Development and validation of simple UV spectrophotometric method for estimation of Itopride Hydrochloride in bulk and tablet dosage form

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ABSTRACT:
A simple, precise and economical spectroscopic method developed for estimation of itopride hydrochloride from bulk drug and tablet dosage form. The UV spectrum of itopride hydrochloride in 0.1NHCl showed λmax at 258 nm. This method was validated for linearity, accuracy, precision, robustness, limit of detection, limit of quantitation. This method showed good linearity in the concentration range of 5-30µg/ml, (obey's Beer-Lambert's law) with regression coefficient (r^2) 0.9995. The recovery was found in the range 110.61 % to 100.88%. Limit of detection (DL) and Limit of quantitation (QL) were found to be 0.69µg/ml and 2.1µg/ml respectively. From results it can be concluded that developed method can be used for analysis of itopride hydrochloride.

KEY WORDS: Itopride hydrochloride, Method development, UV spectroscopy, Validation.

INTRODUCTION:

Itopride hydrochloride is N-[(4-2-dimethylamino ethoxy) phenyl] methyl) 3, 4-dimethoxybenzamide hydrochloride (Figure1). Itopride hydrochloride is a novel gastroprokinetic agent. It is used in treatment of dyspepsia, upper abdominal pain, chronic gastritis and anorexia. Itopride hydrochloride acts as acetylcholine esterase inhibitor and dopamine D_2 receptor inhibitor. Thereby increases acetylcholine concentration and promote gastric motility[1-6].

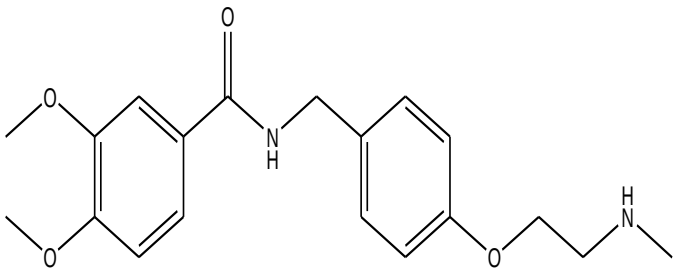

Figure 1. Chemical structure of Itopride hydrochloride

Various analytical methods reported for estimation of itopride hydrochloride as RP-HPLC[7], LC-MS[8], spectrophotometry and spectrofluroimetry[9]. In the present study attempt has been made to develop spectroscopic method for estimation of itopride hydrochloride in bulk and tablet dosage form which is simple, sensitive, precise and reproducible. UV absorption

spectroscopy covers the wavelength in the region 400-200 nm. Itopride hydrochloride was analysed at λmax 258 nm.

MATERIALS AND METHODS:

Chemicals:

Itopride hydrochloride bulk powder was taken as a gift sample from D.K. Pharma Chem. Pvt. Ltd., Thane (MS) India. Itopride hydrochloride 50mg tablet (Ganaton 50mg tablet, Abbott India Pvt. Ltd.) was purchased from local market Malegaon (MS) India. Whatmann filter paper no. 41,0.1N hydrochloric acid.

Instruments:

Analytical balance (Shimadzu: Aux220), Sonicator (Citizon), UV Spectrophotometer (Lab-India 3000+).

METHODS:

Preparation of Standard Stock Solution:

Accurately weighed 10 mg of Itopride hydrochloride in a 100 ml of volumetric flask and dissolved in small quantity of 0.1 N HCl. Make up volume up to 100 ml with 0.1 N HCl. This gives 100 µg /ml (SS).

Analytical method development:

Preliminary identification test for Itopride hydrochloride was done by scanning the Itopride hydrochloride in UV spectrophotometer in 400-200 nm range. From stock solution 2 ml was withdrawn and diluted to 10 ml with 0.1 N Hydrochloric acid to get concentration of 20µg/ml. Then run the sample through UV Visible spectrophotometer.

Analytical method validation[10]:

Linearity:

From the standard stock solution (SS) 0.5, 1 , 1.5, 2, 2.5, 3 ml were withdrawn and volume was made up to 10 ml with 0.1 N Hydrochloric acid to give the concentrations of 5, 10, 15, 20, 25, 30 µg/ml. The absorbance of these solutions was measured against a blank of 0.1N Hydrochloric acid at 258 nm. To obtain calibration curve plot of absorbance verses concentration was taken. The features of calibration curve such as correlation coefficient, slope, and intercept were determined.

Accuracy:

Accuracy of the proposed method was determined by performing recovery study at 80, 100 and 120 % level for Itopride hydrochloride. The recovery study was done by adding pure drug solution to pre-analysed tablet sample solution and concentrations were determined by using calibration graph. Absorbance was measured at 258 nm. Accuracy expressed in terms of percent recoveries. SD and %RSD was calculated.

Precision:

The precision of an analytical method was expressed as the SD and % RSD of the series of measurements. It was ascertained by replicate estimation of standard drugs. It involves intraday and interday precision. For intraday precision nine replicates of the solutions containing Itopride hydrochloride were carried out three times on the same day for three different concentration of Itopride hydrochloride (15, 20, 25 µg/ml).For inter-day precision nine replicates of the solutions were carried out for the three consecutive days at the same concentration level (15, 20, 25 µg/ml).Results were expressed in terms of %RSD.

Robustness:

In this present work robustness study was carried out by changing the (λmax) absorption maxima (± 2nm) that is 256 nm and 260 nm. The % RSD was calculated.

Limit of Detection (DL):

DL is the lowest amount of analyte in a sample which can not necessarily quantitated as an exact value but can be detected. Detection limit is calculated based on standard deviation of response and the slope.

Detection limit is expressed as:

$$DL = \frac{3.3 \times \text{Standard deviation of response}}{\text{Slope of calibration curve}}$$

Limit of Quantitation (QL):

QL can be quantitatively determined with suitable accuracy and precision. Which determines smallest amount of analyte in a sample. The quantitation limit is parameter of quantitative assay for low levels of compound in sample. Quantitation limit is calculated based on standard deviation of response and the slope.

Quantitation limit is expressed as:

$$QL = \frac{10 \times \text{Standard deviation of response}}{\text{Slope of the calibration curve}}$$

Quantitative Analysis of Pharmaceutical tablet Dosage form:

The proposed method was used for the determination of Itopride hydrochloride by using UV spectroscopy in tablet formulation. Marketed twenty tablets of Itopride hydrochloride (50mg) was weighed and powdered. Accurately weighed powder samples equivalent to 10mg of Itopride hydrochloride was dissolved in 0.1N hydrochloric acid and volume was made up to 50 ml in volumetric flask and sonicated for 15 min , then filtered through 0.45µ membrane filter. These solutions were used for determination of concentrations of Itopride hydrochloride in tablet sample solution from calibration graph by UV spectroscopy. Procedure was repeated six times for analysis of sample.

RESULTS AND DISCUSSION:

Itopride hydrochloride is a derivative of benzamide in the class of gastroprokinetic drug. The solubility of itopride hydrochloride was a determined in different polar and non-polar solvents. Drug was found to be freely soluble in distilled water, 0.1N hydrochloric acid, methanol, benzene and ethyl acetate. Sample of itopride hydrochloride was run through UV spectrophotometer in the range of 400-200 nm. Maximum absorbance of Itopride hydrochloride in 0.1 N Hydrochloric acid as a solvent was observed at 258 nm (Figure 2). Therefore it was concluded that the drug has λmax at 258nm.It obeys Beer's Lambert's law in the concentration range 5-30µg/ml.

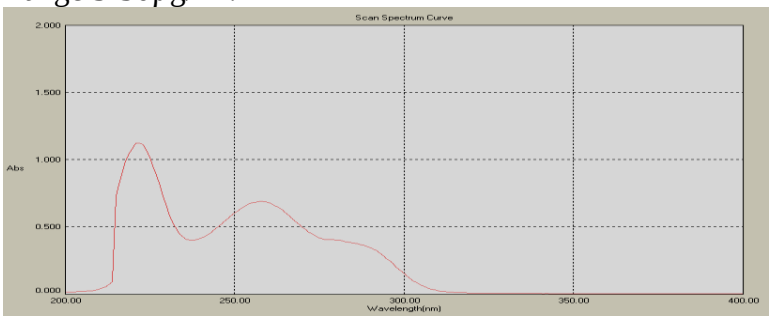

Figure 2 . UV Spectra of Itopride Hydrochloride at 20µg/ml concentration

Method Validation:

Linearity:

The linearity of this method was determined at concentration ranging from 5-30µg/ml. The regression equation was found to be y = 0.0281 x + 0.0297 and correlation coefficient (r^2) 0.9995 (Figure 3, Table 1). This indicate good correlation between concentration and absorbance of drug over the given concentration range.

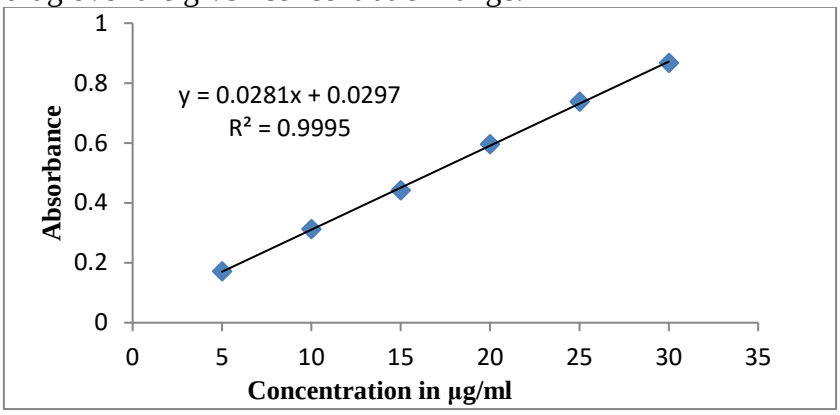

Figure 3. Calibration curve of Itopride Hydrochloride

Table 1. Linearity results

Sr. No	Concentration (µg/ml)	Absorbance			Mean	SD	RSD
		1	2	3			
1	5	0.172	0.167	0.175	0.171	0.0033	1.92
2	10	0.317	0.309	0.311	0.312	0.0041	1.31
3	15	0.447	0.435	0.444	0.442	0.006	1.35
4	20	0.604	0.584	0.597	0.595	0.01	1.68
5	25	0.725	0.751	0.738	0.738	0.013	1.76
6	30	0.857	0.858	0.886	0.867	0.016	1.84

Accuracy:

Mean % recoveries for 80,100 and 120 % were found to be 100.61, 100.88 and 100.87% respectively. %RSD was less than 2% indicates that there was non-interference of excipients used in formulation (Table 2).

Table 2. Results for accuracy

Sr. No.	Recovery added in %	Total Concentration	Abs	Concentration in observed ppm	%amount found	Mean	SD	%RSD
1	80	18	0.542	18.25	101.38	100.61	0.77	0.765
			0.538	18.11	100.61			
			0.534	17.97	99.84			
2	100	20	0.601	20.32	101.6	100.88	0.954	0.945
			0.598	20.25	101.25			
			0.59	19.96	99.8			
3	120	22	0.645	21.92	99.64	100.87	1.29	1.27
			0.652	22.17	100.77			
			0.661	22.49	102.22			

Precision :

The % RSD value for Itopride hydrochloride was found to be less than 2% for repeatability, intraday and inter day showing good precision. So developed method was precise (Table 3, 4).

Table 3 . Intraday Precision results

Sr. No.	Conc. µg /ml (2hrs interval)	Mean % amount found	SD	%RSD
12 pm				
1	15	97.9	0.75	0.766
2	20	100.7	0.561	0.557
3	25	100.96	0.222	0.219
2 pm				
1	15	100.48	1.501	1.49
2	20	99.92	0.53	0.53
3	25	100.05	0.6	0.599
4 pm				
1	15	97.39	1.55	1.59
2	20	99.8	0.626	0.627
3	25	100.29	0.61	0.608

Table 4. Interday precision results

Sr. No.	Conc. µg /ml (2hrs interval)	Mean % amount found	SD	%RSD
	Day 1			
1	15	97.9	0.75	0.766
2	20	100.7	0.561	0.557
3	25	100.96	0.222	0.219
	Day 2			
1	15	98.04	1.31	1.336
2	20	99.72	0.413	0.414
3	25	101.2	0.65	0.642
	Day 3			
1	15	99.72	1.08	1.08
2	20	100.87	1.26	1.24
3	25	101.13	0.351	0.347

Robustness:

The % RSD was less than 2% indicate that there was less influence on absorbance due to small change in wavelength. This shows proposed method was robust (Table 5).

Table 5 . Robustness results

Sr. No.	Conc. µg /ml (2hrs interval)	Mean %amount found	SD	%RSD
λ= 256nm				
1	15	96.55	1.07	1.1
2	20	100.26	0.74	0.738
3	25	101.1	0.8	0.791
λ= 260nm				
1	15	97.97	1.57	1.6
2	20	100.01	0.7	0.699

3	25	100.99	0.932	0.922

DL(Limit of Detection) and QL (Limit of Quantitation):

The sensitivity of the analytical method was determined from, limit of detection (DL) and limit of quantitation (QL) values, which were found to be 0.69 µg/ml and 2.1 µg /ml respectively.

%Assay (Analysis of Itopride hydrochloride marketed tablet formulation):

%Assay for itopride hydrochloride in marketed tablet dosage form was found to be 98.4%(Table 6).

Table 6. Analysis of tablet formulation of Itopride hydrochloride

Sr. No.	Concentration (µg/ml)	Absorbance	Amount obtained	%Amount	% Assay
1	15	0.450	14.95	99.71	98.4 ±1.03
2	15	0.446	14.81	98.73	
3	15	0.438	14.53	96.86	
4	15	0.442	14.67	97.81	
5	15	0.448	14.88	99.24	
6	15	0.443	14.70	98.05	

Validation parameters of UV spectrophotometric method for estimation of itopride hydrochloride were summarized in (Table 7).

Table 7. Summary of validation parameter results

Sr. No.	Parameters	Results
1	Absorption maxima (nm)	258nm
2	Beers Range (µg/ml)	5-30 µg/ml
3	Standard Regression Equation	Y= 0.0281 X + 0.0297
4	Correlation Coefficient(r^2)	0.9995
5	Slope	0.0281
6	Intercept	0.0297
7	% Assay	98.4 ± 1.03
8	Accuracy	
	80%	100.61 ± 0.77
	100%	100.88 ± 0.954
	120%	100.87 ± 1.29
9	Accuracy (%RSD)	Less than 2%
10	Precision (%RSD)	
	Intraday Precision	0.776 (<2%)
	Interday Precision	0.733 (<2%)
11	Robustness	0.975 (<2%)
12	Limit of Detection (DL)	0.69µg/ml
13	Limit of Quantitation(QL)	2.1µg/ml

CONCLUSION :

Itopride hydrochloride indicates good regression values at λmax 258nm. In recovery study results it was found that any small change in drug concentration solution could be accurately determined by the proposed method. Low values of DL and QL showed good sensitivity of developed method. From results it can be concluded that developed method is simple, accurate,

economic, and precise and can be effectively applied for routine analysis of itopride hydrochloride in bulk and tablet formulations.

CONFLICT OF INTEREST: The authors have no conflict of interest to declare.

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