.5 mL of solution from stock 1 & 2.7 mL of stock 2 were taken to produce domperidone- 55 ppm and omeprazole- 27 ppm
- Take 7.5 mL of solution from stock 1 & 3.7 mL of stock 2 were taken to produce domperidone- 75 ppm and omeprazole- 37 ppm

Robustness

- Effect of variation of flow rate

The variation in flow rate was done by injecting at flow rate of 0.8, 1-mL per min. the change in wavelength was done +2 and -2 nm and values were recorded.

- Effect of variation of mobile phase composition

The variation in mobile phase composition was done in proportion of 50:40, 40:50 and values were recorded.

Ruggedness

Ruggedness is the degree to which findings from the analysis of the same sample may be repeated when conducted under a range of standard test settings, including those involving various analysts, facilities, columns, equipment, chemicals, assay temperature levels, different days, etc. (ie from laboratory to laboratory, from analyst to analyst).

Analyst to Analyst variation

Procedure

Different analysts inject the standard solution, and the HPLC injection area was determined. The %RSD for the area of replicate injections was found to be within the specified limits. Chromatogramas re shown in Figure 8.

Column to Column variation

Procedure

The region for injections in HPLC was determined, and several columns were used to inject the standard solution. The %RSD for the area of replicate injections was found to be within the specified limits.

Limit of detection

This is the lowest concentration in a sample that can be detected, but not necessarily quantitated, within the defined experimental parameters.For impurities assays, dose assays

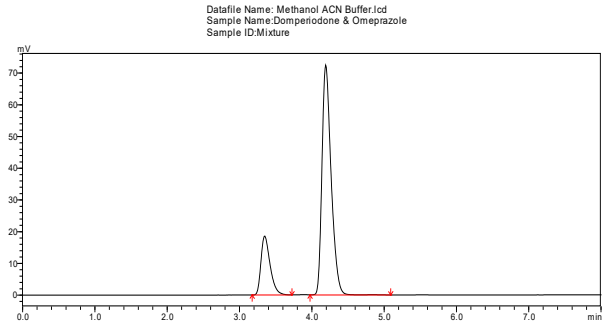

Figure 4: Chromatogram forsystem suitability of omeprazole and domperidone.

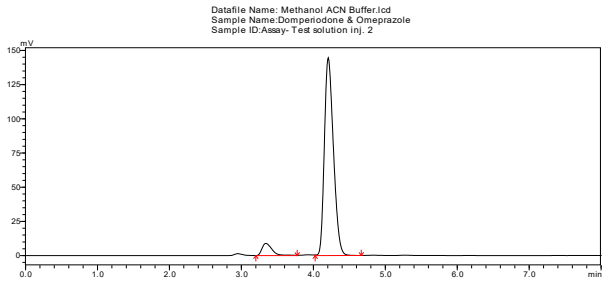

Figure 5: Spectra of accuracy.

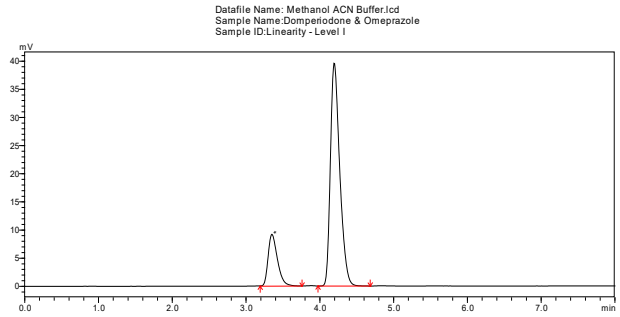

Figure 6: Chromatogram of 25 µg/mL of Omeprazole.

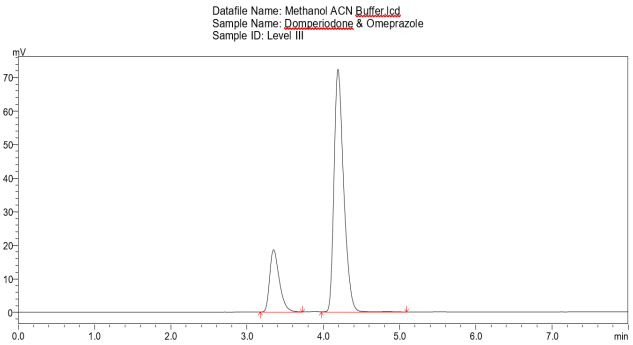

Figure 7: Chromatogram of 25.25 µg/mL of Domperidone.

with low drug levels, and placebo assays, the limit of detection is crucial. On the basis of signal to noise. Chromatogramas re shown in Figure 9 and 10 and limits in Table 6.

Limit of Quantification

This refers to the minimal analyte quantity in a sample which can be identified with reasonable accuracy and precision. According to it, this concentration produces a signal to noise ratio of 10:1. Chromatogramas re shown in Figures 11 and 12 and limits in Table 7.

Specificity

A test's specificity assures that the substance of interest is the source of the signal being evaluated and that excipients, degradation products, impurities, and/or other contaminants are not interfering in any way. The method's particularity was demonstrated by establishing a lack of interference from the diluents blank. In 10 µL of the blank solution was injected into the chromatograph. There should not be any interference with the omeprazole and domperidone peak. Chromatogramas re shown in Figure 13.

Degradation Studies

In forced degradation study the sample is exposed to conditions like heat, light, acidic, alkaline and oxidizing conditions.

RESULTS AND DISCUSSION

Development of a Technique for Estimating Omeprazole and Domperidone[16]

The omeprazole and domperidone were simultaneously determine in bulk and tablet dosage forms. The best peak was shown in below chromatograms. Basedon these trials an optimized method was developed and the chromatogram is shown in Table 1.

Accuracy

From the result shown in the accuracy Table 2 and Figure 5.

Formula for Assay

$$\% \text{ Assay} = \frac{\text{Avg. area of sample solution} \times \text{conc. of std} \times \text{purity} \times 100}{\text{Area of standard solution} \times \text{conc. of sample} \times 100}$$

Domperidone

$$\% \text{ Assay} = \frac{92194 \times 10 \times 100 \times 100}{93237 \times 10 \times 100} = 98.88\%$$

Omeprazole

$$\% \text{ Assay} = \frac{1267071 \times 20 \times 100 \times 100}{1283309 \times 20 \times 100} = 98.73\%$$

Precision

Interday Precision- %RSD calculations of area domperidone
Inter-day Precision- %RSD calculations of area omeprazole

Robustness

- Change in wavelength i.e. $\pm$ 2 nm
- Change in Flow i.e. $\pm$ 0.1 mL/min

Limit of Detection (LoD)

The LoD is determined on basis of standard deviation and slope.

The values of Sy and slope were obtained when creating calibration curve in MS Excel using "SLOPE" and STEYX functions.

$$\text{Omeprazole- LoD} = 3.3(Sy/S) = \frac{3.3 \times 16135}{29822} = 1.78 \text{ ppm}$$

$$\text{Domperidone- LoD} = 3.3(Sy/S) = \frac{3.3 \times 3854}{4028.2} = 3.15 \text{ ppm}$$

Table 3: Interday Precision

Level	*Day 1*	*Day 2*	*Day 3*	*Mean*	*%RSD*
I	149754	150165	150815	150244	0.36
II	227808	228720	229473	228667	0.36
III	307966	308310	309435	308570	0.25

Table 4: Inter-day Precision

Level	*Day 1*	*Day 2*	*Day 3*	*Mean*	*%RSD*
I	525465	524136	523303	524301	0.21
II	831435	828171	826604	828736	0.30
III	1132521	1130121	1130837	1131159	0.11

Table 5: Change in wavelength

	Wavelength	
WL	302 nm	306 nm
Domperidone	250269	235100
Omeprazole	589251	563097

Limit of Quantitation (LoQ)

The LoQ is determined on basis of standard deviation and slope.

$$\text{Omeprazole- LoQ} = 10(Sy/S) = \frac{10 \times 16135}{29822} = 5.41 \text{ ppm}$$

$$\text{Domperidone- LoQ} = 10(Sy/S) = \frac{10 \times 3854}{4028.2} = 9.56 \text{ ppm}$$

Degradation Studies

In forced degradation study the sample is exposed to conditions like heat, light, acidic, alkaline and oxidizing conditions and no degradation is observed.

CONCLUSION

The accuracy and precision of the RP-HPLC technique for the assessment of omeprazole and domperidone were established. Satisfactory findings were achieved after the suggested approach was validated in accordance with ICH recommendations and comparing the measured values with the reference values. Future research study on omeprazole and domperidone may benefit from this research.

Table 6: Change in Flow

	Flow Rate	
Flow	*0.8 mL/min*	*1.0 mL/min*
Domperidone	254805	264618
Omeprazole	601451	620560

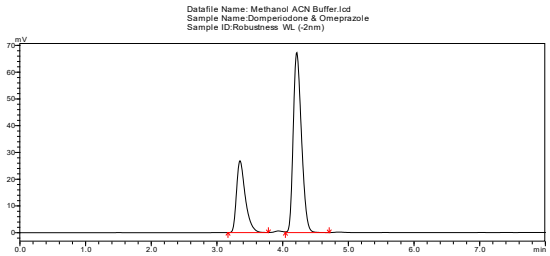

Figure 8: Chromatogram of change in wavelength.

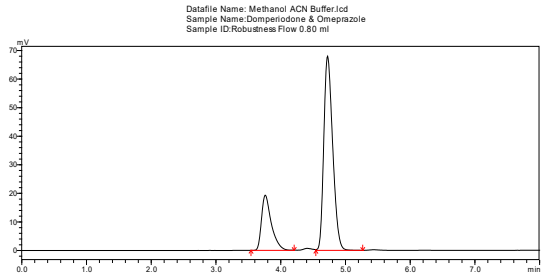

Figure 9: Chromatogram of change in flow rate.

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