IJBPAS, November, 2025, 14(11): 5895-5907

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ECOFRIENDLY MULTIVARIATE SPECTROPHOTOMETRIC ESTIMATION OF ITOPRIDE HYDROCHLORIDE IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS

MISRA M, KOKILAMBIGAI K. S* AND MANIKANDAN K

Department of Pharmaceutical Analysis, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur - 603203, Chengalpattu District, Tamil Nadu, India

***Corresponding Author: Dr. Kokilambigai K S: E Mail: kokilams@srmist.edu.in; kokilampharm@gmail.com**

Received 15th July 2024; Revised 20th Sept. 2024; Accepted 1st Nov. 2024; Available online 1st Nov. 2025

https://doi.org/10.31032/IJBPAS/2025/14.11.9554

ABSTRACT

The primary aim of this study is to develop and verify a simple, reliable, precise, and convenient UV-visible spectroscopic method for determining the quantity of Itopride hydrochloride, following the recommendations outlined in ICH Q2 (R1). The multivariate calibration uses linear regression analysis to determine the relationship between absorbance and concentration at five evenly dispersed wavelengths. Using water as the solvent, Itopride hydrochloride displayed λ_{max} at 258 nm. A linear plot was obtained with a regression coefficient of 0.9993 for the concentrations between 15-45 µg mL^{-1}. The % RSD for intra-day and inter-day precision were found to be 0.157 and 0.194, respectively. The assay was determined and found to be 100.10 % w/w.

Keywords: Itopride hydrochloride, UV-visible spectrophotometry, Multivariate calibration, ICH guidelines

INTRODUCTION

Itopride Hydrochloride, chemically known as N-[[4-[2-(dimethylamino) ethoxy]phenyl]methyl]-3,4-dimethoxybenzamide;hydrochloride. The molecular formula and molecular weight were found to be $C_{20}H_{27}ClN_2O_4$ and 394.9 g mol^{-1} respectively **[1]**. Itopride Hydrochloride is a dopamine D2 and anticholinesterase inhibitor acts as gastroprokinetic medication. Itopride enhances the release of naturally occurring acetylcholine by blocking dopamine D2 receptors on cholinergic neurons after synapse. It also

inhibits the activity of acetylcholinesterase, leading to an accumulation of acetylcholine at receptor sites that respond to acetylcholine **[2]**. One possible element that controls the movement of the gastrointestinal tract is the alteration of peptide levels in the blood, including gastrin, somatostatin, motilin, and cholecystokinin (CCK). Recently, the pharmacological properties of several prokinetic drugs have been studied in regard to their association with hormone levels regulated by the stomach. In a study conducted by Itoh *et al*., cisapride, an agent that acts as a dopamine D2 receptor antagonist and a non-selective serotonin 5-HT1,3,4 receptor agonist, was found to increase plasma motilin and gastrin levels. Similarly, somatostatin and mosapride, a selective serotonin 5-HT4 agonist, were also found to elevate motilin and gastrin levels **[3]**.Literature surveys demonstrate various techniques for estimating itopride hydrochloride, like UV-Visible Spectroscopy (UV) **[4]**, High Performance Liquid Chromatography (HPLC) **[5]**, Mass Spectroscopy **[6]**.

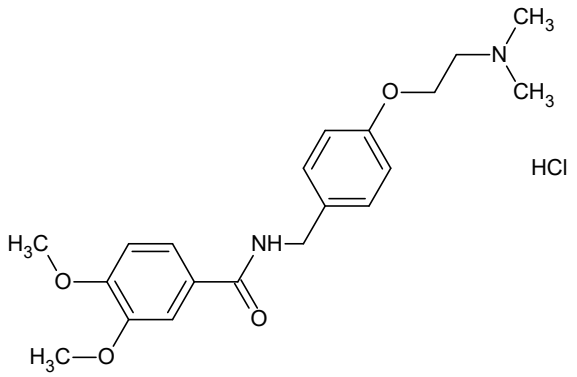

Figure 1: Chemical structure of Itopride hydrochloride

The multivariate technique provides clear accuracy in results, so the results have shown increased accurateness and precision than a conventional UV methods. This method simplifies and converts the result to "m" value as a reliant variable. The analyte's (X) absorbance i.e., Itopride hydrochloride, is measured at five diverse wavelengths surrounding its absorbance maxima (λ = 252, 255, 258, 261, 264 nm). The following formula can be expressed by,

"$A_{\lambda 252} = a\ X\ C_x + \ k_1$ ---------- (1)

$A_{\lambda 255} = b\ X\ C_x + \ k_2$ ---------- (2)

$A_{\lambda 258} = c\ X\ C_x + \ k_3$---------- (3)

$A_{\lambda 261} = d\ X\ C_x + \ k_4$---------- (4)

$A_{\lambda 264} = e\ X\ C_x + \ k_5$---------- (5)"

Equation system from (1-5) represent the analyte's absorbance at specific wavelengths i.e., 252, 255, 258, 261, 264 nm, linear

regression slopes at (a, b, c, d, e), intercepts at (k_1, k_2, k_3, k_4, k_5), and concentration (C_x) respectively. A_T and K_T are the combination of absorbance from the regression equations at five selected wavelengths **[7–14]**.

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

The above equation can be further condensed to

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

$$C_x = \frac{A_T - K_T}{(a+b+c+d+e)} \text{.....(8)}$$

Greenness Evaluation Techniques

The analytical eco scale was developed by the Globally Harmonized System of Classification and Labeling of Chemicals (GHS), which also created a set of pictograms and corresponding signal words **[15]** is predicated on assigning penalty points relying over both quantity and number. The analytical eco scale approach considers each reagent, including its kind and quantity, potential occupational exposure, energy depletion, and waste. Penalty points are eliminated from a 100 point base score.

Analytical eco-scale = 100 - total penalty points……. **[9]**

Comprising five pentagons with separate colour coding, the Green Analytical Procedure Index (GAPI) is a visual representation. At every step of an analytical procedure, the color coding in the pictogram corresponds to three levels of evaluation. GAPI's color coding for greenness runs from green to yellow to red, indicating the minimal, medium, and high environmental implications related with the analytical method, respectively. J. Potka Wasylka provided a succinct overview of GAPI in the year 2018 **[16]**. The third assessment methodology makes use of AGREE metrics' **[17]** special software for assessing the greenness profile. The result of the software is a circle with numbers around the edges that range from 1 to 12 and are oriented clockwise. These figures represent the 12 green analytical chemistry philosophies. Based on the inputs and their weight, the outputs of each of these 12 principles are rated from 0 to 1. This aggregate scale uses the colours red, yellow, and green to show different numbers. Red means zero, dark green means one or close to one, and yellow means a number between red and dark green. A score that represents the level of greenness is produced by adding the 12 principles and the core.

MATERIALS AND METHODS

Materials and reagents required

- Distilled water
- Itopride hydrochloride API was ex-gratis from Ideal Analytical Laboratory, Puducherry
- The dosage form Ganaton 50 mg tablets manufactured by ABOTT LTD, acquired from a local medical shop.

Instrumentation

- UV-Visible double beam spectrophotometer [LAB INDIA 3092]
- Analytical balance
- Micropipette

Analytical method development

Solvent selection

Itopride Hydrochloride shows high solubility in distilled water. Further dilutions of the standard and sample solutions were made by using water as a solvent.

Standard stock solution

Solubilizing 50 milligrams of the active pharmaceutical ingredient in distilled water to obtain 500 µg mL^{-1} serves as the standard stock solution of Itopride hydrochloride. This standard stock solution was used to make an aliquot of solutions with concentrations ranging from 15-45 µg mL^{-1}.

Determination of Absorption maxima

From the standard stock solution, 30 µg mL^{-1} was prepared and scanned in UV spectrophotometry in the region from 200 to 400 nm, to identify the maximum absorbance. The λ_{max} was at 258 nm and is presented in **Figure 2**. The linearity was acquired in concentration limits of 15-45 µg mL^{-1}. The solutions were scanned over various wavelengths about 258 nm in order to reduce the oscillations of the instrument and improve the correlation, wavelength including 252, 255, 258, 261, 264 nm, respectively.

Preparation of sample solution

Ten Itopride hydrochloride tablets were precisely measured and pulverized. In order to obtain 500 µg mL^{-1}, a weight identical to 50 mg has been transferred to a 100 ml standard flask, and it was then further dissolved, diluted, and made up to the mark with water. The resulting filtrate is used for further analysis after filtering.

Method Validation

The above method has been evaluated in accordance with ICH Q2 (R1) guidelines for precision, linearity, sensitivity, and accuracy **[18]**.

Linearity

The standard stock solution of Itopride hydrochloride was used to prepare different concentrations ranging from 15-45 µg mL^{-1}. To eliminate instrumental errors and enhance correlation prepared the solutions were analyzed by scanning their absorbance at the wavelength range centered around their respective maximum absorbance at 252, 255, 258, 261, 264 nm. The absorbances were recorded, and a concentration versus absorbance graph was used to obtain the results. The limit of detection and quantification were calculated using the formula below to determine the method's sensitivity **(Figure 3, Table 1)**.

Table 1: UV Calibration data at five distinct wavelengths

Concentration (µg mL^{-1})	Absorbance				
	252 nm	255 nm	258 nm	261 nm	264 nm
15	0.339	0.351	0.377	0.343	0.310
22.5	0.510	0.524	0.533	0.502	0.461
30	0.651	0.684	0.695	0.659	0.621
37.5	0.808	0.862	0.866	0.824	0.778
45	0.985	1.029	1.047	1.017	0.99

[#]Average of 5 determinations; UV= Ultra Violet

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

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

Where, S stands for standard curve slope, and σ for the standard deviation (SD) of the lowest concentration.

Precision

30 µg mL^{-1} solution was scanned in the UV region from 200 to 400 nm six times in a short period of time on one day for intraday and six different days for interday to evaluate the precision studies.

Accuracy

Assessing the recovery study at 80%, 100%, and 120% was done using the conventional addition technique. To three 100 ml standard flasks, 3 ml of the reference solution was pipetted. Sample solutions of 2.4, 3, and 3.6 ml were added to the same flasks and made up to the mark. After UV scanning these solutions, the recovery percentage was computed.

Assay

By quantifying the absorbance of the tablet solution at a wavelength of 258 nm, the amount of Itopride hydrochloride that is present in the tablet dosage form has been determined.

RESULTS AND DISCUSSION

The λmax of Itopride Hydrochloride was determined to be 258 nm via solvation using distilled water **(Figure 2)**.

This approach is linear within the applied concentration range from 15 to 45 µg mL^{-1}. This linear regression analysis demonstrates a strong linear relationship with R^2 = 0.9991-0.9998. The % RSD values were 0.157 and 0.194 for intra-day and inter-day precision. The LOD and LOQ values were 1.3512 and 4.0948 g mL^{-1}, respectively. Hence, the obtained values were within the ICH validation parameter limits.

Linearity

Linearity at 252, 255, 258, 261, 264 nm was recorded with concentration range from 15-45 µg mL^{-1} **(Figure 3)**, with low relative standard deviation values demonstrates the method accuracy and precision. LOD and LOQ were calculated. The calibration plots were shown in **Figures 4 to 8** and data is presented in **Table 2**.

Precision

The low standard deviation (SD) measurements of the approach show its specificity; percentage RSD for inter-day and intra-day precision was calculated to be 0.157 and 0.194, respectively. It ranges by

less than 2% at every wavelength. The low relative standard deviation percentage shows that this approach is rather exact and accurate **(Figure 9, 10)**.

Recovery

Itopride Hydrochloride has a percentage recovery range from 98.75% to 101.90% w/w as per ICH guidelines **(Figure 11, Table 3)**.

Assay

The UV absorbance of the selected dosage form was recorded at 258 nm. The amount and assay results were 50.05 mg and 100.10 % w/w, respectively with % RSD values as in **Table 4**.

Evaluation of Greenness Profile

The results of greenness profile for the proposed methods were evaluated. The results of analytical scale is shown in **Table 5**, while the results agree metrics and GAPI is depicted in **Figure 12 and Figure 13**.

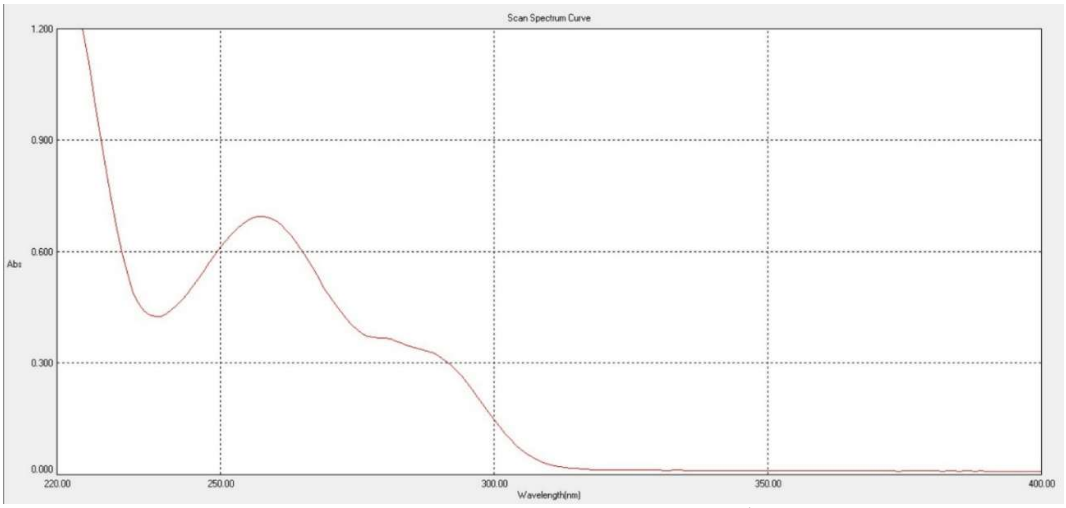

Figure 2: UV spectrum of Itopride Hydrochloride (30 µg mL^{-1}), λ_{max} at 258 nm

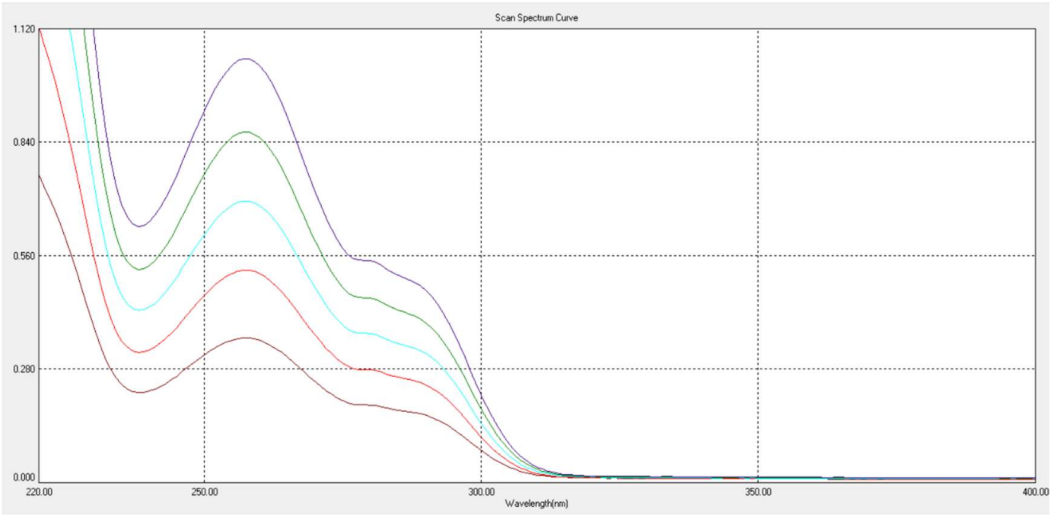

Figure 3: UV Spectrum of Itopride Hydrochloride showing linearity at 258 nm

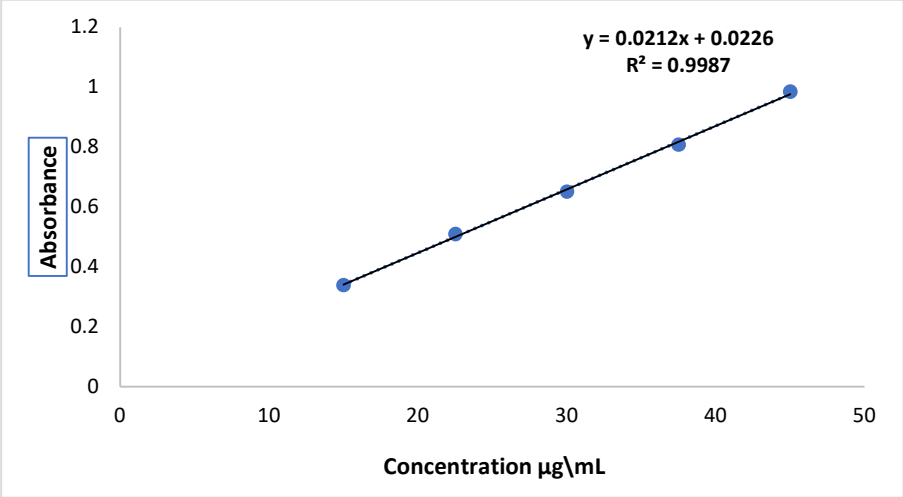

Figure 4: Calibration curve at 252 nm

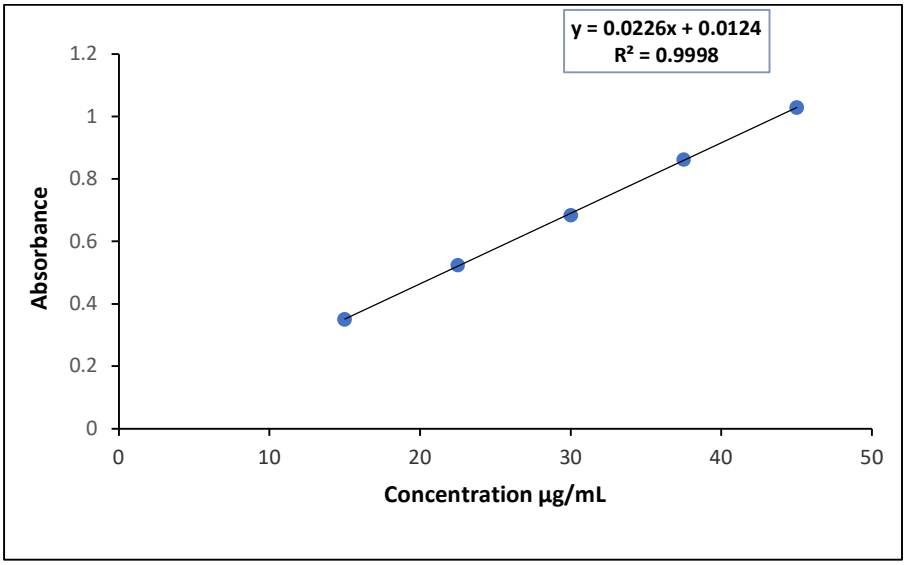

Figure 5: Calibration curve at 255 nm

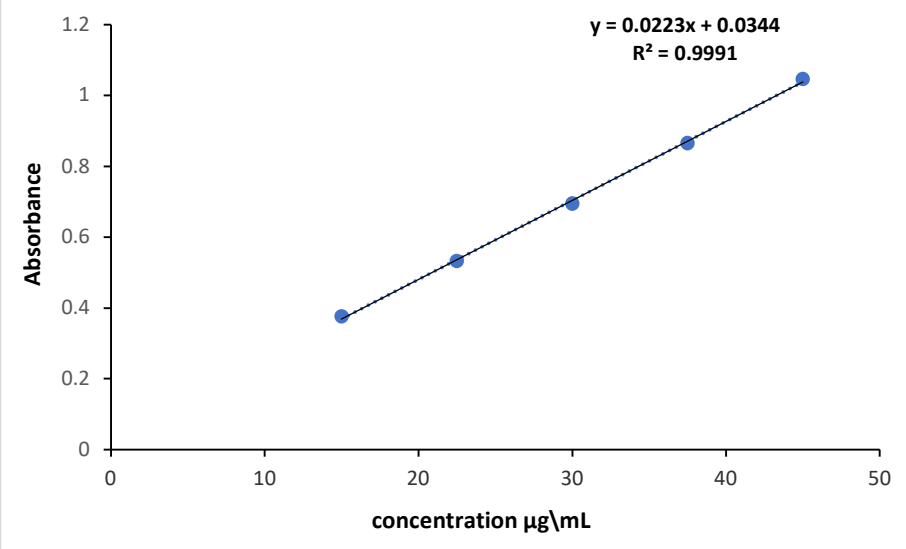

Figure 6: Calibration curve at 258 nm

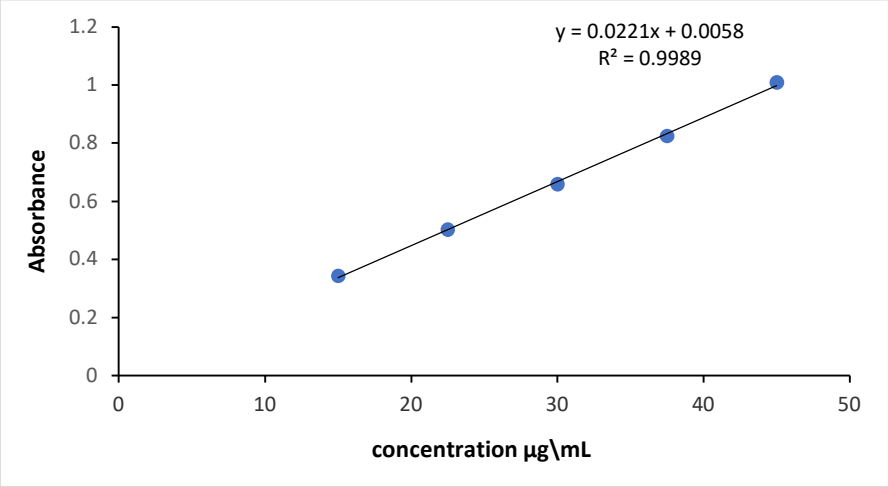

Figure 7: Calibration curve at 261 nm

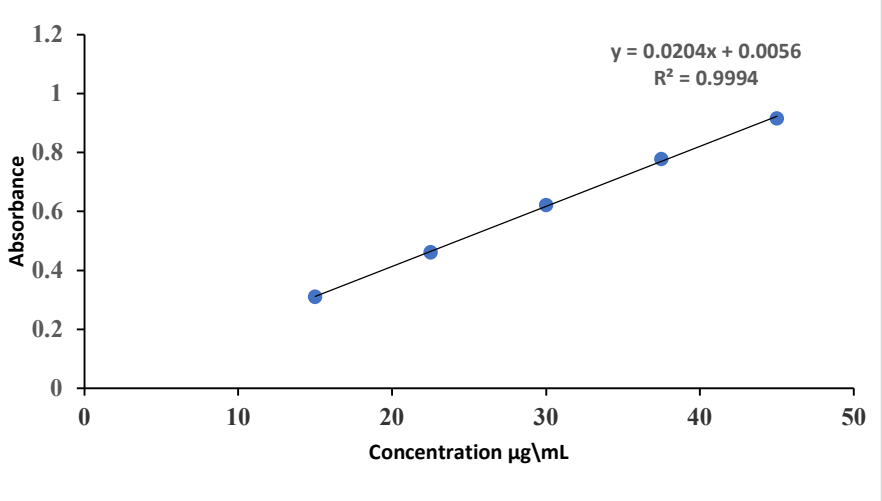

Figure 8: Calibration curve at 264 nm

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

Wavelength (nm)	Regression equation	R^2	LOD (µg mL^{-1})	LOQ (µg mL^{-1})	% RSD
252	y = 0.0212x + 0.0226	0.9987	1.163	4.956	1.5953
255	y = 0.0226x + 0.0124	0.9998	0.622	1.885	0.6171
258	y = 0.0223x + 0.0344	0.9991	1.3512	4.0948	1.2982
261	y = 0.0221x + 0.0058	0.9997	1.5102	4.5765	1.5122
264	y =0.0204x + 0.0056	0.9994	1.1006	3.3352	1.1016

*nm = nanometre; µg mL^{-1} = Microgram per millilitre

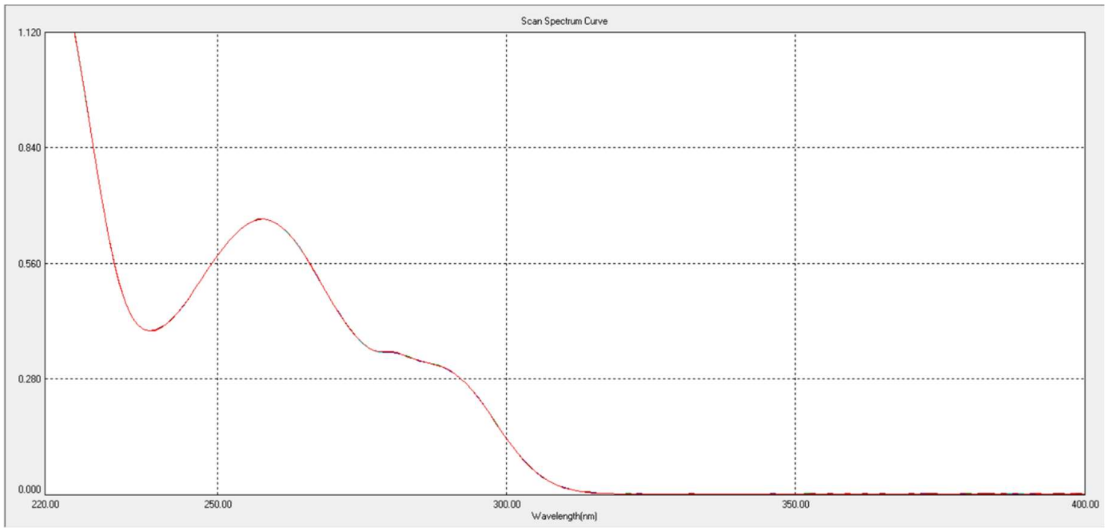

Figure 9: UV spectra showing Intraday precision

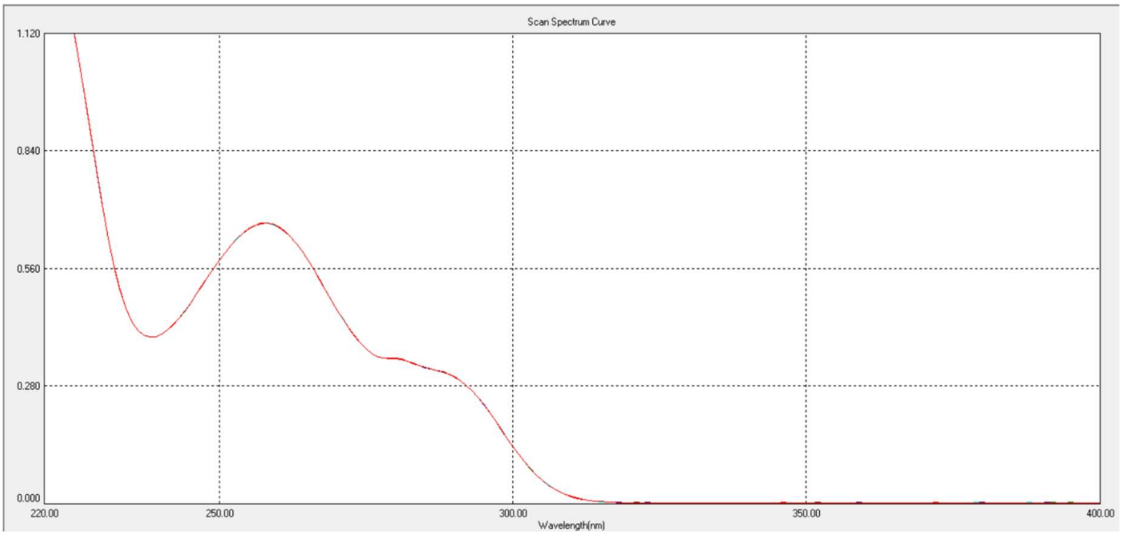

Figure 10: UV spectra showing interday precision

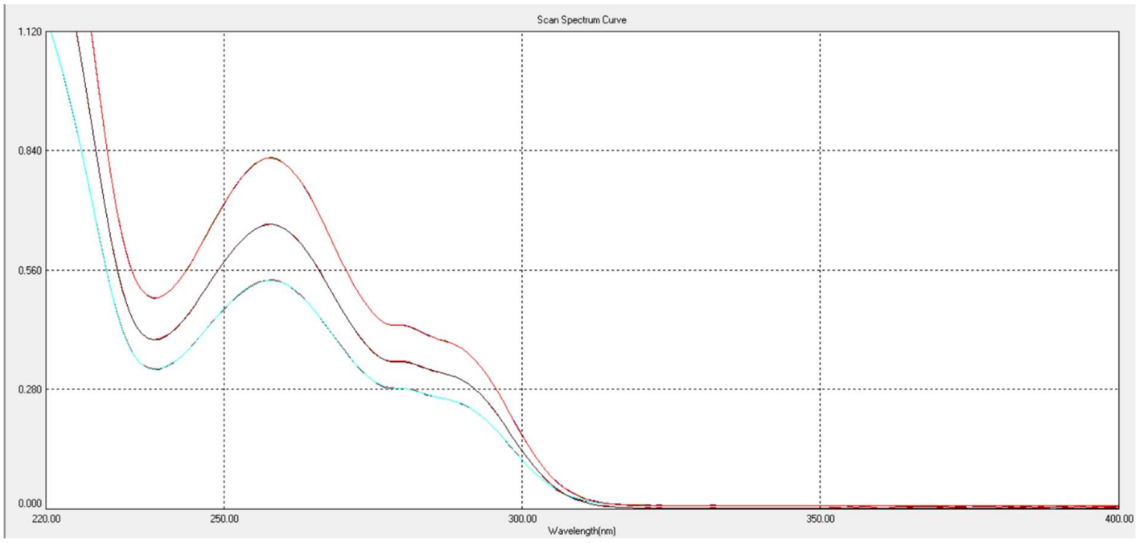

Figure 11: UV spectra showing recovery

Table 3: Recovery Studies

Wavelength (nm)	Amount present (µg mL^{-1})	Amount added (µg mL^{-1})	Amount recovered (µg mL^{-1})	% Recovery
252nm	20	4	23.91	99.63
		10	30.15	100.50
		16	36.21	100.58
255 nm	20	4	23.95	99.79
		10	29.98	99.93
		16	35.97	99.92
258 nm	20	4	23.97	99.88
		10	30.02	100.07
		16	35.98	99.94
261 nm	20	4	24.01	100.04
		10	30.12	100.40
		16	36.12	100.33
264 nm	20	4	23.90	99.58
		10	29.98	99.93
		16	36.01	100.03

Table 4: Assay of Itopride hydrochloride

Label claim (mg)	Amount obtained (mg)	% Assay
50	49.97	99.94
50	50.03	100.06
50	50.15	100.30
Average	50.05	100.10
SD		0.1833
% RSD		0.1831

Table 5: Summary of Eco scale penalty points for the proposed method

Description	Penalty points	Total penalty Points	Score
Distilled water	0	0	100
Instrument	0		
Occupational hazard	0		
Waste	0		

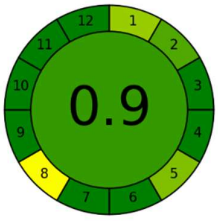

Figure 12: Agree metrics output for the proposed method

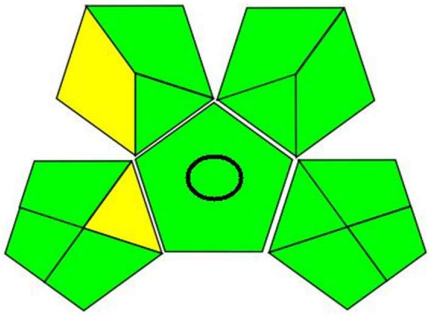

Figure 13: GAPI Pictogram for the proposed method

CONCLUSION

This multivariate analysis is more precise, accurate, sensitive, and economical than a conventional UV-Visible spectrophotometry method for estimating Itopride Hydrochloride. It has been revealed that this multilinear regression analysis is useful for testing both the conventional medication and different Itopride hydrochloride dose formulations. This approach is validated in line with ICH criteria; the values lie within the validation limits. Especially in comparison to costly and sophisticated methods like HPLC and HPTLC, this is a basic working methodology that may be used for routine analysis of itopride hydrochloride in bulk and pharmaceuticals.

ETHICAL STATEMENT

This study does not involve experiments on animals or human subjects.

ACKNOWLEDGMENTS

Authors are thankful to The Chancellor, SRM Institute of Science and Technology, and the management of SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur for providing various sources for carrying out this work.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article exists.

FUNDING SOURCES

There is no funding to report.

REFERENCES

[1] Itopride hydrochloride | C20H27ClN2O4 | CID 129791 - PubChem [Internet]. [cited 2024 Jul 30]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/129791

[2] Lim HC, Kim YG, Lim JH, Kim HS, Park H. Effect of Itopride Hydrochloride on the Ileal and Colonic Motility in Guinea Pig In Vitro. Yonsei Med J. 2008;49(3):472–8.

[3] Katagiri F, Shiga T, Inoue S, Sato Y, Itoh H, Takeyama M. Effects of Itopride Hydrochloride on Plasma Gut-Regulatory Peptide and Stress-Related Hormone Levels in Healthy Human Subjects. Pharmacology. 2006;77(3):115–21.

[4] Gupta KR, Joshi RR, Chawla RB, Wadodkar SG. UV Spectrophotometric Method for theEstimation of Itopride Hydrochloride in Pharmaceutical Formulation. J Chem [Internet]. 2010 [cited 2024 Jul 30];7(SUPPL. 1):S49–54. Available from: https://onlinelibrary.wiley.com/doi/full/10.1155/2010/526891

[5] Thiruvengada Rajan VS, Mohamed Saleem TS, Ramkanth S, Alagusundaram M, Ganaprakash K, Madhusudhana Chetty C. A Simple RP-HPLC Method for Quantitation

of Itopride HCl in Tablet Dosage Form. Journal of Young Pharmacists. 2010 Oct 1;2(4):410–3.

[6] Lee HW, Seo JH, Choi SK, Lee KT. Determination of itopride in human plasma by liquid chromatography coupled to tandem mass spectrometric detection: Application to a bioequivalence study. Anal Chim Acta. 2007 Jan;583(1):118–23.

[7] Kokilambigai KS, Seetharaman R, Kavitha J, Sowndaravel P, Lakshmi KS. Multivariate calibration technique for the spectrophotometric quantification of zaleplon in bulk and pharmaceutical formulations. J Pharm Sci. 2017;9(6):824–9.

[8] Sai Susmitha A, Kokilambigai KS, Lakshmi KS. Spectrophotometric Quantification of Telmisartan Employing Multivariate Calibration Technique in Bulk and Pharmaceutical Formulations. Journal of Research in Pharmacy. 2019;12(4):1799–805.

[9] Madhan S, Kavitha J, Lakshmi KS. Multivariate calibration technique for the spectrophotometric quantification of Ivermectin in pharmaceutical formulation. Asian Journal of pharmaceutical and Clinical research. 2019;12(2):444–51.

[10] Durgadevi P, Kokilambigai KS, Lakshmi KS. First Order Derivative Spectrophotometric Method for the Quantification of Telmisartan employing Multivariate Calibration Technique. Res J Pharm Technol. 2020;13(2):774–80.

[11] Naveena Rani D, Kokilambigai KS, Lakshmi KS. Multivariate calibration technique for the spectrophotometric quantification of Rasagiline in bulk drug and pharmaceutical formulation. Res J Pharm Technol. 2020;13(2):843–9.

[12] Mani Aravinth R, Kokilambigai KS, Anusha Reddy C, Seetharaman R, Lakshmi KS. Spectrophotometric quantification of Granisetron in bulk drug and pharmaceutical formulations employing multivariate calibration technique. Journal of Medical pharmaceutical and allied sciences. 2021 Oct 15;10(4):3435–9

[13] Vimal Ravi, Kokilambigai KS, Seetharaman R, Lakshmi KS. Multivariate UV spectrophotometric quantification of Cilnidipine in bulk drug and pharmaceutical formulations. J Med Pharm Allied Sci. 2022;11(2):4672–8.

[14] Kokilambigai KS, Logesh M, Anusha Reddy C, Seetharaman R, Kavitha J, Lakshmi KS. Multivariate calibration technique for spectrophotometric quantification of Ondansetron in bulk drug and

pharmaceutical formulations. 2022;11(4):4777–82.

[15] Galuszka A, Migaszewski ZM, Konieczka P. analytical ecoscale for assessing the greenness of the analytical procedures. TRAC Trends Anal Chem. 2012;37:61–72.

[16] Plotka- wasylka J. A new tool for the evaluation of the analytical procedure: Green Analytical Procedure Index. Talanta . 2018;181:204–9.

[17] Pena-Pereira F, Wojnowski W, Tobiszewski M. AGREE- Analytical greenness metric Approach and Software. Anal Chem. 2020;92(14):10076–82.

[18] ICH. Harmonized tripartite guideline, validation of analytical procedures: text and methodology. 2005;Q2(R1).