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Research article

Multivariate calibration technique for spectrophotometric quantification of ondansetron in bulk drug and pharmaceutical formulations

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ABSTRACT

The aim of the present work to develop an accurate, simple, sensitive, and validated Ultra violet (UV) spectrophotometric procedure using multilinear regression method for the analysis of Ondansetron in its bulk and commercial dosage form. The multivariate calibration approach was based on equations constructed using linear regression analysis using the correlation between absorbance and concentration at selected five different wavelengths. Ondansetron had a maximum wavelength of 246 nm. The findings were statistically analysed. A linear plot in the concentration range of 5-15 µg/mL, with a regression co-efficient of 0.999 was obtained. The % RSD for intra-day and Inter day precision were 0.0409 and 0.0228, respectively. The assay was determined and found to be 98.38 % – 101.10 % w/w.

Keywords: Ondansetron, Antiemetic agent, UV spectrophotometer, Multivariate calibration, ICH guidelines.

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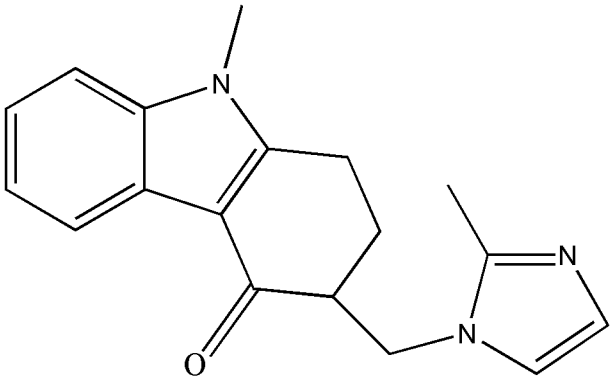
INTRODUCTION

Ondansetron is an antiemetic widely used in the patient's undergoing chemotherapy and radiotherapy. Excitation of 5-Hydroxy tryptamine3 receptors triggers nausea and vomiting. Ondansetron suppresses nausea and vomiting by blocking the action of serotonin at $5HT_3$ receptors[1]. Ondansetron (Figure 1), 1, 2, 3, 9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-yl) methyl]-4H-carbazol-4-one is a carbazole derivative. Chemical Formula is $C_{18}H_{19}N_3O$ and molecular weight is 283.4g/mol[2]. Ondansetron has its receptors both centrally and in GIT. The drug Ondansetron is official in IP, USP and BP[3-5]. The literature surveys revealed various analytical methods like UV and Visible spectrophotometry [6-11], HPLC[12-14], HPTLC[15] and LC-MS/MS[16-17] are available for the assessment in drug formulations and human plasma.

Figure 1: Chemical structure of Ondansetron



The suggested technique has it base on the direct evaluation of Ondansetron with greater accuracy and precision. This technique being simple, and less cost, and it could be used for bulk drugs and their dosage forms. The multivariate standardization method shows the shift of analysis for single species from a single dependent variable to "m" reliant variables. Sensors or wavelengths, for instance, can be included in the calibration model at the same time [18–23].

Under the optimized circumstances, the applied analytical technique will give a great sensitivity, rapidity resolving power, and a low price for quantifiable and regular investigation of an investing admixture.

In this case if the absorbance of an analyte (X) i.e Ondansetron, is scanned at 5 different wavelengths set (λ = 242, 244, 246, 248 and 250 nm), the subsequent formula can be applied for any preferred wavelength.

$$A_{\lambda 242} = a\,X\,C_x + k_1 \quad \text{---------- (1)}$$

$$A_{\lambda 244} = b\,X\,C_x + k_2 \quad \text{---------- (2)}$$

$$A_{\lambda 246} = c\,X\,C_x + k_3 \quad \text{---------- (3)}$$

$$A_{\lambda 248} = d\,X\,C_x + k_4 \quad \text{---------- (4)}$$

$$A_{\lambda 250} = e\,X\,C_x + k_5 \quad \text{---------- (5)}$$

Where A_λ is the analyte's absorbance, a, b, c, d, and e being slopes of the analyte's linear regression functions; while the intercepts are denoted as k1, k2, k3, k4, k5 at the five specified wavelengths, and Cx is the analyte's concentration. The selected five equation systems [(1–5)] listed above can be summarised as follows:

$$A_T = a\,X\,C_x + b\,X\,C_x + c\,X\,C_x + d\,X\,C_x + e\,X\,C_x + K_T \quad \text{---- (6)}$$

The above equation can be further shortened to

$$A_T = C_x\,(a + b + c + d + e) + K_T \quad \text{---------- (7)}$$

Where A_T and K_T is the summation of the absorbance acquired and the totality of intercepts of regression equations at selected five wavelengths respectively. The concentration of the analyte X is computed by following formula.

$$C_x = \frac{A_T - K_T}{(a + b + c + d + e)}$$

MATERIALS AND METHODS

Chemicals and reagents

- Methanol (Gradient grade, Finar Chemicals)
- Analytical grade solvents were used. Ondansetron was obtained as a gift sample from Ideal analytical and Research Institute production, Pondicherry. The marketed tablet formulation used was Zofran, GlaxoSmithKline Pharmaceuticals, India, (Label claim - 4 milligram Ondansetron), procured from the local market.

Instrumentation

- LAB INDIA 3092 UV-Visible double beam spectrophotometer
- Ultra Sonicator Bath
- Analytical balance

Analytical method development

Choice of the solvent

In Methanol, Ondansetron was freely soluble. Hence, it can be used for further dilutions of both standard drug and sample.

Standard stock solution

Ondansetron standard stock solution was prepared by dissolving 100 mg of the standard drug in 50 mL of Methanol and then making up to the mark in a 100 mL standard flask with the same solvent. From this standard stock solution, several concentrations (5-15 µg/mL) of solution were prepared.

Determination of λmax

The standard stock solution was appropriately diluted in methanol to obtain 10 µg/mL. This solution was measured in the Ultra-Violet region from 200 - 400 nm. The λ_{max} was obtained as 246 nm (Figure 2). The linear curve was obtained when the absorbance was plotted against the concentration (Table 1). The solutions were scanned across the range surrounding 246 nm i.e., 242, 244, 246, 248, 250 nm to enhance the correlation and to diminish the instrumental oscillations.

Preparation of sample solution

Accurately weigh and powder 20 tablets of Ondansetron. A weight corresponding to 10 mg was measured into a 10 ml volumetric flask, dissolved and made up to the mark with methanol to obtain 1 mg/mL. This solution was filtered and used for further analysis.

Method Validation

According to ICH Q2B guidelines this method was validated for sensitivity, precision, accuracy, and linearity. [24]

Linearity

The different concentrations over the range of 5-15µg/mL was prepared from the standard stock solution of Ondansetron. In order to minimize the instrumental fluctuations and to improve the correlation these solutions were scanned over a range of wavelength surrounding its absorbance maxima 242, 244, 246, 248, 250 nm. The absorbances were recorded and the standardization graphs were obtained by plotting the concentration vs absorbance. (Figure 3, Table 1).

Table 1: UV calibration data at five distinct wavelengths

Concentration (µg/ml)	Absorbance (Ab)#				
	242 nm	244 nm	246 nm	248 nm	250 nm
5	0.247	0.274	0.295	0.271	0.238
7.5	0.386	0.418	0.451	0.407	0.371
10	0.521	0.556	0.603	0.561	0.482
12.5	0.635	0.698	0.737	0.682	0.597
15	0.759	0.825	0.871	0.812	0.723

Average of 5 determinations; UV= Ultra violet

By calculating the detection limit and quantification limit using the below formulae, the sensitivity of the method was determined.

$$LOD = 3.3\,\sigma/S \quad \text{…… (8)}$$

$$LOQ = 10\,\sigma/S \quad \text{..... (9)}$$

Here, σ is the standard deviation (SD) of the lowermost concentration and

S is the slope of the standard curve.

Precision

To assess the intraday and interday precision, 10 µg/mL solution was scanned six times in a short interval of time in one day for intraday precision and on six different days for interday precision.

Accuracy

Using the standard addition technique, the recovery study for the suggested technique was resolved at 80%, 100%, and 120%. The standard and sample stock solutions was prepared. Pipette out 0.5mL of standard into three standard flasks and add the sample 0.3, 0.5, 0.7 mL into the volumetric flasks above, making up to a capacity of 10 mL with Methanol. The solutions was measured with a UV spectrometer, and the percentage recovery was calculated.

Assay

The amount present in the formulation was calculated by measuring the absorbance of the extracted tablet solution at 246 nm.

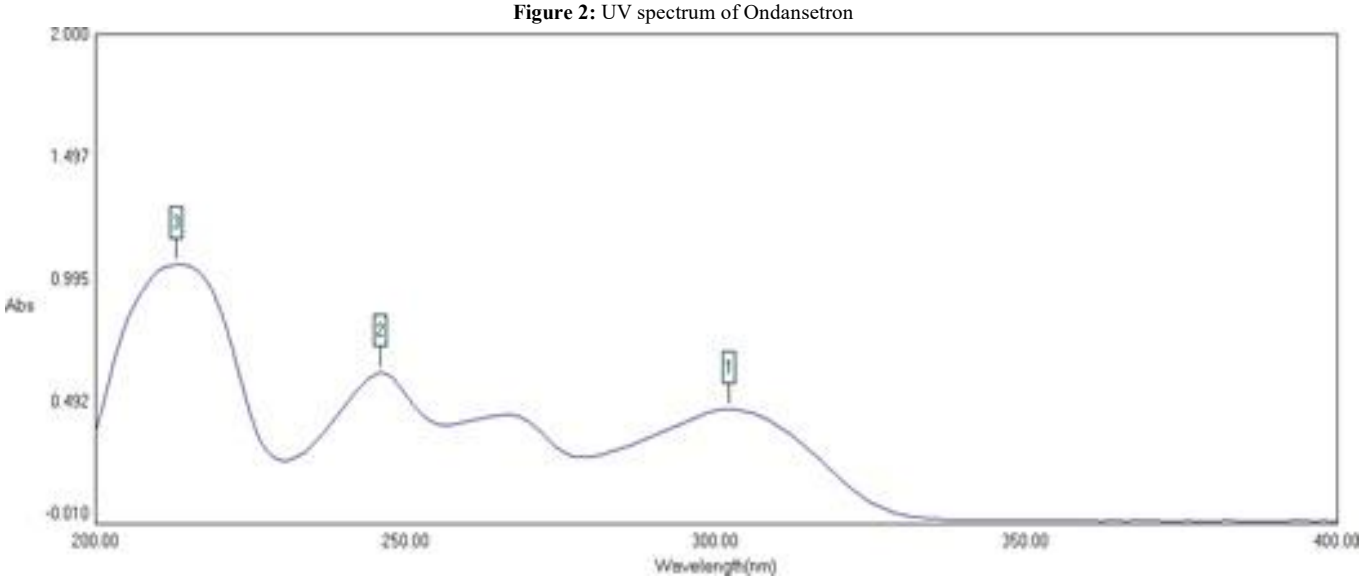
RESULTS AND DISCUSSION

The λmax of Ondansetron was obtained at 246 nm with Methanol as the solvent.

The technique was linear in the designated concentration range over 5-15 µg/mL. The linear regression analysis shows good linear relationship with R^2=0.9999 for all the calibration plots. The equation for the linear regression is Y=0.0575x+0.0162. For precision, the % relative standard deviation was found to be 0.0409 and 0.0228. The LOD and LOQ obtained is 0.016 µg/mL and 0.05 µg/mL respectively. Therefore, according to ICH guidelines each of the

validation parameters were obtained to be within the limits.

Figure 2: UV spectrum of Ondansetron



Linearity

The linearity was recorded at 242, 244, 246, 248 and 250 nm in the concentration range 5-15 µg/mL and depicted in Figure 3 and corresponding calibration curves and residual plots were revealed in Figures 4 to 8 & 9-13 respectively. At each of the wavelength the fewer values of standard deviation show the technique was accurate, precise. The LOD and LOQ were calculated and reported in Table 2.

Figure 3: UV Spectrum showing Ondansetron linearity at 246 nm

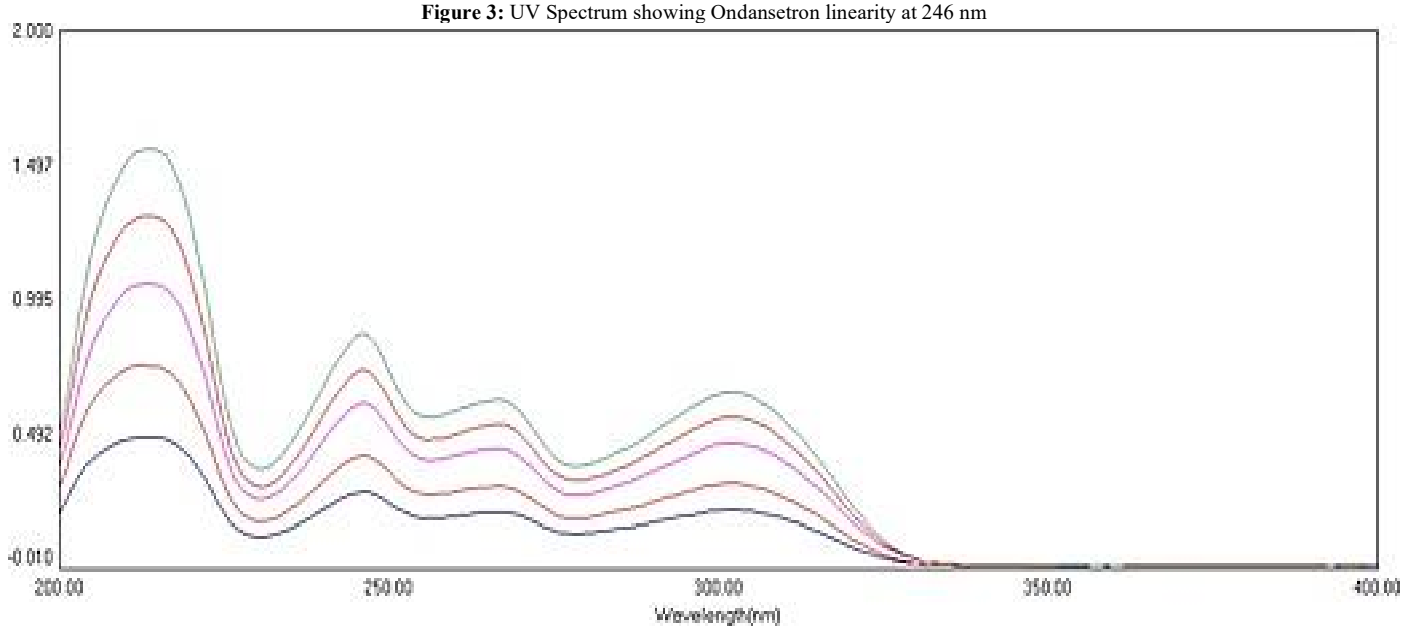


Figure 4: Calibration curve at 242 nm

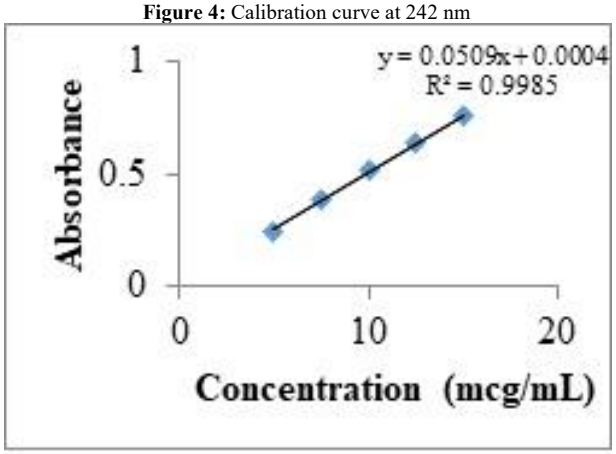


Figure 9: Residual plot at 242 nm

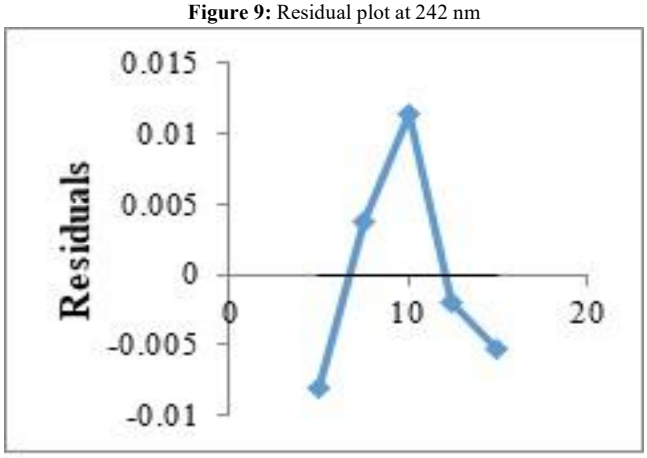


Figure 5: Calibration curve at 244 nm

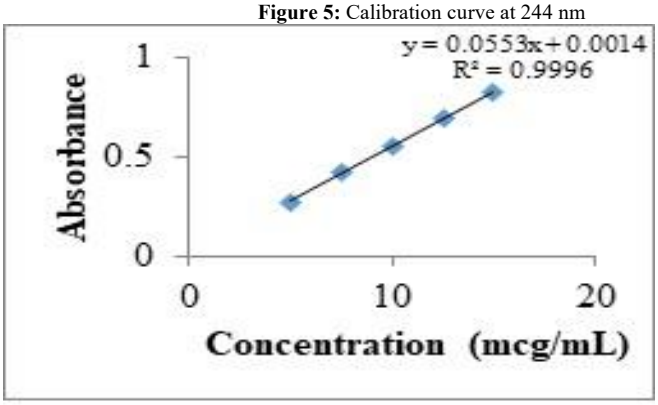


Figure 10: Residual plot at 244 nm

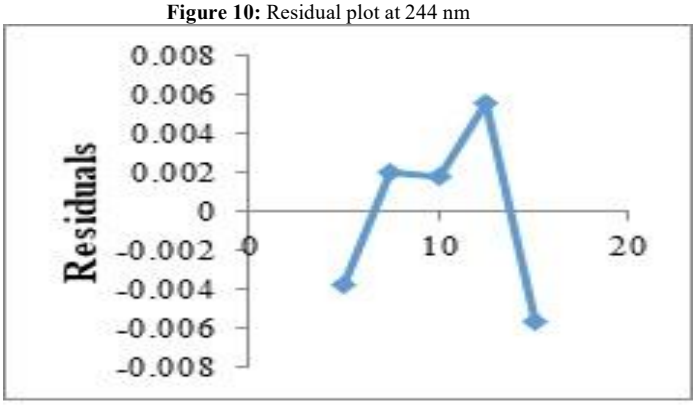


Figure 6: Calibration curve at 246nm

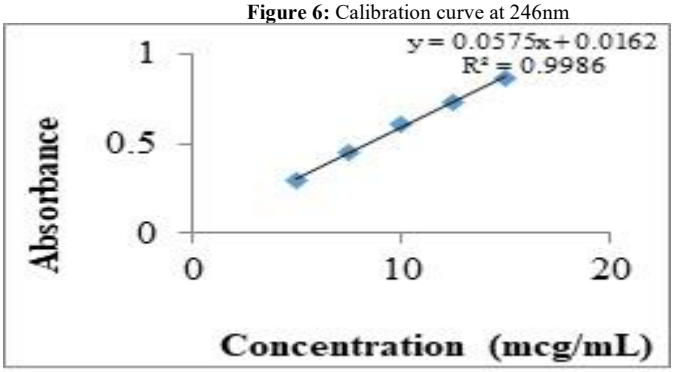


Figure 11: Residual plot at 246nm

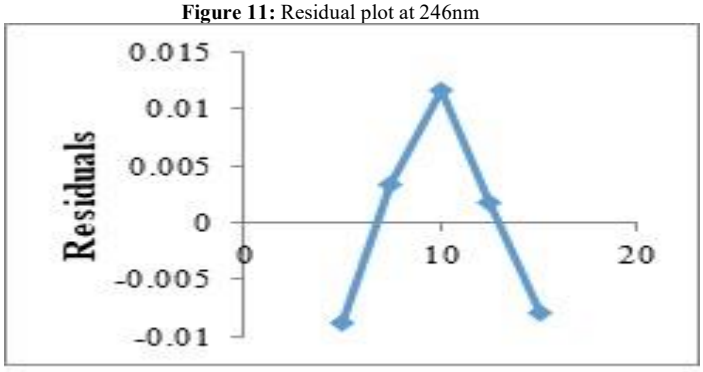


Figure 7: Calibration curve at 248 nm

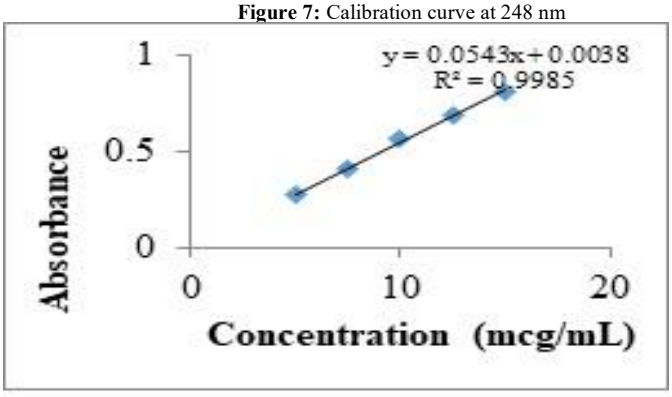


Figure 12: Residual plot at 248 nm

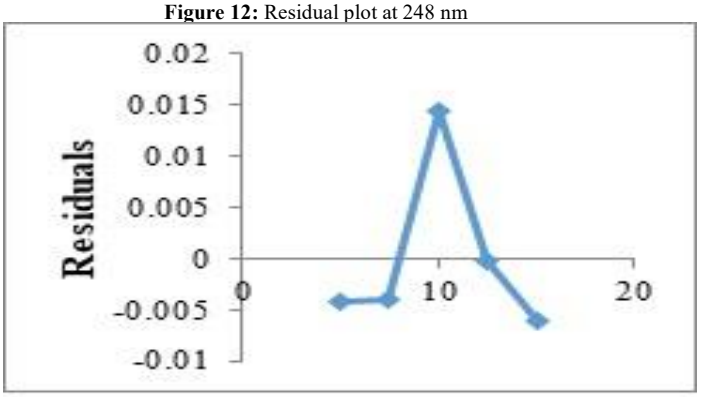


Figure 8: Calibration curve at 250nm

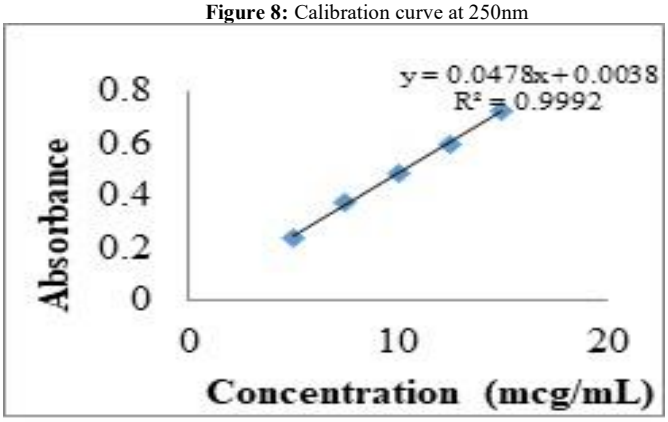


Figure 13: Residual plot at 250 nm

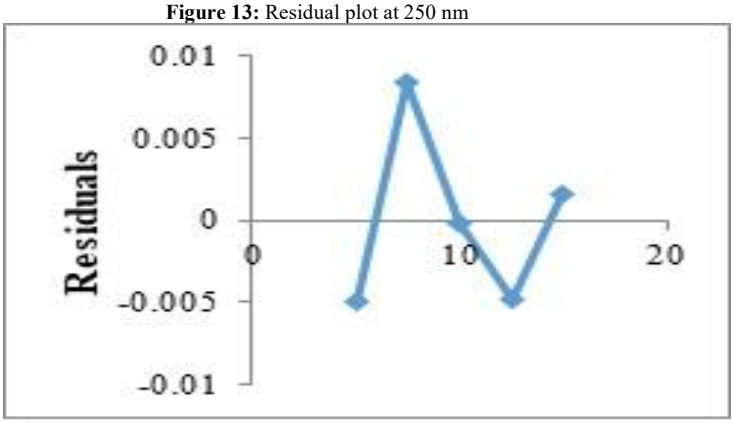


Table 2: Linearity data with LOD and LOQ at selected five wavelengths

Wavelength (nm)	Regression equation	R^2	SD	LOD(µg/mL)	LOQ(µg/mL)	% RSD
242	Y=0.0509x+0.0004	0.9985	0.0011	0.051	0.156	1.7489
244	Y=0.0553x+0.0014	0.9996	0.0005	0.026	0.081	0.9571
246	Y=0.0575x+0.0162	0.9986	0.0002	0.016	0.05	1.6648
248	Y=0.0543x+0.0038	0.9985	0.0004	0.027	0.083	1.7557
250	Y=0.0478x+0.0038	0.9992	0.0004	0.017	0.05	1.3181

*nm = Nanometer; µg/mL = Microgram per millilitre

Precision

The lower value of standard deviation shows that this technique was specific and % RSD for the Intra-day and interday precision obtained at 0.0409 and 0.0228 respectively and it lies within the limit of value less than 2% at each wavelength. The low value of percentage relative standard deviation reveals the suggested technique was accurate and precise (Figure 14, 15).

Figure 14: UV spectra showing intraday precision

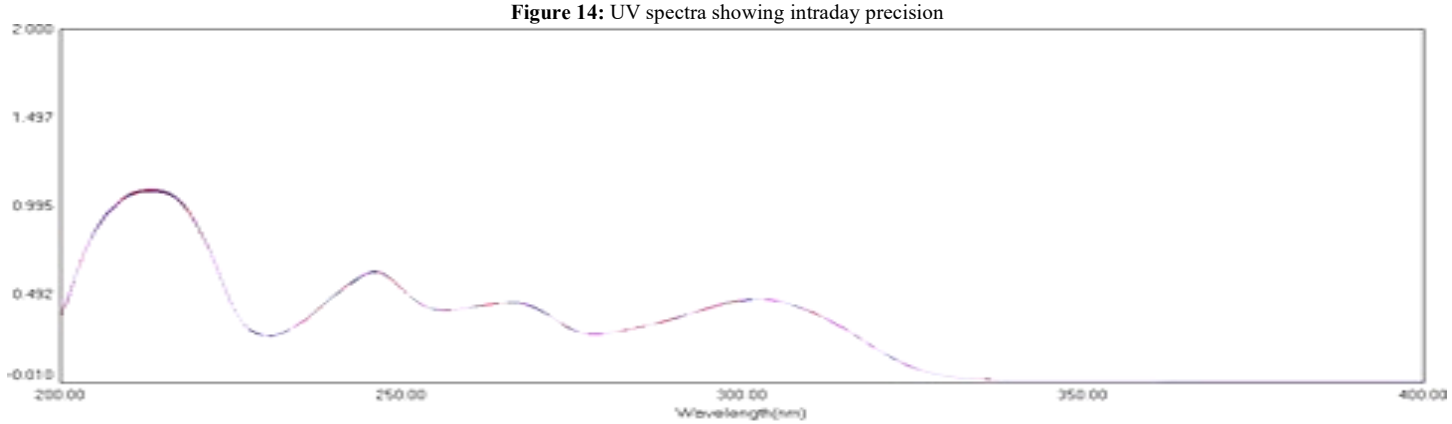
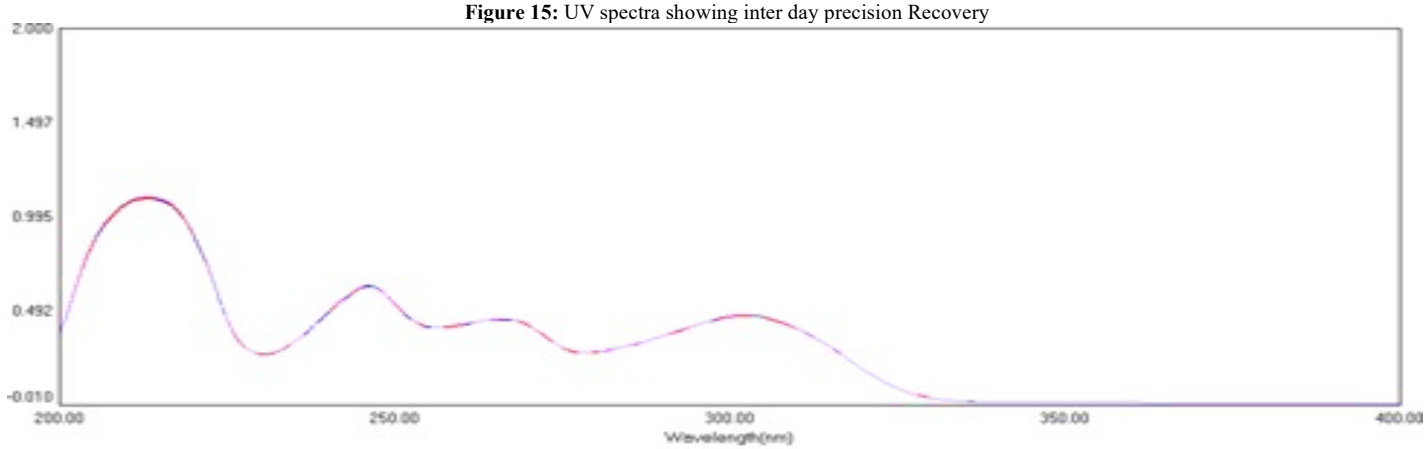


Figure 15: UV spectra showing inter day precision Recovery



As per ICH guidelines the % recovery of the Ondansetron was found from the range of 98.38% - 101.10 % w/w. The recovery was between 97 - 103 % w/w (Figure 16, Table 3).

Figure 16: UV Spectrum showing accuracy of Ondansetron

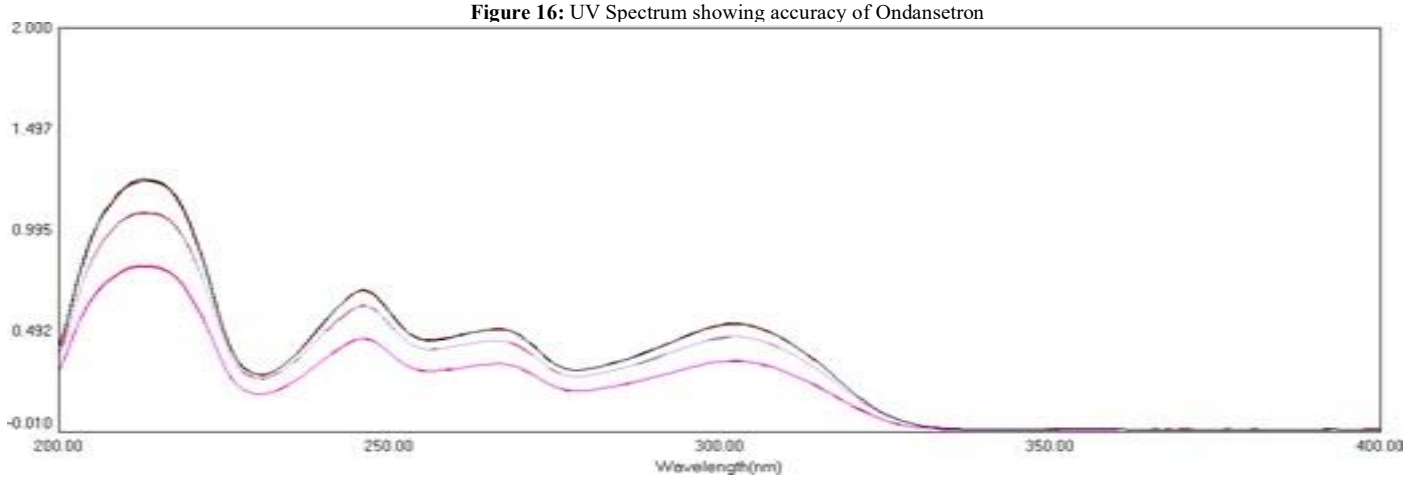


Table 3: Recovery Studies

Wavelength (nm)	Amount present(µg/mL)	Amount added (µg/mL)	Absorbance	Amount recovered (µg/mL)	% Recovery
242	5	3	0.385	7.89	98.63
		5	0.527	10.08	100.80
		7	0.597	11.98	99.85
244	5	3	0.427	7.87	98.38
		5	0.581	10.11	101.10
		7	0.661	11.96	99.67
246	5	3	0.448	7.90	98.75
		5	0.610	10.10	101.0
		7	0.694	11.87	98.92
248	5	3	0.430	7.96	99.5
		5	0.587	9.97	99.7
		7	0.667	11.95	99.58
250	5	3	0.378	7.97	99.63
		5	0.518	10.05	100.5
		7	0.587	11.97	99.75

Assay

The UV absorbance of tablet formulation was documented at 246 nm. The quantity and assay percentage were found to be 4.02 mg and 100.42 % w/w with a % RSD value of as in Table 4.

Table 4: Assay of Ondansetron

Label claim (mg)	Amount obtained (mg)	% Assay
4	3.97	99.25
4	4.09	102.25
4	3.99	99.75
Average	4.02	100.42
SD		1.6073
% RSD		1.6006

CONCLUSION

By using the multilinear regression technique, the newly developed technique was shown to be accurate, specific, sensitive, and repeatable for the evaluation of Ondansetron in its pharmaceutical formulation. The approach was tested using a variety of validation factors, and the results were found to be within ICH recommendations. This approach is a simple working procedure that is comparable to more expensive techniques such as HPLC, and it may be used to analyse Ondansetron in bulk drugs and pharmaceutical formulations on a regular basis.

List of symbols/abbreviations

nm = Nanometer

µg/mL = Microgram per millilitre

ICH = International Conference on Harmonization

UV = Ultraviolet

g/mol = Gram per Mole

GIT = Gastro Intestinal Tract

HPLC = High Performance Liquid Chromatography

HPTLC = High Performance Thin Layer Chromatography

LC/MS = Liquid chromatography-Mass spectrometry

M = Molar

DECLARATIONS

Conflicts of Interest: The authors report on the conflict of interest on the study.

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REFERENCES

1. Rang, Maureen M Dale, 2007. Rang & Dale's Pharmacology, Edinburgh: Churchill Livingstone. 7th Edn. 198.
2. Sean C Sweetman, 2009. Martindale, The complete Drug Reference, 37th ed, Pharmaceutical Press1884.
3. The Indian pharmacopoeia, Government of india, Ministry of health, vol/III: 2014, 2374-2375.
4. The United states Pharmacopoeia. The national formulary. Rockville, MD: The United States Pharmacopeial Convention, Inc., vol/ II, 2018:3028-3029.
5. The British Pharmacopoeia, 6th ed, Stationery office on behalf of the Medicines and Health care Products Regulatory Agency (MHRA), vol.II: 2011, 1598-1600.
6. Sanjay Jain, Maheshwari RK, Rajesh Kumar Nema, et al. 2017. Development and Validation of Simple UV-Spectrophotometric Method of Quantization of Ondansetron Hydrochloride in Solid Dosage Formulations Using Hydrotropic Solubilization Technique. Int. J. Pharm. Sci. Drug Res. 9, 252-255.
7. Biju VM, Naga Gayatri S, Alen Sam Thomas. 2017. A UV Spectrophotometric assay method for available brands of Ondansetron hydrochloride. World J. Pharm. Pharm. Sci. 6, 820-827.
8. Manasa G, Vanitha Prakash K, Mehreen Sultana, et al. 2015. Method development and validation of Ondansetron by UV visible spectrophotometry. Indo Am. J. Pharm. Sci. 2, 1576-1780.
9. Medikonda Sai Kusuma, Mukthinuthalapati Mathrusri Annapurna, Bukkapatnam Venkatesh. 2015. Novel Analytical Techniques for the Determination of Ondansetron Hydrochloride in Pharmaceutical Dosage Forms by Spectrophotometry. J. Chem. Pharm. Sci. 8, 863-866.
10. Kalaichelvi R, Madhava Rao, Manikanta S, Gopinath G, et al 2012. UV Spectrophotometric method for determination of Ondansetron hydrochloride in pure and its formulation. Int. J. Pharm. Pharm. Sci. 4, 151-152.
11. Sradhanjali Patra, Choudhary A, Kar RK, et al, 2007. Spectrophotometric method for Ondansetron hydrochloride. Indian J. Pharm. Sci. 69, 840-841.

12. Juluri Krishna Dutta Tejaswi, 2020. Estimation of Ondansetron hydrochloride by RP-HPLC. EPRA International Journal of Research and Development. 5(5). 29-34.
13. Santosh V. Gandhi, Madhuri S Rathi, Atul P Chaudhari, 2018. Development and validation of stability indicating HPLC method for estimation of Ondansetron hydrochloride. Indo Am. J. Pharm. Sci. 5, 584-591.
14. Mani Aravinth R, Kokilambigai K. S, Anusha Reddy C, Seetharaman R, Kavitha J, Lakshmi. K. S, 2021. Spectrophotometric quantification of Granisetron in bulk drug and pharmaceutical formulations employing multivariate calibration technique. Journal of Medical Pharmaceutical and Allied Science V 10 - I 4, Pages – 3435 - 3439. doi: 10.22270/ jmpas.V10I4.1442.
15. Mushabbar basha MD, Praveena B, Srinidhi M, et al, 2013. Method Development and Validation of Ondansetron in bulk And Pharmaceutical Dosage Form by stability- indicating RP-HPLC method. Int. J. PharmTech Res. 5, 86-98.
16. Chauhan A, Arora PK, Yadav A, et al, 2013. Development and validation of HPTLC method for the estimation of Ondansetron hydrochloride in bulk drug and tablet dosage forms. J. Pharm. Res. 12, 61-65.
17. Gaurav Ninama, Rashmin Patel, Mrunali Patel, et al, 2017. Solid phase extraction liquid chromatography mass spectrometry method with electrospray ionization for the determination of Ondansetron in human plasma: Development and validation consideration. Arabian J. Chem. 10, 3135-3141.
18. Roberto F, Moreira, Myriam Salvadori C, et al, 2010. Development and validation of a rapid and sensitive LC-ESI-MS/MS method for Ondansetron quantification in human plasma and its application in comparative bioavailability study. Biomed. Chromatogr. 24. 1220-1227.
19. Kokilambigai KS, Seetharaman R, Kavitha J, et al, 2017. Multivariate Calibration technique for the spectrophotometric quantification of Zaleplon in bulk and pharmaceutical formulations. J. Pharm. Sci. Res. 9, 824-829.
20. SaiSusmitha A, Kokilambigai KS, Lakshmi KS, 2019. Spectrophotometric quantification of Telmisartan employing multivariate calibration technique in bulk and pharmaceutical formulations. Res. J. Pharm. Technol. 12, 1799-1805.
21. Madhan S, Kavitha J, Lakshmi KS, 2016. Multivariate calibration technique for the spectrophotometric quantification of Ivermectin in Pharmaceutical Formulation. Asian J. Pharm. Clin. Res. 12, 444-451.
22. Durgadevi P, Kokilambigai KS, Lakshmi KS, 2020. First order derivative spectrophotometric method for the quantification of Telmisartan employing Multivariate Calibration Technique. Res. J. Pharm. Technol. 13, 774-780.
23. Naveena Rani D, Kokilambigai KS, Lakshmi KS, 2020. Multivariate calibration technique for the spectrophotometric quantification of Rasagiline in bulk drug and pharmaceutical formulation. Res. J. Pharm. Technol. 13, 843-849.
24. Q2R1 ICH guidelines for analytical method development. Available at: ich.org/ fileadmin/Public_Web_Site/ ICH_Products /Guidelines/ Quality/Q2_R1/Step4/Q2 _R1__Guideline.

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