UV Spectrophotometric Method for the Estimation of Itopride Hydrochloride in Pharmaceutical Formulation

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Abstract: Three simple, precise and economical UV methods have been developed for the estimation of itopride hydrochloride in pharmaceutical formulations. Itopride hydrochloride in distilled water shows the maximum absorbance at 258.0 nm (Method A) and in first order derivative spectra of the same shows sharp peak at 247.0 nm, when $n = 1$ (Method B). Method C utilises area under curve (AUC) in the wavelength range from 262.0-254.0 nm for analysis of itopride hydrochloride. The drug was found to obey Beer-Lambert's law in the concentration range of 5-50 $\mu\text{g/mL}$ for all three proposed methods. Results of the analysis were validated statistically and recovery studies were found to be satisfactory.

Keywords: Itopride hydrochloride, UV spectroscopy, Derivative spectroscopy, Area under curve.

Introduction

Itopride hydrochloride¹ (ITH) is a gastroprokinetic agent. It increases the release of acetylcholine (Ach) through dopamine D₂ receptor antagonistic action, resulting in enhancement of gastrointestinal motility. Chemically it is *N*-[4-[2-(dimethylamino)ethoxy]-benzyl]-3,4dimethylbenzamide hydrochloride (Figure 1). Literature survey reveals HPLC determination of itopride hydrochloride in tablet formulation^{2,3}, RP-HPLC in human serum^{4,5,6}. Extractive spectrophotometric⁷ determination of itopride hydrochloride in bulk and formulation which involves the formation of coloured chloroform extractable complex of drug with bromocresol green in acidic medium. The complex in chloroform has showed absorption maxima at 419.8 nm, spectrophotometric estimation based on coloured complex with methyl orange⁸, HPTLC⁹. The above method requires costlier solvents or tedious extraction procedure before its application. Hence an attempt has been made to develop new uv methods for its estimation in pharmaceutical formulation with good accuracy, simplicity, precision and economy so as to utilises it in routine analysis of the drug.

Figure 1. Structure of itopride hydrochloride HCl

Experimental

A Shimadzu UV 1100 series Spectrophotometer was used with 1 cm matched quartz cells.

Standard solutions

A standard stock solution of ITH in distilled water was prepared having a concentration 600 $\mu\text{g/mL}$. A 5.0 mL portion of stock solution was further diluted with water in a 100.0 mL volumetric flask up to mark to get final concentration 30 $\mu\text{g/mL}$.

The standard solution of ITH (30 $\mu\text{g/mL}$) was scanned in the range of 400-200 nm (Method A) in 1.0 cm cell against solvent blank and spectra was recorded, the absorbance maxima was observed at 258.0 nm (Figure 2). The drug followed the Beer's- Lambert's law in concentration range of 5-50 $\mu\text{g/mL}$ for all three methods and correlation coefficient was 0.9999, 0.9998 and 0.9999 for Method A, B and C respectively. Using the A (1%, 1 cm) of ITH the concentration of sample solutions can be determined.

Figure 2. Zero order spectrum of itopride hydrochloride in water

The first order derivative spectra at $n=1$ (method B) showed a sharp peak at 247.0 nm (Figure 3). The absorbance difference calculated at $n = 1$ ($dA/d\lambda$) is calculated by the inbuilt software of the instrument which was directly proportional to the concentration of the standard solutions. The calibration curve of $dA/d\lambda$ against concentration of the drug showed linearity, which are used for the estimation of the drug. The AUC (Area under Curve) method (method C) involves the calculation of integrated value of absorbance with respect to the wavelength λ_1 and λ_2 .

Figure 3. First order spectrum ($N=1$) of itopride hydrochloride in water

Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis was selected by entering the wavelength range over which the area has to be calculated. The wavelength range from 262.0-254.0 nm was selected which showed good linearity between area under curve and concentration. Different concentration of the standard drug (5-50 µg/mL) were prepared and scanned in the spectrum mode from the wavelength range 400-200 nm (Figure 4). Calibration curve was plotted as area under curve against concentration, and the estimation of the drug was done using the curve.

Figure 4. Area under curve of itopride hydrochloride in water

Sample solutions

An accurately weighed quantity of tablet powder equivalent to about 30 mg ITH was transferred to 50.0 mL volumetric flask, sonicated for 15 min with distilled water and diluted up to the mark with distilled water to get a stock solution of 600 µg/mL. The solution was then filtered through Whatmann filter paper (no. 41). A 5.0 mL of the filtrate was further diluted in a 100.0 mL volumetric flask with distilled water to get concentration of 30 µg/mL of ITH (on labelled claim basis). The absorbance of the resulted solution was read and the amount of ITH was calculated by taking A (1%, 1 cm) at 258.0 nm (method A), by first order derivative spectroscopy at 247.0 nm (method B) and by Area under Curve (AUC) (Method C). Calculation was done by using following formulae for method A, for method B and C comparison with standard for its derivative absorbance and area were done respectively.

By A (1%, 1 cm)

$$\% \text{ of labelled claim} = \frac{\text{Absorbance} \times \text{Dilution factor} \times \text{Avg wt}}{A (1\%, 1 \text{ cm}) \times \text{Wt. taken} \times \text{Label claim}} \times 100$$

Table 1. Optical characteristics and other parameters

Parameters	Method A	Method B	Method C
λ_{Max} , nm / wavelength range Beer-	258.0	247.0	262– 254.0
Lambert's range, µg/mL	5-50	5-50	5-50
Correlation coefficient, r	0.9999	0.9998	0.9999
Regression equation $Y = mx + c$			
a. Slope, m	0.0338	0.001	0.25923
b. Intercept, c	0.00057	0	0.00638
LOD	0.5022	1.5068	0.2511

Where, X is concentration in µg/mL & Y is absorbance unit, A is zero order derivative spectrum method with $n = 0$, B is first order derivative spectrum method with $n = 1$, C is the AUC method.

Table 2. Estimation itopride hydrochloride in tablet formulation

Method	Tablet formulation	% Label claim*	S. D., $\pm$ *	S. E.*
A	T1	100.25	0.58	0.25
	T2	100.11	0.53	0.24
B	T1	100.32	0.60	0.26
	T2	100.02	0.53	0.23
C	T1	99.69	0.29	0.14
	T2	99.40	0.41	0.18

Where, A is zero order spectrum method, B is first order derivative spectrum method with $n = 1$, C is the AUC method. T1 and T2 are two different brands of tablet formulations. * The results are the mean of five readings ($n = 5$).

Results and Discussion

All the methods A, B and C for the estimation of itopride hydrochloride in tablet dosage form were found to be accurate, simple and reproducible. Beer-Lambert's law was obeyed in the concentration range of 5-50 $\mu\text{g/mL}$ in all three methods. The values of standard deviation were satisfactory and the recovery studies were close to 100%. All these methods were validated according to ICH guidelines for accuracy, precision, stability, linearity and range and ruggedness

Accuracy

Recovery studies were carried out at four different levels by adding the pure drug (5.04, 10.08, 15.12 and 20.16 mg) to previously analyzed tablet powder sample. From the amount of drug found, percentage recovery was calculated and the results of analysis of recovery studies are shown in Table 3a.

Table 3a. Recovery study data (Accuracy)

Method	Tablet formulation	Amount of pure drug added, mg	Amount of pure drug recovered, mg	% Recovery*	S.D., $\pm$	S.E
A	T1	5.04	5.05	100.20	0.63	0.32
		10.08	10.10	100.20		
		15.12	15.24	100.79		
		20.16	20.01	99.26		
B	T1	5.04	5.01	99.40	0.50	0.25
		10.08	10.04	99.60		
		15.12	15.13	100.07		
		20.16	20.27	100.54		
C	T1	5.04	5.04	100.00	0.24	0.12
		10.08	9.96	99.81		
		15.12	15.18	100.39		
		20.16	20.19	100.15		

Where, A is zero order spectrum method, B is first order derivative spectrum method with $n = 1$, C is the AUC method. T1 is the brand of the tablet formulation. *The results are the mean of three readings at each level of recovery.

Precision

Inter-day precision

It was done by analysing the solutions by same analyst on alternate days till 5th day. The % RSD is shown in Table 3b.

Intra day precision

It was done by analysing the solutions by same analyst within a day. The % RSD is shown in Table 3b.

Table 3b. Summary of validation parameters

Parameters	Results		
	Method A	Method B	Method C
Intra day precision			
Amount found*	100.41±0.25	100.52±0.05	99.44±0.24
RSD, %	0.25	0.05	0.24
Inter day precision			
Amount found*	100.02± 0.48	100.52 ± 0.05	99.05 ± 0.48
RSD, %	0.48	0.05	0.48
Ruggedness, RSD%	0.35	0.82	0.24

Where, A is zero order spectrum method, B is first order derivative spectrum method with $n = 1$, C is the AUC method. * (Mean % $\pm$ S. D)

Linearity and range

Accurately weighed quantities of tablet powder equivalent to 80, 90, 100, 110 and 120% of label claim of ITH were taken in a series of 50 mL volumetric flasks and dilutions were made as under sample solution. The graphs of % label claim vs. absorbance were plotted for method A (Zero order spectrum method) & method B (first order derivative spectrum method) and % label claim vs. area under curve was plotted for method C (AUC method). All the three methods were found to be linear.

Ruggedness

It was done by analysing the samples solutions by three different analysts. The % RSD by proposed methods is shown in Table 3b.

Stability

The samples were kept under following conditions viz.

- 1) Refluxed for 3 h after addition of 30.0 mL of 0.5 N NaOH (Alkali)
- 2) Refluxed for 3 h after addition of 30.0 mL of 0.5 N HCl (Acid)
- 3) At 50 °C after addition of 30.0 mL of 6% H₂O₂ for 24 h (Oxide 6%)
- 4) At 60 °C for 24 h (Thermal Degradation)
- 5) At 75% humidity for 24 h (Humidity)
- 6) At UV exposure for 24 h (UV)
- 7) Exposed to direct Sun Light for 6 h (Photochemical)

After the specified period, the results were analysed by proposed methods and are shown in Table 4. The present three methods have also been subjected to stress conditions to assess the stability of the drug under various conditions. Though conclusive evidence about its un-stability can be predicated from the results but it can be seen that drug was found to be susceptible to alkaline, oxide and humidity conditions as indicated by different percent label claim as compared to normal condition by proposed methods.

Table 4. Results of specificity studies

Sample (Sample Treated)	% Label claim		
	Method A	Method B	Method C
Alkali	105.22	103.27	105.55
Acid	101.25	100.23	100.54
Oxide 6%	92.98	90.29	92.85
Thermal Degradation	100.86	99.65	99.99
Humidity	96.56	95.93	96.36
UV	100.41	99.85	99.73
Photochemical	100.23	100.01	99.56

Conclusion

From the statistical comparison of results, were found to satisfactory and validated. Hence these methods can be useful in the routine analysis of ITH in bulk drug and formulations.

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