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MULTIVARIATE REGRESSION OF SPECTROPHOTOMETRIC QUANTIFICATION OF ITOPRIDE IN BULK PHARMACEUTICAL PRODUCT

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INTRODUCTION

In the gastrointestinal system, itopride hydrochloride1 (ITH) stimulates or promotes motility. By operating as a dopamine D2 receptor antagonist, it boosts the release of acetylcholine (Ach), which stimulates gastrointestinal motility. It minimally raises prolactin levels, does not lengthen the Q-T interval, and cannot cross the blood-brain barrier due to its high polarity, hence it does not result in any CNS-related adverse drug reactions [1]. As indicated in **Figure 1**, it is N-[4-[2-(dimethyl amino) ethoxy]-benzyl]-3,4-dimethylbenzamide hydrochloride.
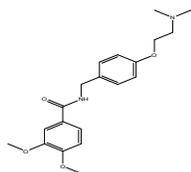
The primary purpose of itopride hydrochloride (IH) is to treat GERD. IH additionally serves to treat non-ulcer dyspepsia, chronic gastritis, anorexia, heartburn, nausea, and vomiting, as well as gastrointestinal symptoms produced by diminished gastrointestinal motility [2].

A few scientific techniques have been published for the evaluation of Itopridein pharmaceutical goods, according to a review of the literature. These methods include UV [2, 3] and HPLC [4], either singly or in combination, HPLC alone [5] HPTLC [6, 7], GC-MS, LC-MS [8], RP-HPLC [9-12], and spectrofluorimetry [13].

Figure 1 : Molecular Structure Of Itopride

Since the suggested method directly measures ATX and has been proven with greater accuracy and precision than the conventional UV-Visible evaluation, there is a higher level of confidence in the results. The individual result is diminished by this multivariate standardization procedure, which converts it to a "m" value as a dependent variable. If the absorbance of an analyte (X), i.e., ATX, has been observed at 5 different wavelengths (λ = 266, 268, 270, 272, and 274 nm), the formula below may be applied for any given wavelength.

$$A_{\lambda 310} = a \, X \, C_x + \ k_1 \ \text{-------------------} (1)$$

$$A_{\lambda 312} = b \, X \, C_x + \ k_2 \ \text{--------------} (2)$$

$$A_{\lambda 314} = c \, X \, C_x + \ k_3 \text{-------------} (3)$$

$$A_{\lambda 316} = d \, X \, C_x + \ k_4 \text{--------------------} (4)$$

$$A_{\lambda 318} = e \, X \, C_x + \ k_5 \text{---------------------} (5)$$

Where K1, K2, K3, K4, and K5 are the intercepts at the five given wavelengths, Aλ denotes the absorbance of the analyte, a, b, c, d, and e constitute the slopes of the linear regression functions for the analyte, and Cx denotes the analyte concentration. The total of the five formula sets (1–5) listed above is as follows:

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

The above equation can be further condensed to

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

A_T and K_T are the summations of the absorbance acquired cum totality of intercepts of regression equations at selected five wavelengths, respectively. The following formula computes the concentration of the analyte X.

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

EXPERIMENTAL:

Chemicals and solvents employed:

- Distilled Water
- The marketed tablet formulation (Ganoton tablets - 50mg of Itopride, by Abbott India Limited) was purchased from the local market.

Solubility:

- Extremely readily soluble in distilled water

Instrumentation:

- UV-Vis double beam Spectrophotometer (Lab India UV-3092)
- Electronic balance (SHIMADZU BL-220H)
- Ultra-Sonic Bath (ILE, ILTC)

METHOD DEVELOPMENT:

Selection of solvent:

Distilled Water, the compound that was used to dissolve the drug during analysis, was shown to be easily soluble in itopride.

Preparation of standard stock solution:

Itopride was precisely weighted at 50 mg and then transferred into a 50 mL volumetric flask. After adding 10 mL of the solvent, the mixture was sonicated for 15 minutes. The compound was added to the final volume, which was then well mixed (1 mg/mL). 5 mL more of the aforementioned solution were pipetted out and put into 50 mL volumetric flask, where the total volume was brought up to the required level using the solvent and thoroughly mixed. The resulting solution was subsequently diluted with the solvent to produce concentrations between 16 and 24 g/mL.

Determination of λ_max:

For attaining a concentration of 20 g/mL, the itopride standard stock solution was diluted using the solvent. This solution was examined in the 400–200 nm UV spectrum. The Itopride UV spectra are displayed in Figure 2.

Preparation of sample solution:

The average weight of 20 Itopride tablets (Ganoton tablets, label claim: 50 mg of Itopride) was determined. The tablets were split apart, their contents were extracted in a mortar, and they were mixed thoroughly. From the combined contents, a weight equal to 50 mg of Itopride was measured, dissolved in 5 mL of solvent using sonication for 15 minutes, and the volume is then increased to 50 mL using the solvent. It was well blended

and filtered before use. For further analysis, the filtrate was appropriately diluted.

METHOD VALIDATION:

According to the ICH Q2(R1) procedure, the created technique was verified to look at the validation parameters like linearity, sensitivity, precision, and accuracy.

Linearity:

The itopride standard stock solution was diluted with the solvent to yield concentrations of 16, 18, 20, 22, and 24 g/mL. Using five distinct wavelengths selected to be close to the drug's maximum (258 nm), namely 254, 256, 258, 260, and 262 nm, respectively, the absorbance of the produced concentrations was measured **(Table 1)**, and the overlay UV spectra demonstrating linearity is shown in **Figure 3**. In order to build a linear correlation and get rid of the instrumental variations, this was done. The correlation coefficient values for the developed linear regression equations were calculated for each of the five selected wavelengths **(Table 2)**. **Figure 3** shows the calibration graphs that were created at five different wavelengths.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

By determining the limit of detection (LOD) and limit of quantification (LOQ) values using the slope and intercept values of the linear

regression line, the sensitivity of the developed technique was ascertained **(Table 2)**.

Precision:

By measuring the absorbance of the linearity solution at the 100% concentration level (20 g/mL) at each of the five chosen wavelengths, intraday and interday precision tests were carried out. 6 times per day (intraday precision) and three distinct days (interday precision) were used to scan the chosen concentration. **Tables 3** through 6 contain the absorbance measurements made at the chosen wavelengths for intraday and interday precision investigations, and **Figure 4** shows the overlay UV spectra for these two types of studies. Calculations were made to get the Standard deviation (SD) and percentage relative standard deviation (% RSD) values.

Assay:

At 258 nm, the extracted sample solution's absorbance was measured. The formulation's drug content was determined, and the assay findings are summarized in **Table 7**.

Accuracy (Recovery studies):

At doses of 50%, 100%, and 150%, recovery trials using the conventional addition method were conducted to evaluate the precision of the suggested method. From the prepared stock solutions of the standard and sample, 0.08 mL of the sample solution was pipetted into three separate 10 mL volumetric flasks. Then, 0.08, 0.12, and 0.16 mL of the standard stock solution were added, respectively, to the volumetric flask above. With the aid of distilled water, the final volume was brought into compliance. The recovery percentage numbers were computed. The recovery study findings were listed in **Table 8** with its overlay UV spectra shown in **Figure 5**.

RESULTS AND DISCUSSION:
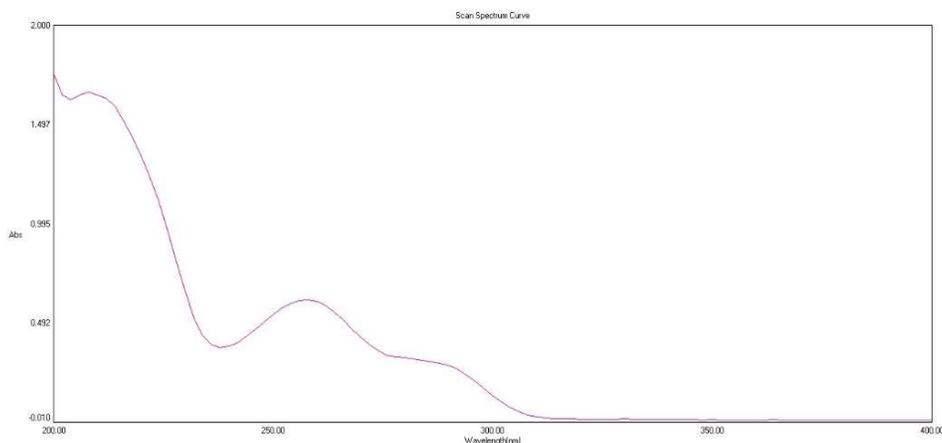
Itopride's absorption peaks were seen at 258 nm.

Figure 2: UV Spectra of Itopride

Linearity:

According to reports, the devised approach is linear over the stated concentration range of 16 to 24 g/mL. For each of the five specified wavelengths of 254, 256, 258, 260, and 262 nm, a linear regression equation was created. The obtained correlation coefficient values were discovered to be more than 0.998.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The computed LOD and LOQ values were discovered to vary from 0.34 to 0.53 g/mL and 1.05 to 1.6 g/mL, respectively.

Precision:

Precision experiments were conducted both within and between days. % RSD values for intraday and interday precision were determined to be far below the ICH recommendations' 2% acceptability threshold, at 1.59-1.70 and 1.43-1.55, respectively. The accurate nature of the developed technique is shown by the low estimated% RSD value.

Assay:

Itopride concentration in the tablet formulation was determined based on the sample solution's absorbance at 258 nm. The drug's assay percentage was determined to be 99.58 % w/w, and the estimated RSD was discovered to be less than 2 %.

Recovery:

The drug's percentage recovery was calculated and found to be within the ICH protocol's limit of 97 to 103% w/w, ranging from 99.35 to 102.9% w/w. The process can therefore be considered accurate.

CONCLUSION:

For the evaluation of itopride in capsule formulation, the established straightforward and quick UV spectrophotometry aided Multivariate calibration technique was found to be linear, sensitive, accurate, and exact. The absorbance of the medication is measured at five separate selected wavelengths, making the multivariate calibration procedure more accurate than the previous published techniques. When the current methodology for quantifying itopride was compared to other methods that have been published, the current methodology was shown to be more sensitive with lower reported LOD and LOQ values. According to ICH criteria, every one of the validation parameters evaluated was found to be within the allowed range. Itopride is available in a number of different dosage forms, including tablets, injectables, and powders for inhalation, and the discovered approach can be expanded to quantify the medication in these other forms as well. Therefore, a quick and easy approach based on mathematical concepts was created. This method was proven to be more predictable

than the other spectrophotometric methods, and it is highly advised for use in routine quality control analyses of itopride in the pharmaceutical formulations.
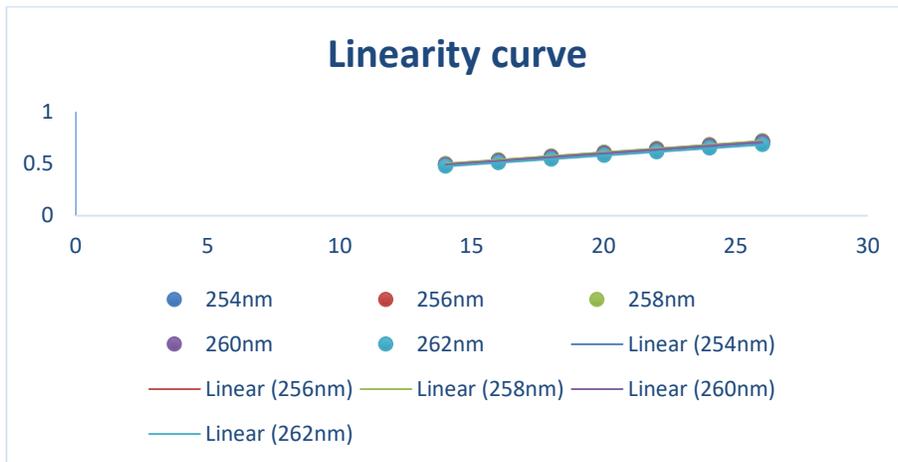

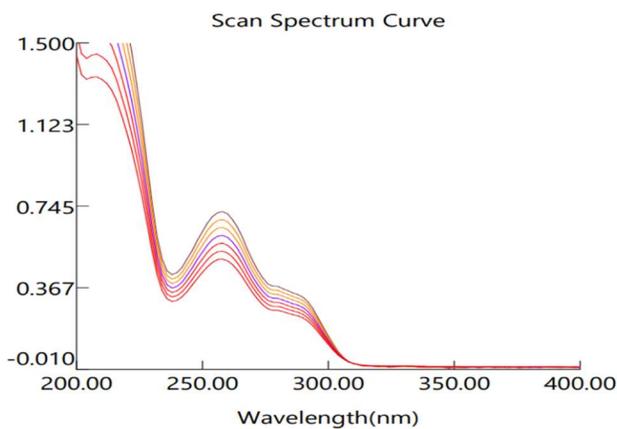**Figure 3: UV Spectrum of itopride showing linearity 258 nm**

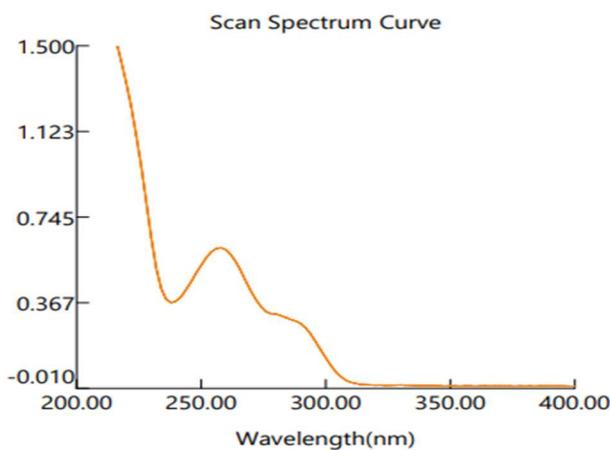**Figure 3 A: Calibration graph at five selected wavelengths**

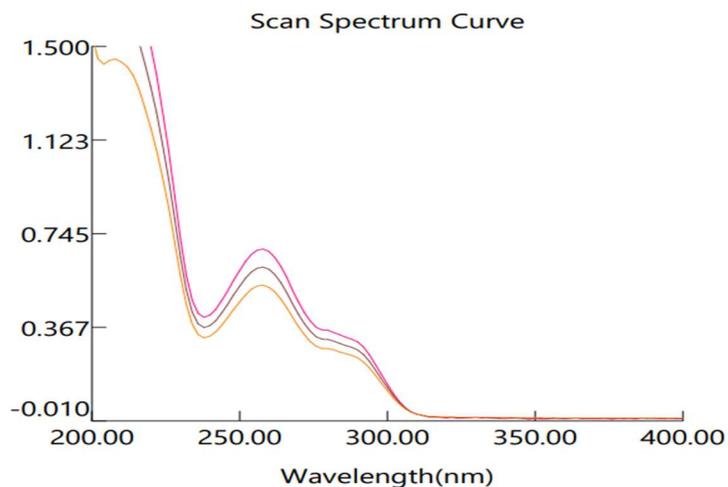**Figure 4: Overlay UV Spectra of itopride showing inter and intraday precision studies.**

Figure 5: Overlay UV Spectra of itopride showing accuracy

Table 1: Multivariate UV calibration at five selected wavelengths

Concentration (µg/mL)	Absorbance (nm)				
	254	256	258	260	262
14	0.485	0.498	0.501	0.492	0.475
16	0.520	0.534	0.537	0.529	0.510
18	0.556	0.570	0.574	0.565	0.544
20	0.591	0.606	0.610	0.601	0.579
22	0.626	0.642	0.647	0.637	0.614
24	0.661	0.678	0.683	0.674	0.649
26	0.697	0.715	0.720	0.710	0.683

Table 2: Linearity data showing system suitability parameters at the selected wavelengths

Wavelength (nm)	Regression equation	r^2	Average of Slope	SD of Intercept	LOD (µg/mL)	LOQ (µg/mL)
254	y = 0.0179x + 0.2323	0.9985	0.01782	0.000846759	0.15	0.47
	y = 0.0177x + 0.2341	0.9984				
	y = 0.0178x + 0.2334	0.9984				
	y = 0.0178x + 0.2331	0.9983				
	y = 0.0179x + 0.232	0.9985				
256	y = 0.0179x + 0.2496	0.9982	0.01788	0.000432435	0.079	0.24
	y = 0.0178x + 0.2505	0.9981				
	y = 0.0179x + 0.2501	0.9981				
	y = 0.0179x + 0.2498	0.9981				
	y = 0.0179x + 0.2494	0.998				
258	y = 0.0184x + 0.2418	0.999	0.01834	0.001173456	0.21	0.63
	y = 0.0183x + 0.2427	0.999				
	y = 0.0182x + 0.2442	0.999				

	y = 0.0184x + 0.2416	0.999				
	y = 0.0184x + 0.2413	0.9987				
260	y = 0.0181x + 0.2387	0.9998	0.01812	0.000593296	0.10	0.32
	y = 0.0182x + 0.2375	0.9998				
	y = 0.0181x + 0.2379	0.9999				
	y = 0.0181x + 0.2386	0.9998				
	y = 0.0181x + 0.2389	0.9997				
262	y = 0.0174x + 0.2316	0.9997	0.01738	0.000694982	0.13	0.39
	y = 0.0174x + 0.2304	0.9997				
	y = 0.0174x + 0.2307	0.9998				
	y = 0.0174x + 0.2309	0.9998				
	y = 0.0173x + 0.2321	0.9998				

Table 3: Absorbance values for intraday precision studies

Concentration (µg/mL)	Number of repetitions	Absorbance				
		254nm	256 nm	258 nm	260 nm	262 nm
100	1	0.591	0.606	0.610	0.601	0.579
	2	0.591	0.606	0.610	0.601	0.579
	3	0.590	0.606	0.609	0.600	0.579
	4	0.590	0.605	0.609	0.600	0.579
	5	0.590	0.605	0.609	0.600	0.578
	6	0.590	0.605	0.609	0.600	0.578

Table 4: Absorbance values for interday precision studies

Concentration (µg/mL)	Number of repetitions	Absorbance				
		254 nm	256 nm	258 nm	260 nm	262 nm
50	1	0.485	0.498	0.501	0.492	0.475
	2	0.485	0.498	0.501	0.492	0.475
	3	0.484	0.497	0.500	0.491	0.474
100	1	0.591	0.606	0.610	0.601	0.579
	2	0.591	0.606	0.610	0.601	0.579
	3	0.590	0.606	0.609	0.600	0.579
150	1	0.697	0.715	0.720	0.710	0.683
	2	0.696	0.714	0.719	0.709	0.682
	3	0.697	0.715	0.720	0.710	0.683

Table 5: Interday precision study

Concentration (µg/mL)	Description	254 nm	256 nm	258 nm	260 nm	262 nm
50	Mean	0.484666667	0.497666667	0.500666667	0.491666667	0.474666667
	SD	0.00057735	0.00057735	0.00057735	0.00057735	0.00057735
	%RSD	0.11	0.11	0.11	0.11	0.12
100	Mean	0.590333333	0.6055	0.6093	0.60033	0.578666667
	SD	0.000516398	0.000547723	0.000516398	0.000516398	0.000516398
	%RSD	0.08	0.09	0.08	0.08	0.089

150	Mean	0.696666667	0.714666667	0.719666667	0.709666667	0.696666667
	SD	0.00057735	0.00057735	0.00057735	0.00057735	0.00057735
	%RSD	0.08	0.08	0.08	0.08	0.08

Table 6: Intraday precision study

Concentration (µg/mL)	Description	254 nm	256 nm	258 nm	260 nm	262 nm
100	Mean	0.590333333	0.6055	0.6093	0.60033	0.578666667
	SD	0.000516398	0.000547723	0.000516398	0.000516398	0.000516398
	% RSD	0.08	0.09	0.08	0.08	0.089

Table 7: Assay of Itopride in marketed pharmaceutical formulation

Label claim (mg)	Amount estimated (mg)	%Assay
10.0	9.98	99.80
	10.01	100.10
	9.97	99.70
	10.03	100.3
	9.96	99.6
Average		99.90
SD		0.2608
% RSD		0.26

Table 8: Recovery studies

Wavelength (nm)	Conc. levels (%)	Sample Conc. Present (µg/mL)	Standard Conc. (µg/mL)	Final Conc. (µg/mL)	Amount recovered (µg/mL)	% Recovery
254	50	12	2	14	1.84	99.46
	100	12	8	20	8.15	100.25
	150	12	14	26	13.65	99.61
256	50	12	2	14	1.97	99.90
	100	12	8	20	7.98	99.96
	150	12	14	26	13.76	99.73
258	50	12	2	14	2.89	102.9
	100	12	8	20	7.89	99.81
	150	12	14	26	14.91	101.01
260	50	12	2	14	1.97	99.9
	100	12	8	20	7.61	99.35
	150	12	14	26	14.97	101.07
262	50	12	2	14	1.82	99.40
	100	12	8	20	7.99	99.98
	150	12	14	26	13.96	99.95

ETHICAL STATEMENT

There are no subjects who are humans or creatures in this study's trials.

A DISAGREEMENT OF INTEREST

There are no fiscal conflicts of interest that might affect this content.

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