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ORIGINAL PAPER

Stability-Indicating Spectrofluorimetric Method for Determination of Itopride Hydrochloride in Raw Material and Pharmaceutical Formulations

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Abstract A simple, sensitive and rapid spectrofluorimetric method for determination of itopride hydrochloride in raw material and tablets has been developed. The proposed method is based on the measurement of the native fluorescence of the drug in water at 363 nm after excitation at 255 nm. The relative fluorescence intensity-concentration plot was rectilinear over the range of 0.1–2 μg/mL (2.5×10^{-7}–5.06×10^{-6} mole/L), with good correlation (r=0.9999), limit of detection of 0.015 μg/mL and a lower limit of quantification of 0.045 μg/mL. The described method was successfully applied for the determination of itopride hydrochloride in its commercial tablets with average percentage recovery of 100.11±0.32 without interference from common excipients. Additionally, the proposed method can be applied for determination of itopride in combined tablets with rabeprazole or pantoprazole without prior separation. The method was extended to stability study of itopride. The drug was exposed to acidic, alkaline, oxidative and photolytic degradation according to ICH guidelines. Moreover, the method was utilized to investigate the kinetics of the alkaline, acidic and oxidative degradation of the drug. A proposal for the degradation pathways was postulated.

Keywords Stability-indicating · Itopride · Spectrofluorimetry · Native fluorescence · Excitation · Emission · Rabeprazole · Pantoprazole

Introduction

Itopride hydrochloride(ITP, Fig. 1), *N*-{*p*-[2-(Dimethylamino) ethoxy]benzyl}veratramide hydrochloride, is a substituted benzamide that has been used for its prokinetic and antiemetic actions [1]. Several analytical methods were reported for the determination of itopride in pharmaceutical preparations and human plasma, including, spectrophotomety [2–9], electrochemical method [10], TLC [11, 12], liquid chromatography with ultraviolet detection [13–17], chemiluminescence detection [18], fluorescence detection [19–21] and tandem-mass spectrometry [22, 23].

Spectrofluorimetry has been widely used in the determination of pharmaceutical compounds because it is a highly sensitive, selective, easily operated and economical technique. To the best of our knowledge, up till now nothing has been published concerning the spectrofluorimetric determination of ITP. Moreover, the proposed method has the advantage of being sensitive and economic for the determination of the studied drug either alone or in the presence of its co-formulated drugs, rabeprazole and pantoprazole without need of the tedious procedures of HPLC methods. Also, the present study was extended to establish the inherent stability of ITP under different stress conditions such as, alkaline, acidic, oxidative and photolytic conditions according to ICH guidelines [24].

Experimental

Apparatus

*The fluorescence spectra and measurements were recorded using a Perkin-Elmer UK model LS 45luminescence spectrometer, equipped with a 150 Watt Xenon arc lamp, the excitation and emission wavelengths were 255 and 363 nm respectively. Slit width for both monochromators were set at 10 nm, and the photomultiplier voltage was set to auto. Quartz cell 1 cm was used.

A Consort NV P901 pH Meter calibrated with standard buffers was used for pH measurements. CAMAG UV-lamp

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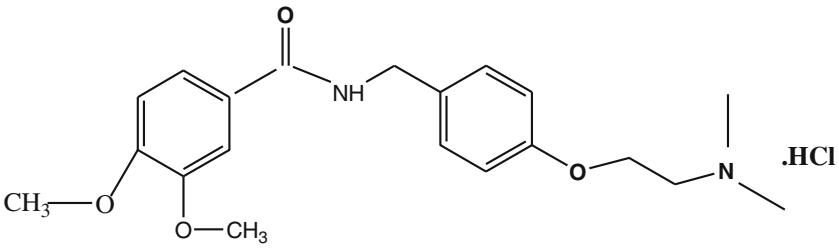
Fig. 1 Structural formula of itopride hydrochloride



(S/N 29000), dual wavelength (254/366), 2×8W (Muttenz, Switzerland) was used in the photo-stability study.

Materials and Reagents

*All chemicals used were of analytical grade, and distilled water was used throughout the study:

- Itopride, pure sample obtained from Chemipharm Company, Cairo, Egypt, and was used as received. Its purity was found to be 100.32 % according to the comparison method [16].
- Rabeprazole sodium, pure sample obtained from Sigma Pharmaceutical Company, Cairo, Egypt, and was used as received. Its purity was found to be 99.88 % according to the comparison method [16].
- Pantoprazole sodium sesquihydrate, pure sample obtained from Sigma Pharmaceutical Company, Cairo, Egypt, and was used as received. Its purity was found to be 99.55 % according to the comparison method [16].
- Ganaton® tablets (batch # 96717/3J), labeled to contain 50 mg of itopride/tablet, produced by Kahira Pharm. and Chem. Ind. Co., Cairo, Egypt, and was purchased from local pharmacy.
- Prepared tablets composed of itopride 150 mg, rabeprazole 20 mg or pantoprazole 40 mg, talc powder 20 mg, maize starch 15 mg, lactose 15 mg and 10 mg magnesium stearate per tablet or capsule.
- Methanol, acetonitrile, propanol and acetone were obtained from Sigma-Aldrich (Germany).
- Acetate buffer solutions (0.2 M), covering the pH range of 3.6–5.6 and borate buffer solutions (0.2 M), covering the pH range of 6.5–9.5 (BDH, UK).
- Sodium dodecyl sulfate (SDS, 95 %), cetrimide (CTAB, 99 %)(Winlab, UK), carboxymethyl cellulose (CMC) produced by El Nasr Chem.Co., Cairo, Egypt, tween-80 (Adwic Co., Egypt), 0.5 % aqueous solution of each was prepared.
- Hydrochloric acid (10 M), sodium hydroxide, hydrogen peroxide (30 %), toluene, chloroform and ammonia solution, were obtained from El Nasr Chem.Co., Cairo, Egypt.
- Silica gel sheets 60 F_{254} (5 cm×10 cm with 0.2 mm thickness were obtained from Sigma-Aldrich (Germany).

Standard Stock and Working Solutions

Stock standard solutions of 100.0 μg/mL of ITP, rabeprazole and pantoprazole were prepared in distilled water. Working standard solutions of 10.0 μg/mL of each drug was prepared by further dilution with distilled water as appropriate.

Procedures for Calibration Graph

Transfer accurately measured aliquots of the working solution so that the final concentration is in the range of 0.1–2 μg/mL into a series of 10.0 mL volumetric flasks and completed to the volume with distilled water. The fluorescence intensity was measured at 363 nm after excitation at 255 nm. The relative fluorescence intensity was plotted against the final concentration of the drug. Alternatively the corresponding regression equation was derived.

Procedures for Tablets

- An accurately weighed quantity of ten pulverized Ganaton® tablets equivalent to 10.0 mg of ITP was transferred into 100.0 mL volumetric flask and sonicated with 80.0 mL of water for 30 min. The volume was completed to the mark with water and filtered. The solution was further diluted with the same solvent to obtain solution containing 10.0 μg/mL of itopride. The procedure described under "Construction of calibration graph" was followed. The nominal content of tablets was calculated using the calibration graph or the corresponding regression equation.
- For prepared tablets: An accurately weighed quantity of mixed contents of ten prepared tablets equivalent to 150 mg of ITP with 20 mg of rabeprazole or 40 mg of pantoprazole were transferred into 100.0 mL volumetric flask and sonicated with 80.0 mL of water for 30 min. The volume was completed to the mark with water and filtered. The solutions were further diluted with the same solvent to obtain the desired concentrations. The procedure described under "Construction of calibration graph" was followed. The nominal content of tablets was calculated using the calibration graph or the corresponding regression equation.

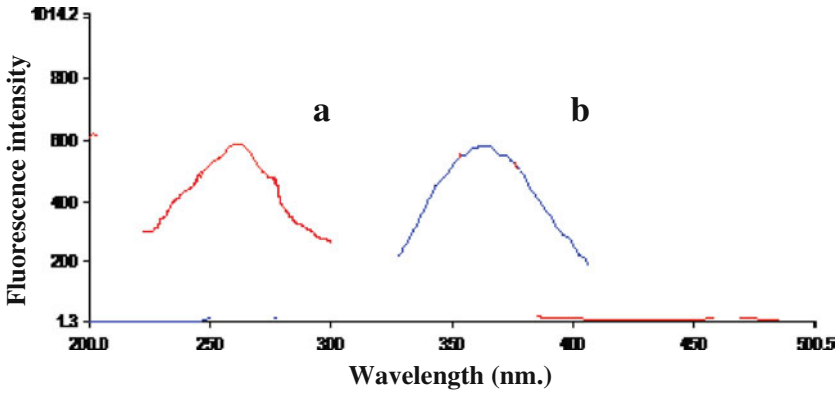


Fig. 2 (**a**) Excitation and (**b**) Emission spectra of (1.5 μg/mL) of ITP in water

Procedures for Stability Studies

**Acidic and Alkaline Degradation Study* 250 μg/mL of ITP was transferred into 25.0 mL volumetric flask, add 5.0 mL of 2 M HCl or 5.0 mL of 2 M NaOH, boil for 10, 20, 30, 40, 50, 60 min. Cool, neutralized to pH 7 with either 2 M NaOH or 2 M HCl respectively. The solutions were completed to the volume with water. Transfer 1.5 mL into 10.0 mL volumetric flask and complete to the volume with water (final concentration 1.5 μg/mL), the general recommended procedure was followed.

**Oxidative Degradation Study* 250 μg/mL of ITP was transferred into 25.0 mL volumetric flask, add 5.0 mL of 15 % hydrogen peroxide, heat at 80 °C for 10, 20, 30, 40, 50, 60 min. Cool, complete to the volume with water. Transfer 1.5 mL into 10.0 mL volumetric flask and complete to the volume with water (final concentration 1.5 μg/mL), the general recommended procedure was followed.

**Photolytic Degradation Study* 15 μg/mL of ITP was transferred into 10.0 mL volumetric flask and complete to the volume with either methanol or distilled water (final concentration 1.5 μg/mL). The flasks were exposed to UV-light at wavelength of 254 nm for 24 h under the UV-lamp, where the distance between the source and the sample solution was 15 cm. The fluorescence intensity was measured after 24 h.

Results and Discussion

Itopride was found to exhibit an intense native fluorescence in aqueous solution at 363 nm emission after excitation of 255 nm (Fig. 2). This property allows us to develop a new spectrofluorimetric method for determination of ITP in its dosage forms either alone or even in the presence of co-formulated drugs like, pantoprazole and rabeprazole without interference. Different experimental parameters were studied:

*Effect of pH

The influence of pH on the native fluorescence of ITP was studied by adding 2 mL of acetate buffer (pH 3.6–5.6), borate buffer (pH 6.5–9.5), 0.1 M NaOH and 0.1 M HCl. It was found that, addition of buffer decreases the fluorescence intensity of ITP. So, no buffer was used throughout the work.

*Effect of different organized media

The effect of different surfactants on the native fluorescence of ITP was studied by adding 1.0 mL of aqueous solution of each one to 0.5 μg/mL of the drug solution. Different surfactants like, SDS (anionic surfactant), cetrimide (cationic surfactant), tween 80 (non-ionic surfactant) and carboxymethylcellulose(CMC) were tried. It is obvious from the results that, the presence of surfactants cause no effect as in case of CMC or decrease the fluorescence of the studied drug especially by adding tween 80 as shown in (Fig. 3). So, no surfactant was added throughout the work.

*Effect of diluting solvent

Different diluting solvents were tried such as water, methanol, propanol, acetonitrile and acetone. It was found that propanol, acetonitrile and acetone decrease the fluorescence intensity of ITPthis may be attributed to change in the medium polarity that may result insome sort of

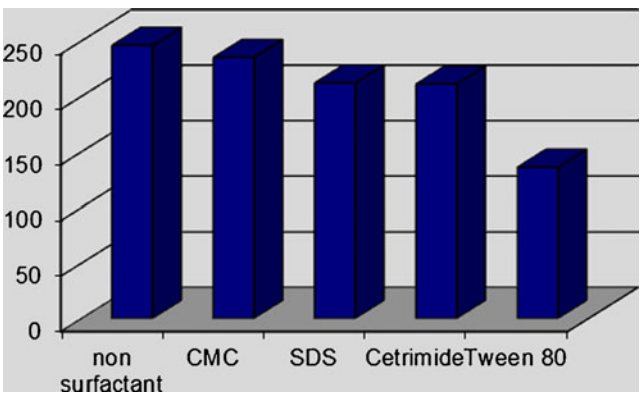


Fig. 3 Effect of various surfactants on the fluorescence intensity of ITP(0.5 μg/mL) in water

Table 1 Analytical performance data for the spectrofluorimetric determination of itopride

Parameter	Results
Wavelength (λex./λem.)(nm)	255/363
Linearity and range (μg/mL)	0.1–2
Limit of detection(LOD)(μg/mL)	0.015
Limit of quantification(LOQ)(μg/mL)	0.045
Intercept (*a*)	36.24
Slope (*b*)	396.90
Correlation coefficient (*r*)	0.9999
S.D. of residuals (S*y/x*)	2.99
S.D. of intercept (S*a*)	1.80
S.D. of slope (S*b*)	1.53
% RSD	1.25
% Error	0.41

*Where: *Sy/x* standard deviation of the residuals, S_b standard deviation of the slope, *Sa* standard deviation of the intercept, %Error=% RSD/√n

physical interaction between these solvents and the excited singlet state of the drug molecules. On the other hand, methanol increases the fluorescence intensity but shifts the peak (peak maxima at 345 nm) but without reproducibility in the results. So water was used as diluting solvent since it gave the highest fluorescence intensity and lowest blank reading with reproducible results.

*Effect of time

The fluorescence intensity of ITP was measured at different times. It was found that the fluorescence intensity was immediately developed and not affected by time for 24 h.

*Effect of temperature

The effect of temperature was studied in the range 40–100 °C using a thermostatically controlled water bath. It was found that, increasing the temperature causes decreasing of fluorescence intensity. It may be due to the collision between the excited singlet state and the solvent molecules causes loss of energy. So, the fluorescence intensity of ITP was measured at room temperature (25 °C).

Validation of the Method

The validity of the method was checked by testing linearity, LOD, LOQ, accuracy, repeatability, precision and specificity according to ICH recommendations [24].

Table 2 Application of the proposed method to the determination of ITP in pharmaceutical formulations

Preparation	Proposed method			Comparison method [16]	
	Conc.added of Itopride(μg/mL)	Conc. Found (μg/mL)	Recovery (%)	Conc.added (μg/mL)	Recovery(%)
Ganaton tablets (50 mg/tablet)	0.5	0.502	100.40	0.5	99.40
	1.0	0.997	99.77	1.0	101.50
	1.2	1.202	100.16	2.0	99.67
$\overline{X}$ ± SD	100.11±0.32			100.19±1.14	
t	0.12 (2.77)*				
F	12.91 (19)*				
Prepared tablets with pantoprazole	Proposed method			Comparison method [16]	
	0.75	0.763	101.70	0.5	99.53
	1.5	1.495	99.69	1.0	100.45
	1.8	1.812	100.68	2.0	100.58
$\overline{X}$ ± SD	100.69±1.01			100.18±0.57	
t	0.75 (2.77)*				
F	3.08 (19.0)*				
Prepared tablets with Rabeprazole	Proposed method			Comparison method [16]	
	0.75	0.762	101.55	0.5	100.60
	1.5	1.514	100.92	1.0	99.20
	1.8	1.822	101.19	2.0	101.80
$\overline{X}$ ± SD	101.22±0.32			100.53±1.30	
t	0.88 (2.77)*				
F	16.95 (19.0)*				

*Each result is the average of three separate assays

*Values between brackets are the tabulated t and *F* values at $p=0.05$ [26]

*Nominal content of itopride in Ganaton® tablets=50.055 mg/tablet

*Nominal content of itopride in prepared tablets with pantoprazole= 151.03 mg/tablet

*Nominal content of itopride in prepared tablets with rabeprazole= 151.83 mg/tablet

Linearity and Range

Assessment of linearity of the assay method was performed by analyzing nine sets for the drug ($n=9$ for standard calibration plot), the fluorescence vs. concentration plot was linear over the concentration range of 0.1–2 μg/mL (2.5×10^{-7}–5.06×10^{-6} mole/L). Linear regression analysis of the data gave the following equation:

$$RFI = 36.24 + 396.90\ C$$

Where RFI is the relative fluorescence intensity and C is the final concentration of ITP in μg/mL. Statistical analysis of the data gave small values of the standard deviation of residual (Sy/x), the standard deviation of the intercept (Sa), the standard deviation of the slope (Sb) and the percentage of relative error (% Er) are all shown in Table 1.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) was determined by evaluating the smallest concentration that can be detected and was found to be 0.015 μg/mL. The limit of quantification (LOQ) was determined by establishing the smallest concentration that can be measured, below which the calibration graph is non linear, it was found to be 0.045 μg/mL according to ICH Q2R1 recommendations [24]:

$$LOQ = 10\ Sa/b \quad LOD = 3.3\ Sa/b$$

Where

Sa standard deviation of the intercept of the calibration curve.
b slope of the calibration curve.

Accuracy and Precision

To prove the accuracy of the proposed method, the results of the assay of the proposed method of the drug in pure form and in its pharmaceutical formulations were compared with the results obtained by the comparison method [16].

Table 3 Results of the degradation study of ITP under different stress conditions

Degradation condition	Reaction rate constant (K, min^{-1})	Half life time ($t_{1/2}$)
*Acidic degradation (2 M HCl, 100 °C).	0.021	33 min
*Alkaline degradation (2 M NaOH, 100 °C).	0.0025	4.6 h
*Oxidative degradation (15 % H_2O_2, 80 °C).	0.024	28.90 min

Intraday Precision

It was performed through replicate analysis of three concentrations of the studied drug on three successive times. The small value of relative standard deviation indicating good precision within day.

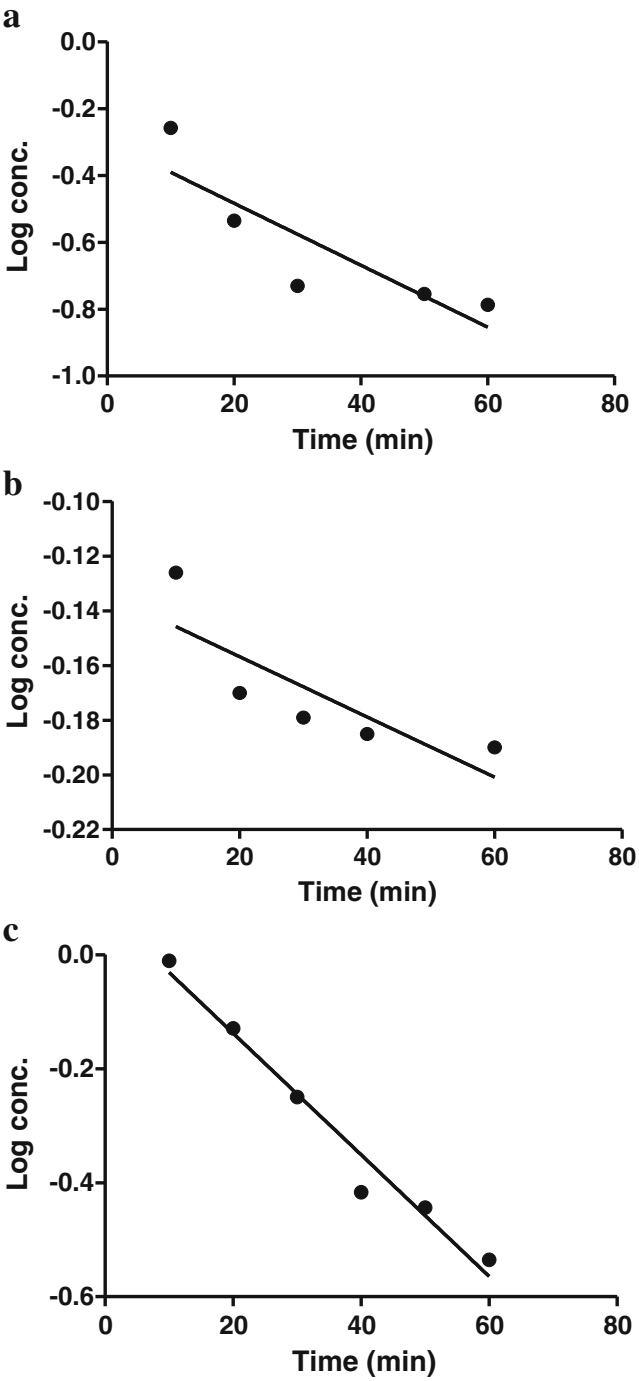


Fig. 4 Effect of time on 1.5 μg/mL of ITP by: **a** boiling with 2 M HCl. **b** boiling with 2 M NaOH. **c** heating at 80 °C with 15 % H_2O_2

Interday Precision

Interday precision was carried out through replicate analysis of three concentrations of the studied drug on three successive days. The small value of relative standard deviation indicating reasonable repeatability and intermediate precision of the proposed method.

Specificity

The specificity of the proposed method was proven by its ability to determine ITP in its pharmaceutical formulations without interference from the common excipients or the co-formulated drugs, pantoprazole and rabeprazole, the results were summarized in Table 2.

Results of Stability Studies

The stability indicating capability of the proposed method was demonstrated by accelerated degradation of ITP by acidic degradation using 2M HCl, alkaline degradation using 2 M NaOH, oxidative degradation using 15 % H_2O_2 for different times and photolytic degradation for 24 h. The apparent first order degradation rate constant and half life time was calculated as shown in Table 3. The acidic, alkaline and oxidative degradation causes gradual decrease of fluorescence intensity and was found to be time dependent, as illustrated in (Fig. 4). Also the effect of UV light on the stability of ITP was studied by exposing ITP in water and methanol as a diluting solvent to UV lamp at 254 nm for 24 h. It was found that, a minor degradation of the aqueous

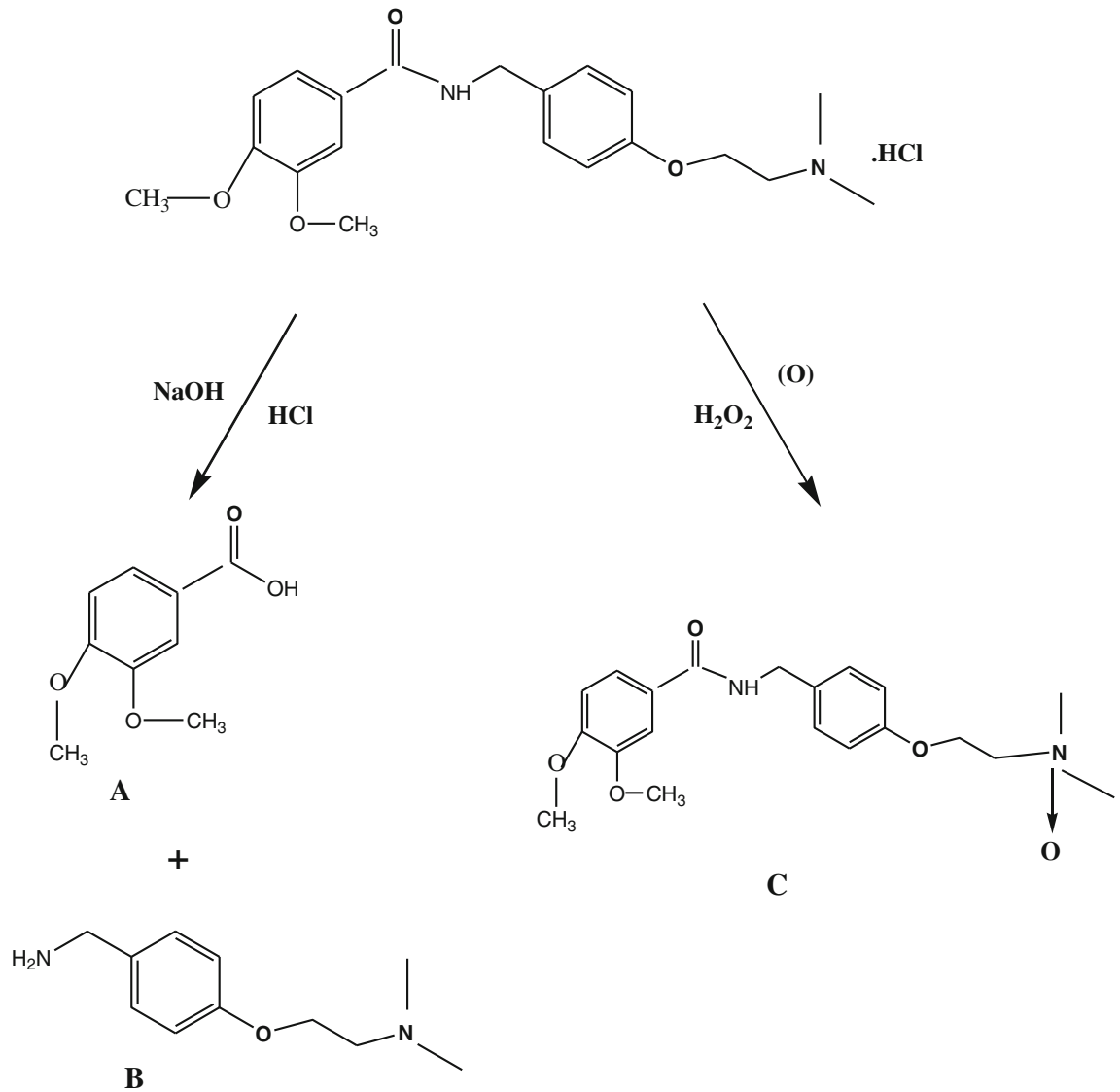


Fig. 5 The proposal pathway of ITP degradation

solution of the drug occurred with slight decrease of fluorescence intensity due to the photolytic oxidation of the nitrogen atom (product **C**). On the other hand, the methanolic solution of the drug shows no degradation when irradiated for the same period. This can be explained by the fact that, polar solvents tend to increase the degradation of drug molecules that produce degradates which are more polar than the original drug, and non-polar solvents enhance the degradation of polar compounds that produce less polar degradates [25]. The acidic and alkaline treatments of ITP are expected to cleavage the amide group (producing product **A** and **B**) resulting in decrease of the native fluorescence intensity of the drug due to the decrease of the conjugation of the structure of the drug. While the oxidative and the photolytic degradation cause oxidation of the nitrogen atom producing more polar product(nitrogen oxide, product **C**) resulting in decrease of the native fluorescence of ITP. The proposal pathway was postulated as shown in (Fig. 5). Thin layer chromatography after the exposure of ITP to acidic, alkaline, oxidative and photolytic degradation was applied using silica gel sheets 60 F_{254} (5 cm × 10 cm with 0.2 mm thickness) and mobile phase composed of toluene: methanol: chloroform: 10 % ammonia solution (5: 3: 6 : 0.1), visualized under UV lamp at 254 nm [17]. For acidic and alkaline degradation R_f was 0.31, for oxidative and photolytic degradation was 0.48 and for intact drug was 0.54. From the results of R_f value indicate that the products of acidic, alkaline, oxidative and photolytic degradation are more polar than the intact dug which confirm the proposal pathway.

Conclusion

A sensitive, rapid and economic spectrofluorimetric method was developed for determination of itopride in its raw material and pharmaceutical formulations. The spectrofluorimetric technique, by virtue of its high sensitivity, was not used before for the determination of itopride. The simplicity of the method allows the successful determination of the studied drug in its tablets either alone or in presence of co-formulated drugs without prior separation and does not require the elaborate treatment associated with chromatographic methods. Moreover, the method was extended to the stability study of the drug under different stress conditions.

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