

DEVELOPMENT AND VALIDATION OF FIRST DERIVATIVE SPECTROSCOPY METHOD FOR SIMULTANEOUS DETERMINATION OF ONDANSETRON AND METOCLOPRAMIDE IN COMBINED DOSAGE FORM

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ABSTRACT

The present work describes a validated first derivative Spectrophotometric method for simultaneous estimation of Ondansetron Hydrochloride and Metoclopramide Hydrochloride in tablet formulation. The first derivative spectroscopy was performed on a double beam UV Visible spectrophotometer using methanol as a solvent. Absorbances were recorded at 266 nm (ZCP of ONDA) and 253 nm (ZCP of METO) for METO and ONDA, respectively. The selected spectrophotometric conditions were found to quantitatively determine Ondansetron Hydrochloride and Metoclopramide Hydrochloride in a combined dosage form without any physical separation. Linearity was found over the range of 2-10 µg/ml for Ondansetron Hydrochloride and 2-15 µg/ml for Metoclopramide Hydrochloride. The values of Limit of Detection were found to be 0.257 µg/ml for Ondansetron Hydrochloride and 0.241 µg/ml for Metoclopramide Hydrochloride. The values of Limit of Quantification were found to be 0.78 µg/ml for Ondansetron Hydrochloride and 0.73 µg/ml for Metoclopramide Hydrochloride. The proposed method was found to be fast, accurate, precise, reproducible and rugged and can be used for simultaneous analysis of these drugs in combined tablet formulations.

Keywords: Ondansetron Hydrochloride, Metoclopramide Hydrochloride, Derivative Spectroscopy

INTRODUCTION

Ondansetron (ONDA) hydrochloride is chemically 1, 2, 3, 9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-yl) methyl]-4H-carbazol-4-one, monohydrochloride, dihydrate.

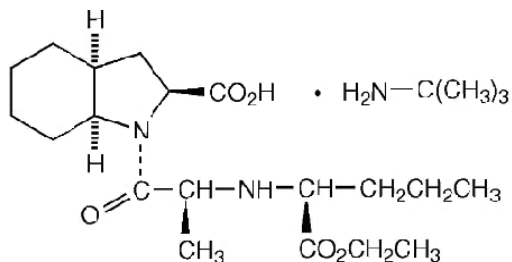


Fig. 1: Structure of Ondansetron Hydrochloride

It represents the class of selective 5HT₃ antagonists which is commonly employed as anti-emetic in combination with anti-ulcer and anti-cancer agents¹⁻⁷. Literature survey revealed that very few analytical methods have been reported for the estimation of ONDA which includes HPLC⁸⁻¹³ and HPTLC¹⁴. Second drug, Metoclopramide (METO) a hydrochloride salt of 4-amino-5-chloro-N-[2-(diethylamino)ethyl]-2-methoxybenzamide, represents the class of orally active Dopamine Antagonist employed in the management of gastro-esophageal reflux disease, nausea and vomiting¹⁵⁻²⁰.

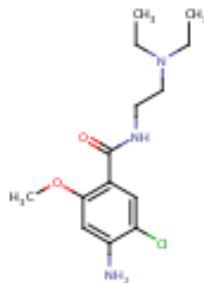


Fig. 2: Structure of Metoclopramide Hydrochloride

The individual determination of METO has been carried out in formulations by Spectroscopy²¹⁻²⁴, HPLC²⁵⁻³² and Flow Injection Analysis³³⁻³⁴. Literature review did not reveal any method for simultaneous determination of ONDA and METO in combined pharmaceutical dosage form. So, we decided to work towards development and validation of simple, sensitive, accurate, precise, rugged and economic method for simultaneous determination of these drugs in combined dosage forms. The present work describes a validated first derivative spectroscopy method for simultaneous determination of these drugs in combined dosage form.

MATERIALS AND METHOD^{35,36}

Instrumentation

A double-beam UV-Visible spectrophotometer, model UV-1700 (Shimadzu, Japan) having two matched cells with 1-cm light path

Chemicals and Reagents

Authentic samples of Ondansetron (ONDA) and Metoclopramide (METO) were supplied by Cambridge Ltd. (Mehsana, India) and LINCOLN Pharmaceutical Ltd. Methanol (AR, Finar laboratories) was used as solvent.

Preparation of Solutions

Standard stock solution (100 µg/ml)

Accurately weighed ONDA (10 mg) and METO (10 mg) were transferred to two separate 100 ml volumetric flask. 50 ml methanol was added to the flask. The drug was dissolved with sonication and the final volume was adjusted with methanol up to the mark to prepare a 100 µg/ml stock solution of both drugs.

Working standard solution

Use the Standard stock solution (100 µg/ml) of ONDA and METO as working standard solutions.

Sample solution

Twenty tablets were weighed and powdered. A quantity of powder equivalent to about 8 mg of ONDA and 20 mg of METO was weighed accurately and transferred to a 10 ml measuring flask. Drug powder was dissolved in methanol with sonication for 20 minutes. The solution was filtered through Whatman filter paper No. 41 and residues were washed thoroughly with methanol. The filtrate and

washings were combined in a 100 ml volumetric flask and diluted to the mark with methanol.

Selection of wavelengths having the maximum absorbance and wavelength of ZCP for estimation of ONDA and METO

The working standard solutions were scanned in the range of 200 nm to 400 nm against methanol as a blank. Maximum absorbance was obtained at 246 nm and 277 nm and the ZCP were found to be at 266 nm and 253 nm for ONDA and METO, respectively.

Calibration curve (Linearity)

Accurately measured working standard solution of ONDA (0.2, 0.4, 0.6, 0.8, and 1.0 ml) and METO (0.2, 0.4, 0.6, 1.0, and 1.5 ml) was transferred to separate 10 ml volumetric flasks and diluted up to the mark with Methanol. Absorbance was measured at ZCP of ONDA (266 nm) and ZCP of METO (253 nm) for METO and ONDA respectively using methanol as a blank. The calibration curve was constructed by plotting absorbance versus concentration corresponding to each working standard solutions. Each reading was average of three determinations. A calibration curve was plotted over a concentration range from 2-10 µg/ml for ONDA and 2-15 µg/ml for METO.

Accuracy and precision

Accuracy was determined in terms of percentage recovery. The proposed method was applied to determine ONDA and METO in tablets. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the tablets with three different concentrations of standards. Precision was determined in terms of intra-day and inter-day precision.

Estimation of ONDA and METO in its formulation

Test solution from tablets which contain ONDA (2.0, 3.0 and 4.0 µg/ml) and METO (5.0, 10.0 and 15.0 µg/ml) were prepared as described above and solutions were analyzed as described under the proposed method.

Method validation^{37,38}

Solution stability

Sample solutions were kept at 25°C and 2-8°C for 24 h and three days, respectively. Assay of initial time period was compared with these two time periods. The falls in the assay values were evaluated. The difference between assays should not be more than 2 % for formulation, and 0.5% for API.

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation proportional to the concentration of analyte in samples within a given range.

The range of analytical method is the interval between upper and lower level of analyte including levels that have been demonstrated to be determining with precision and accuracy using the method. The linear responses of drugs were determined by analyzing five independent levels of the calibration curve. Result should be expressed in terms of correlation co-efficient.

Precision

The precision is measure of either the degree of reproducibility or repeatability of analytical method. It provides an indication of random error. The precision of an analytical method is usually expressed as the standard deviation, Relative standard deviation or coefficient of variance of a series of measurements.

Repeatability (Precision on replication)

It is a precision under a same condition (Same analyst, same apparatus, short interval of time and identical reagents) using same sample. Method precision experiment was performed by preparing the standard solution of drug for six times and analyzed as per the proposed method.

Intermediate precision (Reproducibility)

It expresses within laboratory variations as on different days analysis or equipment within the laboratory.

Intra-day and inter-day precision

Variation of results within same day is called Intra-day precision and variation of results amongst days is called Inter-day precision.

Intra-day precision of the proposed method was evaluated by assaying freshly prepared solutions of both drugs in triplicate at three different concentrations on the same day.

Inter-day precision was evaluated by using freshly prepared solutions of both drugs in triplicates at three different days.

Accuracy (% Recovery)

Accuracy of an analysis is determined by systemic error involved. It is defined as closeness of agreement between the actual (true) value and analytical value and obtained by applying test method for a number of times. Accuracy may often be expressed as % Recovery by the assay of known added amount of analyte. It is measure of the exactness of the analytical method. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the tablets with three different concentrations of standards.

Limit of Detection

It is the lowest amount of analyte in sample that can be detected but not necessarily quantified under the stated experimental conditions. It is expressed as signal to noise ratio of 2:1 or 3:1.

Limit of detection can be calculated using following equation as per ICH guidelines.

$$\text{LOD} = 3.3 \times N/S$$

Where, N is the standard deviation of the absorbance of the drug and S is the slope of the corresponding calibration curve.

Limit of Quantification

It is the lowest concentration of analyte in the sample that can be determined with the acceptable precision and accuracy. It is expressed as signal to noise ratio of 10:1.

Limit of quantification can be calculated using following equation as per ICH guidelines.

$$\text{LOQ} = 10 \times N/S$$

Where, N is the standard deviation of the absorbance of the drug and S is the slope of the corresponding calibration curve.

Analysis ONDA and METO in Combined Dosage Forms

Pharmaceutical formulation of ONDA and METO was purchased from local pharmacy. The responses of formulations were measured at 253 nm and 266 nm in first derivative spectra for quantification of ONDA and METO. The amounts of ONDA and METO present in sample solution were determined by fitting the responses into the regression equation for ONDA and METO.

RESULTS AND DISCUSSION

The proposed method can determine ONDA and METO in combined dosage form and the validity of this method was confirmed in accordance with the ICH guidelines. In proposed method absorbance were recorded at 253 nm and 266 nm, Zero Crossing Points of METO and ONDA, for ONDA and METO in their first derivative spectra, respectively (Fig.-3(a) and 3(b)).

The calibration graphs for ONDA and METO were constructed by plotting the absorbance versus their corresponding concentrations, good linearity was found over the range 2-10 µg/ml for ONDA and over 2-15 µg/ml for METO. Results obtained by applying the RP-HPLC method showed that the concentrations of ONDA and METO can be simultaneously determined in prepared mixtures. The proposed method has been applied to the assay of ONDA and METO in pharmaceutical dosage form. The validity of the method was

further assessed by applying the standard addition technique (Table 1). The results obtained indicate that the additives present do not interfere with analysis of the studied mixtures. The optical and

regression characteristics and validation parameters are reported in Table 2. Results of application of proposed method on pharmaceutical formulation are given in Table 3.

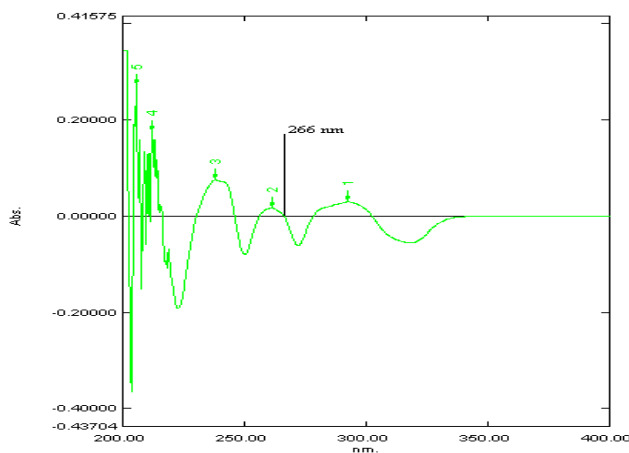


Fig. 3 (a): A typical First order derivative Spectrogram of Ondansetron Hydrochloride

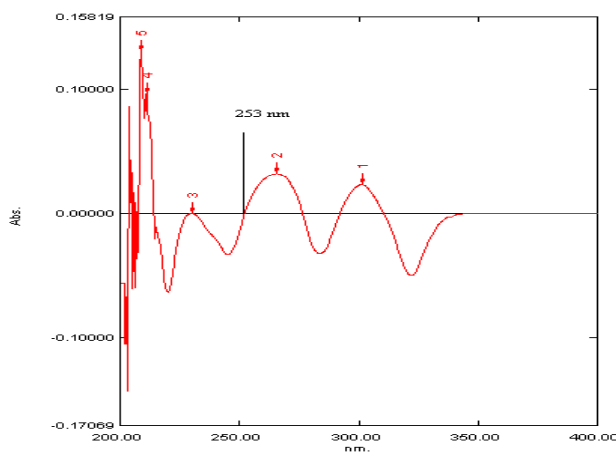


Fig. 3(b): A typical First order derivative Spectrogram of Metoclopramide

Table 1: Data of Recovery study

Drug	Amount taken ($\mu\text{g/ml}$)	Amount added ($\mu\text{g/ml}$)	Amount found ($\mu\text{g/ml}$)	% Recovery \pm S.D. (n=3)
ONDA	2	4	5.85	97.59 \pm 1.156
	2	6	8.02	100.27 \pm 0.636
	2	8	10.01	100.11 \pm 1.347
METO	2	2	3.96	99.16 \pm 1.666
	2	4	6.0	100.0 \pm 1.469
	2	8	10.21	102.11 \pm 1.503

Table 2: Method validation Parameters

Parameters	ONDA	METO
Calibration range	2-10 $\mu\text{g/ml}$	2-15 $\mu\text{g/ml}$
Detection limit	0.257 $\mu\text{g/ml}$	0.241 $\mu\text{g/ml}$
Quantification limit	0.78 $\mu\text{g/ml}$	0.73 $\mu\text{g/ml}$
Slope	-0.003	0.003
Intercept	0.0000	0.00
Mean	100	102.8889
Standard deviation	1.086534	1.025598
Coefficient of variance	1.086534	0.996802
Correlation coefficient	0.998	0.998
Intraday RSD, %	0.967-1.666	0.813-1.773
Inter-day RSD, %	0.732-1.666	0.837-1.739

Table 3: Application of the proposed method to the pharmaceutical dosage forms

ONDA			METO		
Amount taken ($\mu\text{g/ml}$)	Amount found ($\mu\text{g/ml}$)	% Amount Found \pm S.D. (n=3)	Amount taken ($\mu\text{g/ml}$)	Amount found ($\mu\text{g/ml}$)	% Amount Found \pm S.D. (n=3)
2	1.96	98.33 \pm 1.666	5	5.011	100.22 \pm 1.387
3	2.93	97.77 \pm 1.111	7.5	7.6	101.33 \pm 1.777
4	4.01	100.27 \pm 1.734	10	10.022	100.2 \pm 1.503

CONCLUSION

From the results obtained by applying the suggested procedures, it is obvious that the proposed method is accurate, precise, simple, sensitive, selective, robust and rapid and can be applied successfully in routine analysis for the estimation of ONDA and METO in pharmaceutical formulations without interference from commonly encountered excipients and additives.

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