Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2017, 9(21):35-39
(http://www.derpharmachemica.com/archive.html)

Accelerated Stability Indicating Method Development and Validation of Misoprostol by UV-Spectrophotometry

Jambulinngam M[1*], Ananda kumar K[1], Sasirehka A[1], Selvi R[1], Sivapriya K[1], Sindhuja V[1], Abdul Lathiff MKM[1], Ananda Thangadurai S[2]

[1]*Department of Pharmaceutical Analysis Swamy Vivekanandha College of Pharmacy, Elayampalayam, Tiruchengode Namakkal District, Tamilnadu-637205, India*
[2]*Department of Pharmaceutical Analysis, University of Tabuk, Kingdom of Saudi Arabia*

ABSTRACT

A simple, sensitive and reproducible method was developed using UV spectrophotometer for the determination of Misoprostol in a drug substance and pharmaceutical dosage form. It is applicable for the quantification of and assay of drug substances. The study was carried out at 275 nm. The developed method was validated for linearity, accuracy, precision, Limit of Detection (LOD) and Limit of Quantification (LOQ) as per International Conference on Harmonization (ICH) guidelines. Misoprostol was subjected to different experimental stress conditions of oxidative acid, base, hydrolytic, thermal and photolytic degradation. It was found to be stable in oxidative acid, oxidative base and hydrolytic conditions. The method developed was validated as per ICH guidelines and can be used in both bulk and pharmaceutical dosage forms.

Keywords: Misoprostol, UV-Spectrophotometer, Ethanol

INTRODUCTION

Misoprostol is chemically 7-[(1R, 2R, 3R)-3-hydroxy-2-[(E)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopentyl] heptanoic acid. It is used as an antiulcer drug and also for the prevention of Nonsteroidal Anti-inflammatory Drugs (NSAID)-induced gastric ulcers. It acts upon gastric parietal cells, inhibiting the secretion of gastric acid. Oxytocin has long been used as the standard agent for labor induction, but it does not work well when the cervix is not yet riped. Misoprostol may also be used in conjunction with oxytocin [1,2]. It causes Prostaglandin E1 myometrial contractions by interacting with specific receptors on myometrial cells [3-5]. So far several studies for the estimation of misoprostol have been carried out. Some of them are Ultra Performance Liquid Chromatography (UPLC) [6,7], Reverse Phase High Performance Liquid Chromatography (RP-HPLC) [8,9] and HPLC [10-12]. Survey of literature reveals that there is no accelerated stability indicating studies for Misoprostol and in combination. Therefore we have established a new technique of analysis in bulk and pharmaceutical dosage form (Figure 1).

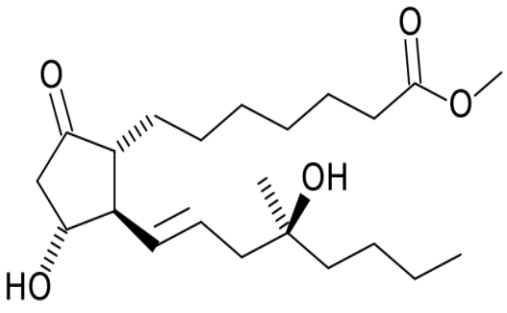

Figure 1: Structure of misoprostol

MATERIALS AND METHODS

UV-Spectrophotometer parameters and apparatus

Perkin Elmer equipped with λ=25 UV-Spectrophotometer, Digital pH meter Elico, India) and Electronic balance (Shimadu, Japan) were used for this study.

Drug samples

The standard pure misoprostol and marketed formulation were used for the estimation and were procured from Sai Mira Pvt. Ltd, Chennai and local market respectively. The label claim for the drug misoprostol was 200 mg.

Reagents and solutions

Ethanol, hydrochloric acid, sodium hydroxide and hydrogen peroxide of analytical grade reagent were procured from Merck India Pvt. Ltd, Mumbai.

Preparation of the misoprostol standard and sample solution

Solvent: Ethanol was used as a solvent for the study.

Standard solution preparation: (Misoprostol 50 µg/ml)

Weighed accurately 100 mg of misoprostol and dissolved in 70 ml of ethanol in a 100 ml clean, dry, standard volumetric flask and the volume was made up to 100 ml with ethanol to obtain a concentration of 1000 µg/ml. The 10 ml of the above stock solution was pipetted and transferred into a 100 ml clean, dry, standard flask and the volume was made up to 100 ml with ethanol to obtain the concentration 100 µg/ml, further dilution was made using 5 ml from the above solution to 10 ml with ethanol in 10 ml clean dry standard flask. The prepared solution was scanned from 200-400 nm.

Sample solution preparation

Weighed 20 tablets and powdered. A quantity of powder equivalent to 100 mg was taken in a 100 ml standard flask and dissolved in 70 ml of ethanol and the volume was made to 100 ml with ethanol. Diluted 5 ml of the above solution with ethanol to obtain a 10 µg/ml and the absorbance was measured at 275 nm using a reagent blank. The results were shown in Table 1.

Table 1: Sample preparation

S. No.	Brand name	Label claim	Amount present	Percentage purity
1.	Misoprost	200	0.2087	104%

Method validation

Linearity

From the prepared standard stock solution, different dilutions were made ranges from 1, 2, 3, 4, and 5 (µg/ml) respectively using ethanol and absorbance for the above prepared solutions were done at 275 nm. A plot of sample concentration vs absorbance was done. The regression of the plot was computed by least square regression method. The slope and intercept of standardization curve was found to be y=0.063x (R^2=0.951). The results were shown in Table 2 and Figure 2.

Table 2: Linearity studies

Concentration (µg/ml)	Absorbance (nm)
1 µg/ml	0.0228
2 µg/ml	0.1025
3 µg/ml	0.1829
4 µg/ml	0.2472
5 µg/ml	0.3433

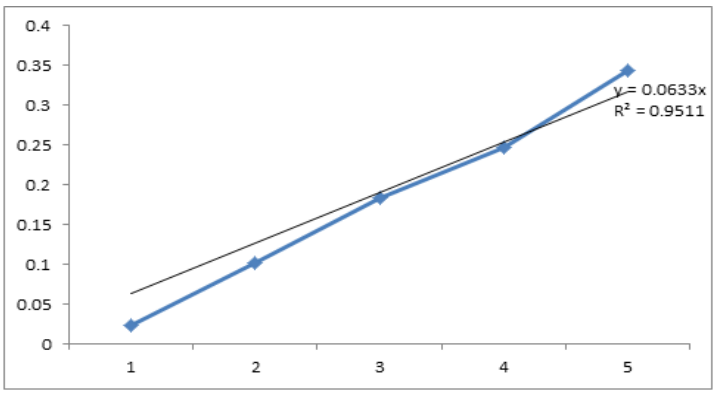

Figure 2: Linearity graph

Accuracy

The accuracy of developed method was confirmed by recovery studies at three different levels 50%, 100% and 120% and which was carried out by adding a known amount of standard drug to preanalyzed sample solutions. The percentage of recovery for the each level lies between 99% and 101%. The results were shown in Table 3.

Table 3: Accuracy studies

Drug	% Level	Amount present (mg)	Amount added (mg)	Absorbance (n=3)	Amount recovered (g)	% Recovery
Misoprostol	80%	200	8	0.066	207	99.51%
	100%	200	12	0.1320	209	98.58%
	120%	200	16	0.1460	215	99.53%

Precision

Method precision: The sample solutions were prepared as per standard method and the absorbance was measured for the solution. The % Relative Standard Deviation (RSD) was found to be within the specified limit. The results of method precision were shown in Table 4.

Table 4: Precision studies (inter day)

S. No.	Misoprostol (4 µg/ml)		
	After 1 h	After 3 h	After 6 h
1	0.025	0.027	0.031
2	0.030	0.034	0.032
3	0.027	0.031	0.037
AVG	0.082	0.092	0.1
S.D	0.002517	0.003512	0.003215
%R.S.D	0.0839	0.117066	0.107166

Intermediate precision (Analyst to analyst variability): The standard solution of 4 µg/ml was prepared as per test method by two different analysts and intermediate precision study was performed. The results were shown in Table 5.

Table 5: Precision studies (Intermediate)

S. No.	Misoprostol	
	Analyst 1 (4 µg/ml)	Analyst 2 (4 µg/ml)
1	0.022	0.019
2	0.022	0.019
3	0.023	0.018
4	0.022	0.019
5	0.022	0.019
6	0.022	0.019
Average	0.133	0.113
S.D	0.000408	0.000402
%R.S.D	0.0068	0.0067

LOD and LOQ: From the linearity plot the LOD and LOQ are calculated: LOD=4.98 µg/ml; LOQ=15.10 µg/ml

Acceptance criteria: The % RSD should be less than 2%.

Limit of Detection and Limit of Quantification (LOD and LOQ): The LOD and LOQ were calculated by using the following formula based upon the linearity data. The results were shown in Table 5.

$$\text{LOD}=3.3\,\frac{\sigma}{s}$$

$$\text{LOQ}=\frac{10\,\sigma}{s}$$

Where, σ=Standard deviation of the response, S=Slope of the calibration curve of the Analyte.

Robustness

A study was conducted to determine the change in effect of wavelengths at 273 nm and 278 nm respectively. The results were shown in Table 6.

Table 6: Robustness

S. No.	Absorbance	Absorbance
	At 278 nm	At 273 nm
1	0.2378	0.1372
2	0.238	0.138
3	0.227	0.142
Mean	0.2342	0.139
S.D	0.0062	0.0025
%RSD	0.2098	0.0857

Stability indicating analytical methods

The international conference on harmonization entitled testing of drug related substances and products requires stress testing to be carried out to eliminate the inherent stability characteristics of active pharmaceutical ingredients. Forced degradation studies were carried out using parameters such as acid hydrolysis, alkali hydrolysis, dry heat condition, oxidative degradation and photolytic degradation (Table 7).

Table 7: Forced degradation studies

Condition	Time	% Degradation
0.1 N NaOH (1 ml)	60 min	10.8
	90 min	12.06
3 N HCl (1 ml)	60 min	10.5
	90 min	14.3
Dry heat 70°C	48 h	14.7

Acid hydrolysis

To the 3 ml of stock solution containing 1000 µg/ml of misoprostol, 1 ml of 3N hydrochloric acid was added in 10 ml clean, dry, standard volumetric flask and the volume was made with ethanol to 10 ml. Then the volumetric flask was kept under normal condition for 90 min. After 60 min from the dilution made, 1 ml of solution was taken out from this flask, neutralized with alkali and diluted with ethanol in a 10 ml clean dry, standard flask to get the concentration of 30 µg/ml. For the preparation of blank, 0.5 ml of solution of 3 N hydrochloric acid and 5 ml solution of 3 N sodium hydroxide were diluted with ethanol in 10 ml of volumetric flask. After 90 min, 1 ml of the solution was pipetted out from the flask and the above procedure was repeated as per test and standard.

Alkaline hydrolysis

To the 3 ml of stock solutions containing 1000 µg/ml of Misoprostol, 1 ml of 0.1 N sodium hydroxide was added in a 10 ml volumetric flask and the volume was made with ethanol. Then the volumetric flask was kept at normal condition for 90 min. After 60 min from the dilution made, 1 ml of solution was pipetted out from flask, neutralized and made up to 10 ml with ethanol and the further dilution was carried out to achieve the appropriate concentration (30 µg/ml). This solution was taken in cuvette. For the blank, 0.5 ml solution of 0.1 N hydrochloric acid and 0.5 ml solution of 0.1 N sodium hydroxide was diluted with ethanol in 10 ml of volumetric flask. After 90 mins 1 ml of the solution was pipetted out from the flask and the above procedure was repeated as per test and standard.

Dry heat induced degradation

A sample solution of Misoprostol was taken in a Petri plate and exposed to a temperature of 70°C for 48 h in a hot air oven. After 48 h, 10 mg of the sample was taken and diluted with ethanol. From this solution, further dilution was carried out to achieve the concentration of 30 µg/ml and the solution was taken for the analysis.

Oxidative degradation

To the 1.5 ml of the prepared solution containing misoprostol concentration of 1000 µg/ml, 1 ml of 30% w/v of hydrogen peroxide was added in 10 ml volumetric flask and the volume was made with ethanol which was kept at room temperature for 15 min. For the blank, 1 ml of 30% v/v of hydrogen peroxide was kept at ambient condition for overnight in 10 ml of volumetric flask. Both standard and blank solutions were heated on boiling water bath to remove excess of hydrogen peroxide. After 15 min, dilutions were made from the stock solutions to achieve the concentration of 30 µg/ml. Then the solutions were analyzed further.

RESULTS AND DISCUSSION

A precise method has been developed and validated for the drug Misoprostol. The standardization curve was linear over the concentration range of 1-5 µg/ml. The LOD and LOQ values were found to be 4.9 µg/ml and 15.10 µg/ml. Stress degradation studies were established for Misoprostol by subjecting it to acid, base, oxidation, and dry heat induced degradation. The stress samples were assayed and results shown were within the range when compared against a standard drug. The UV-Spectrophotometer method developed was considerably suitable for routine analysis (Figure 3).

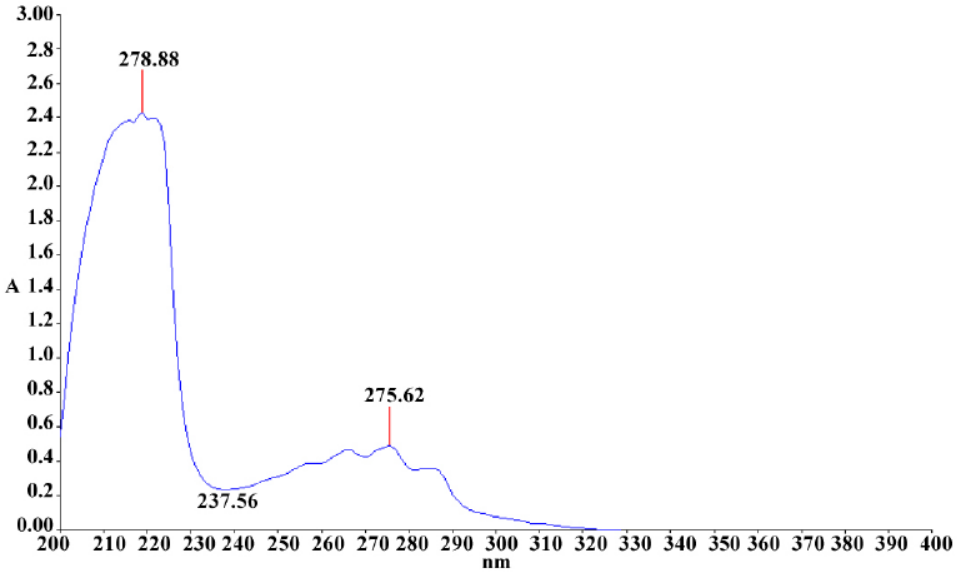

Figure 3: Method development UV-Spectrophotometry

CONCLUSION

The method developed by UV-Spectrophotometer for the estimation of misoprostol in bulk and pharmaceutical dosage form was found to be simple, precise, accurate and reproducible. The Statistical analysis of the developed method showed minimal deviation and all the validation parameters were well within the specified limit. Hence the proposed method can be successfully applied for the analysis of misoprostol in bulk and formulation. Forced degradation studies of misoprostol have revealed that the drug mostly degraded in thermal medium compared to the other stress conditions.

ACKNOWLEDGEMENT

We would like to express our thanks to Sai Mira Pharmaceuticals Pvt Ltd. We are greatly thankful to the Principal, Swamy Vivekananda College of Pharmacy, Elayampalayam, Tiruchengode Namakkal Dist for having provided the necessary facilities to carry out this research work.

REFERENCE

[1] F.R. William, C.F. Kevin, *Am. J. Obstet. Gynecol*., **1998**, 179, 1175-1180.
[2] G.L. Zelger, P. Anne-Lise, P. Furrer, S. Rudaz, *Int. J. Gynecol. Obstet*., **2013**, 4, 71-76.
[3] Cooper, *Int. J. Gynecol. Obstet*., **2011**, 118(11), 1292-1301.
[4] Easmin, *Mymensingh Med. J*., **2012**, 20(4), 566-9.
[5] Mittal, *Int. J. Gynecol. Obstet.*, **2011** 1.12(1), 34-39.
[6] M. Dias, S.S. Simoe, A.C. Ajenjo, *J. Pharm. Biomed. Anal*., **2012**, (17), 198-201.
[7] M.J. Dias, M. Wanare, A.S. Welankiwar, J. Kumar, *J. Pharm. Biomed. Anal*., **2012**, 71, 198-201.
[8] N. Yamsani, *Int. J. Chem. Pharm. Anal*, **2015**, 3, 1.8413-1.8416.
[9] K.E. Pravallika, B. Jagadeesh Naidu, P. Ravi, *Der Pharma Leteri.*, **2016**, 8(19), 92-99.
[10] D. Chavan, M. Damle, *Int. J. Adv. Pharm. Sci*., **2015**, 8(4), 3434-3443.
[11] K. Patidar, J. Mehta, N. Vyas, V. Patel, N. Kshatri, *J. Anal. Method*., **2009**, 13.
[12] S.R. Dhaneshwar, V.K. Bhusari, *Der Chemica Sinica*., **2010**, 1(2), 110-118.