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METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF MISOPROSTOL BY UV-SPECTROPHOTOMETRY

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ABSTRACT

A UV spectrophotometric technique for quantifying misoprostol in bulk and pharmaceutical formulations has been developed and validated. Distilled water is utilized as solvent because misoprostol dissolves in it. Misoprostol was dissolved in distilled water and scanned in the UV range (200–400nm). The λ_{max} (absorption maxima) of the drug was found to be 208nm. In the concentration range of 1 to 5 g/ml, Beer's law holds true. Accuracy, precision, linearity, robustness, LOD, and LOQ were all validated in the devised approach. With a correlation coefficient of 0.9980, linearity was achieved in the 1-5g/ml range. 2.0924g/ml and 6.3406g/ml, respectively, were found to be the LOD and LOQ. As a result of the method's high reproducibility and repeatability, it may be used for routine analysis of misoprostol in bulk.

KEYWORDS: Misoprostol, UV spectrophotometric method, Validation, ICH.

INTRODUCTION

Misoprostol is a synthetic prostaglandin E_1 analogue used to prevent gastro duodenal damage brought on by no steroidal anti-inflammatory drugs (NSAIDs).Chemically it is methyl7-[(1*R*,2*R*,3*R*)-3-hydroxy-2-[(*E*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopentyl]heptanoate. Literature revealed that several several methods have been reported for the simultaneous quantification of misoprostol and mifepristone but no analytical method using UV spectrophotometer for the individual quantification of misoprostol is reported. The objective of this study was to develop and validate a simple, economic, precise, and reproducible UV spectrophotometric method for the estimation of misoprostol.

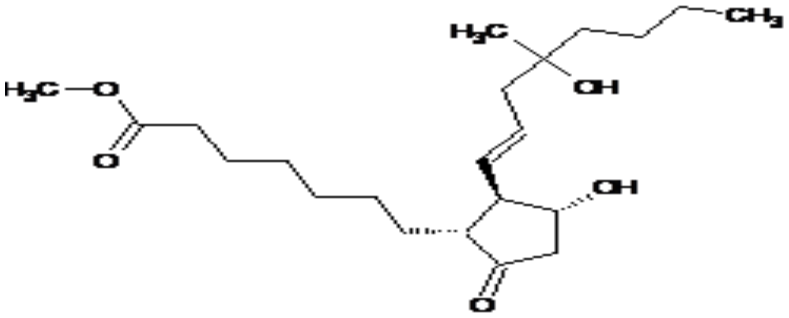


Figure I: - Chemical structure of Misoprostol.

MATERIALS AND METHODS

Instruments

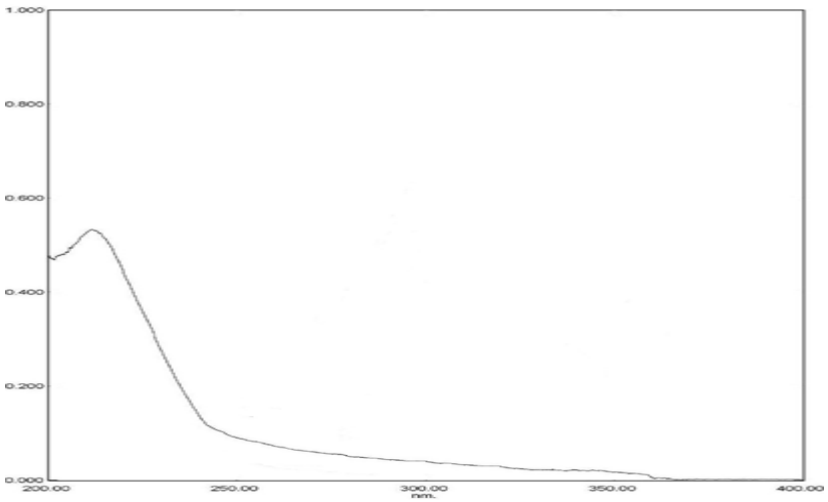
UV-Vis Double Beam Spectrophotometer (Systronics) was used to determine the absorbance. Weighing was carried out using analytical balance (Shimadzu BL-220H) and sonicator (Citizen Digital Ultrasonic Cleaner) was utilized for sonication.

MATERIALS

Misoprostol (purity: 98.40%) was a gift sample from Sai Mira Pvt.Ltd, Chennai. Tablet containing misoprostol (Misoprost 200) was procured from Chaitra Medical Centre; Kasaragod. Distilled water was used as solvent in the present study.

Preparation of standard and test solutions

The standard stock solution of pure misoprostol (1 mg/ml) was prepared by dissolving 100mg misoprostol in 100ml distilled water in a 100ml volumetric flask and sonicated for 30 minutes. This solution was further diluted with distilled water to get various working solutions.



Absorption maxima of misoprostol is at 208nm

Procedure for calibration curve

The standard solutions were prepared by the proper dilution of the primary stock solution. All the measurements were performed at room temperature. The absorbance of the solutions containing Misoprostol was determined in the UV range 200-800nm using an appropriate blank. The λ_{max} was found to be 208nm. The spectrum of Misoprostol was as shown in figure II. For linearity study, dilutions made for Misoprostol in the range of 1 to 5 µg/ml concentrations were prepared by diluting the stock solution with distilled water. The calibration curve was established at this wavelength by plotting a graph between absorbance and concentration. The standard calibration was as shown in figure II.

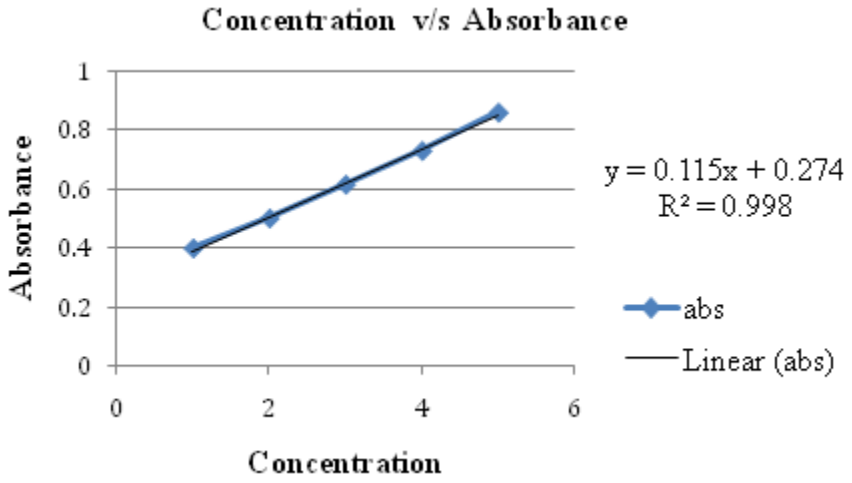


Figure II: Standard calibration curve for analysis of Misoprostol at 208 nm.

Preparation of sample solution

For the estimation of drugs in the commercial formulation, 20 tablets were weighed. Their average weight was determined and was grounded to fine powder using a glass mortar and pestle. Tablet powder equivalent to about 100mg of misoprostol was transferred to 100ml volumetric flask, volume was made up to the mark with distilled water and sonicated for 30 min.The resulting solution was mixed and filtered using filter paper. This solution was used for further analysis.

Table I: Determinations of Active Ingredients in Tablets.

Sample	Label claimed (mg)	Amount found(mg) per tablet	% label claim *
Misoprostol	200	198.75±0.871	98.33±0.129

(Average of Three Determinations)*

Validation of method parameters

Precision

Assay of method precision (intra-day precision) was evaluated by carrying out three independent assays of test samples of Misoprostol. The intermediate precision (inter-day precision) of the method was also evaluated using two different analysts, systems and different days in the same laboratory.

Intraday Precision (Repeatability) results of Misoprostol

Table II.

Day	Reps	Weight of sample (g)	Absorbance	% Purity
1	1	0.0847	0.518	97.85
	2	0.0849	0.529	99.69
	3	0.0849	0.519	97.81
	Avg		0.522	98.36
	SD		0.0061	1.1575
	% RSD		1.1653	1.1768
2	1	0.0849	0.517	97.43
	2	0.0847	0.529	99.92
	3	0.0847	0.518	97.85
	Avg		0.5213	98.4
	SD		0.0061	1.3330
	% RSD		1.2772	1.3546
3	1	0.0849	0.517	97.43
	2	0.0849	0.519	97.81
	3	0.0847	0.518	97.85
	Avg		0.518	97.75
	SD		0.0010	0.1451
	% RSD		0.1930	0.1490

Table 4: Intermediate Precision Results of Misoprostol.

Max.wavelength (nm)	206	210
Absorbance	0.610	0.623
	0.611	0.621
	0.610	0.621
Avg	0.610	0.621
SD	0.0007	0.0014
% RSD	0.1147	0.2254

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Linearity

The aliquots of concentration ranging 1-10 µg/ml were prepared in triplicate, but linearity was found to be between 1-5µg/ml concentrations. The calibration graphs were obtained by

plotting the absorbance versus the concentration data and were treated by linear regression analysis.

Table III.

Concentration (µg/ml)	Absorbance
1	0.398
2	0.500
3	0.615
4	0.730
5	0.860

Accuracy (recovery test)

The accuracy of the method is the closeness of the measured value to the true value for the sample. Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts to the tablet. The recovery was performed by preparing concentration 3 µg/ml of Misoprostol standard solution. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The % recovery of the added pure drug was calculated as % recovery = [(*Ct*–*Cs*)/*Ca*] x 100, where *Ct* is the total drug concentration measured after standard addition; *Cs*, drug concentration in the formulation sample; *Ca*, drug concentration added to formulation. The results were as shown in Table II.

Table IV: Results of Recovery.

Reps	Concentration (%)	Origin level (µg/ml)	Amount added (µg/ml)	Absorbance	% recovery	Mean % recovery	% RSD
1	80	1	0.8	0.484	102.50	101.25	1.23
2		1	0.8	0.485	100.0		
3		1	0.8	0.485	101.25		
1	100	3	3	0.675	100.0	100.22	1.34
2		3	3	0.674	101.66		
3		3	3	0.674	99.0		
1	120	5	6	0.893	100.0	99.83	0.16
2		5	6	0.893	99.83		
3		5	6	0.891	99.66		

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ of Misoprostol were determined by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines. LOD and LOQ values were calculated using the relation,

LOD=3.3δ /S

LOQ=10 δ /S

Where, δ= standard deviation; S=slope of the curve

Table III: Regression and validation parameters of Misoprostol.

Sr. No.	Parameter	Result
Regression parameters		
1	Slope	0.115
2	Intercept	0.274
3	Standard Regression Equation	y=0.115x+0.274
4	Correlation Coefficient (R^2)	0.998
Validation parameters		
1	Absorption maxima(nm)	208nm
2	LOD (µg/ml)	2.0924
3	LOQ (µg/ml)	6.3406
4	Linearity range (µg/ml)	1-5
5	Accuracy(% Recovery ±SD)	99.83±0.16

RESULTS AND DISCUSSION

The development of a simple, rapid, sensitive, and accurate analytical method for the routine quantitative determination of samples will reduce unnecessary tedious sample preparations, the cost of materials and labor. Misoprostol is a UV-absorbing molecule with specific chromospheres in the structure that absorb at a particular wavelength and this fact was successfully employed for their quantitative determinations using the UV spectrophotometric method. The absorption spectrum of Misoprostol in distilled water was shown in Figure I. Calibration curve data was constructed in the range of the concentrations of 1-10µg/ml, but Beer's law obeyed in concentration range of 1-5µg/ml. The regression equation was found to be y = 0.115x +0.274. The correlation coefficient (r^2) of the standard curve was found to be greater than 0.998. The λ_{max} of the drug for analysis was determined by taking scans of the drug sample solutions in the entire UV region.

Performing replicate analyses of the standard solutions was used to assess the accuracy, precision, and reproducibility of the proposed method. The selected concentration within the calibration range was prepared in distilled water and analyzed with the relevant

calibration curve to determine the intra and inter day variability. The proposed method can be successfully applied for assay in tablet dosage forms without any interference. The assay showed that the drug content of this product to be in accordance with the labelled claim (Table I). The recovery of the analyte of interest from a given matrix can be used as a measure of the accuracy of the method (Table IV). The obtained results demonstrate the precision of the proposed method for the determination (Table II).

CONCLUSION

A spectrophotometric method for quantifying Misoprostol in formulation samples has been developed and validated. The assay is selective, precise, accurate and linear over the concentration range studied. LOD was approximately 2.0924µg/ml in formulation and the LOQ was found to be 6.3406µg/ml. In summary, the proposed method can be used for the drug analysis in routine quality control.

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