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Novel validated stability-indicating methods for determination of itopride hydrochloride

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ABSTRACT

Two novels simple, accurate, sensitive and reproducible methods have been developed and subsequently validated for the determination of itopride Hydrochloride in presence of its acidic and oxidative-degradates, as stability-indicating studies. In the first method, zero-crossing technique was adopted for determination of the investigated drug in presence of its acidic and oxidative-degradates, by the use of derivative and derivative ratio spectrophotometry, respectively. The second method was based on isocratic high-performance liquid chromatography (HPLC) separation of itopride Hydrochloride from its acidic and oxidative-degradates on a reversed phase ODS columns, utilizing (methanol: phosphate buffer at pH = 4.0) (35:65, v/v) as a mobile phase. The flow rate was 1.0 $ml.min^{-1}$ and the samples were detected by using UV detector at 258 nm and fluorescence detector 291/342nm as excitation/emission wavelengths. All the proposed methods were validated according to the International Conference on Harmonization (ICH) guidelines and successfully applied for determination of the itopride Hydrochloride in pure form, in laboratory prepared mixtures and in pharmaceutical preparations. The obtained results were statistically compared to the manufacturer's method of analysis for itopride Hydrochloride and no significant differences were found.

KEYWORDS

Itopride hydrochloride derivative spectrophotometry;
Ratio spectra;
HPLC;
Stability indicating study.

INTRODUCTION

Itopride Hydrochloride, N-[[4-(2-dimethylamino-ethoxy) phenyl] methyl]-3,4-dimethoxy-benzamide hydrochloride, occurs as white to off-white crystalline powder, very soluble in water, methanol and sparingly soluble in acetic Acid[1], having a molecular formula $C_{20}H_{26}N_2O_4$,HCl with molecular weight = 394.9[2-4]. It increases the release of acetylcholine (Ach.) through dopamine D_2-receptor antagonistic action also inhibits decomposing released acetylcholine[5-8].

The ICH-guidelines[9] requires performing stress-testing of the drug substance that can help in identifying the likely degradation-products, also can be useful in

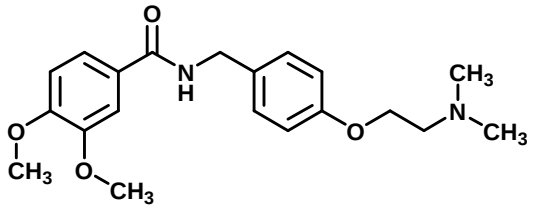


Figure 1 : Chemical structure of itopride

establishing the degradation-pathways and validating the stability-indicating power of the analytical procedures used. Moreover, validated stability-indicating method should be applied in the stability study[10]. Stability-indicating methods can be specific one that evaluates the drug in the presence of its-degradation products, excipients and additives[11]. In this perspective, few analytical methods employed for the determination of the investigated drug, including colorimetry[12], spectrophotometry[13-17], high performance liquid chromatography[13,18-24] and high performance thin layer chromatography[20,25,26].

The present work establishes new simple, accurate, rapid and reproducible technique stability indicating spectrophotometric and chromatographic methods for the determination of itopride hydrochloride in the presence of its acidic and oxidative-degradates, which can be used for the routine quality control analysis of these drugs in raw material and pharmaceutical formulations and for stability studies.

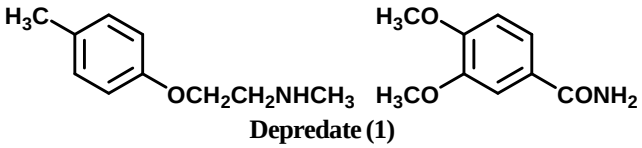
MATERIALS AND METHODS

Chemicals and reagents

Itopride hydrochloride authentic drug was kindly provided by ORBIT IMPEX, and certified to contain 98.50 %. Ganaton® tablets: batch number: 76583/3J/L, manufactured by El kahira-Pharmaceuticals, A.R.E., under license of Abbott's pharmaceutical company. Each tablet was labeled to contain 50 mg of itopride hydrochloride.

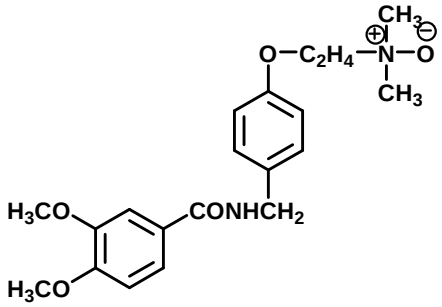
Acetonitrile, methanol and bi-distilled water (Riedel-dehaen, Sigma-Aldrich, Germany), Hydrochloric acid (Adwic), aqueous 5.0M, sodium hydroxide (Adwic) aqueous, 5.0M, chloroform (Adwic), hydrogen peroxide 30% (E. Merck, Germany), O-phosphoric acid (Adwic) triethylamine (Fluka) and TLC aluminium plates pre-coated with silica gel 60 F_{254} (E.Merck).

All chemical and reagents used through this work



Depredate (1)

Itopride hydrochloride acidic degradation



Depredate (2)

Itopride hydrochloride oxidative degradate

are of spectroscopic and chromatographic analytical grade. Bi-distilled water is used throughout the whole work and is indicated by the word 'water'.

Instruments

A double-beam Shimadzu (Japan) UV-VIS Spectrophotometer (UV-1601 PC), model TCC-240 A; connected to an IBM compatible computer and HP 695 C DeskJet printer is used. The bundled software is UVPC personal spectroscopy software version 3.7 (Shimadzu). The spectral bandwidth is 2 nm and the wavelength scanning speed was 2800.0 nmmin^{-1}. The absorption spectra of the reference and the test solutions are recorded in1.0-ml quartz cells at 25.0°C, using '$\Delta\lambda$ = 4 nm and scaling factor of 10 for computing first derivative (D^1)' and '$\Delta\lambda$ = 8 nm and scaling factor of 100 for third (D^3) derivatives'.

The HPLC (Agilent Hewlett Packard series) instrument was equipped with a model series 1100 pump, manual injector Agilent 1100 series, 20 µl loop and a UV-visible wavelength detector Agilent 1100 series. The HPLC (Agilent Hewlett Packard series) instrument was equipped with a model series 1200 pump, manual injector Agilent 1200 series, 20 µl loop and a fluorescence wavelength detector Agilent 1200 series. The chromatographic separation was performed using (150×4.6 mm I.D.) Agilent eclipse XDB C18 and C25 columns (5µm particle size) at ambient temperature. Ultrasonic vibrator, (J.P Selecta'S-a; CD 300513 Espain). Disposible membrane filters, 0.45µm, (Agilent

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3150-0576).

A (Jenway 3510, UK) pH-meter, equipped with combined glass electrode for pH adjustment.

Standard solutions

Standard solutions of the studied drugs

Stock standard solutions of itopride hydrochloride, having concentration of (20.0 $\mu g.ml^{-1}$) were prepared in water, which is used also as working standard solutions.

Standard solution of degradates

(1) Standard solution of acidic-degradate

Stock standard solution of itopride hydrochloride acidic-degradate was prepared by refluxing 0.005 gm of itopride hydrochloride with 10.0 ml of 5.0 N HCl for 3 hours at 100°C, cooling, neutralizing the media with 5.0 M NaOH and making volume to 250 ml with water, which is used also as working standard solutions.

(2) Standard solution of oxidative-degradate

Stock standard solution of itopride hydrochloride oxidative-degradate was prepared by refluxing 0.005 gm of itopride hydrochloride with 20.0 ml of 30% H_2O_2 for 2 hours at 100°C, cooling, and making volume to 250 ml with water, which is used also as working standard solutions, which is used also as working standard solutions.

Complete degradation was checked by using TLC system; silica gel 60 F254 plates and chloroform: methanol (50: 50, v/v) as a developing system.

Procedures

Spectrophotometric determination of itopride hydrochloride in presence of its acid and oxidative-degradates:

(1) First (D^1) and third (D^3) derivative spectrophotometric method

From standard working solution of itopride hydrochloride, aliquots were transferred into a series of 10 ml volumetric flasks, and diluted to volume with water to obtain a concentration range of 1.0-12.0 $\mu g.ml^{-1}$ and 3.0-12.0 $\mu g.ml^{-1}$, respectively. The values of the first (D^1) and the third (D^3) derivative spectrophotometry amplitudes were computed for the investigated drug in presence of its acid and oxidative-degradates, at 247.20 nm and 298.20 nm, respectively. Those values were then plotted versus corresponding concentrations; and the regression equation was then computed.

(2) First (DR^1) and third (DR^3) derivative ratio spectrophotometric method

Calibration curve was performed by transferring aliquots of itopride hydrochloride stock standard solution into a series of 10 ml volumetric flasks, and diluting to volume with water to obtain a concentration range of 1-12 $\mu g.ml^{-1}$ and 3-12 $\mu g.ml^{-1}$, respectively. The spectra of acidic and oxidative-degradated solutions having concentration 6.0 and 7.0 $\mu g.ml^{-1}$ were scanned and stored in the instrument PC as a devisors. The spectra of itopride hydrochloride were divided separately by the devisor's spectra, then the first (DR^1) and the third (DR^3) derivative of the ratio spectra were computed for the investigated drug in presence of its acid and oxidative-degradates, at 254.20 nm and 301.40 nm, respectively. Those values were then plotted versus corresponding concentrations; and the regression equation was then computed.

(3) Chromatographic determination of itopride hydrochloride in presence of its acid and oxidative-degradates

Chromatographic determination of itopride hydrochloride in presence of its acid and oxidative-degradates, using UV-HPLC:

Stationary phase, XDB C18 column (5μm, 150×4.6 mm), methanol: phosphate buffer 'pH 4.0' in a ratio (35:65, v/v) with a flow rate was 1.0 $ml.min^{-1}$ as 'degassed and filtered' mobile phase and UV detection at 258 nm, were the chromatographic conditions adopted. Construction the calibration curve was performed by transferring aliquots of itopride hydrochloride stock standard solution into a series of 10 ml volumetric flasks and diluting with the mobile phase to the volume, having a concentration range of 1.0-7.0 $\mu g.ml^{-1}$. Under the previously mentioned chromatographic conditions, 20.0-μl volume from each solution was injected in triplicate, the average peak area obtained for each concentration was plotted versus concentration and the regression equation was then computed.

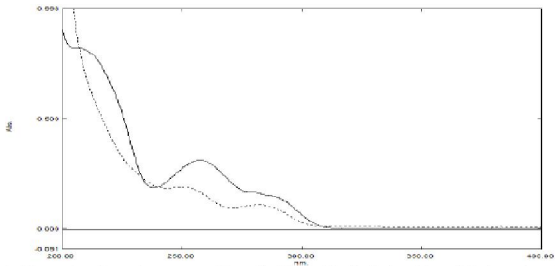

Figure 2a : Zero order absorption spectra of itopride hydrochloride (-) and its acidic-degradates (…), [8.00µg.ml^{-1} each]

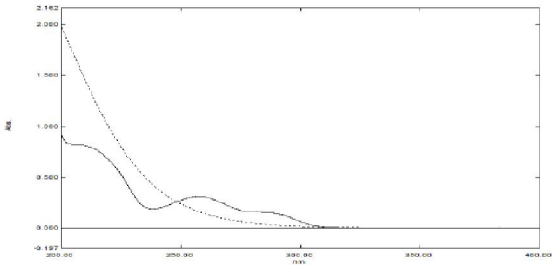

Figure 2b : Zero order absorption spectra of itopride hydrochloride (-) and its oxidative-degradates (…), [8.00 µg.ml^{-1} each]

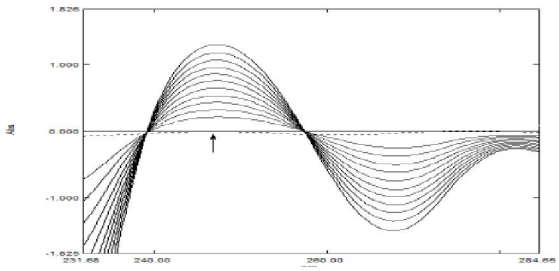

Figure 3a : First derivative spectra (D^1) of itopride hydrochloride (-) and its acid-degradates (…)

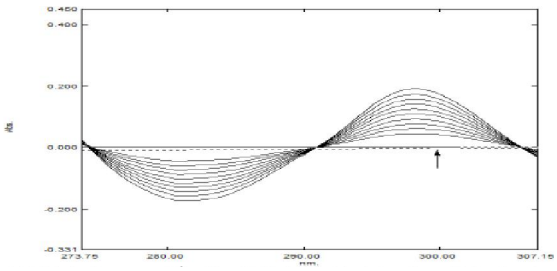

Figure 3b : Third derivative spectra (D^1) of itopride hydrochloride (-) and its oxidative-degradates (…)

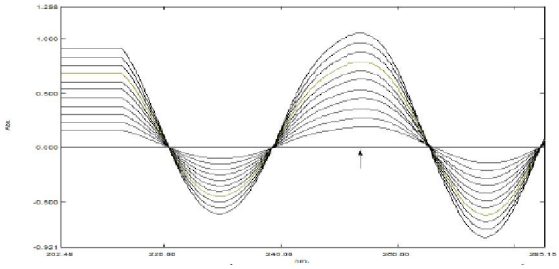

Figure 4a : First derivative of ratio spectra (DR^1) for different concentrations (2.0- 12.0 µg.ml^{-1}) of itopride hydrochloride at 254.40 nm, using 6.0 µg.ml^{-1} of its acidic-degradates as a divisor

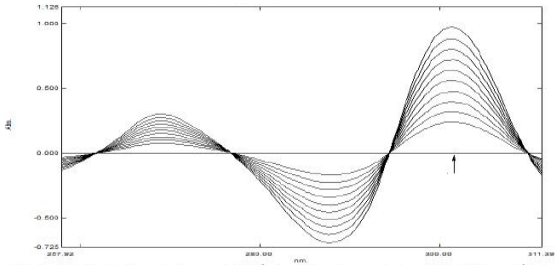

Figure 4b : Third derivative of ratio spectra (DR^3) for different concentrations (3.0- 12.0 µg.ml^{-1}) of itopride hydrochloride at 301.40 nm, using 7.0 µg.ml^{-1} of its oxidative-degradates as a divisor

(4) Chromatographic determination of itopride hydrochloride in presence of its acid and oxidative-degradates, using fluorescence-HPLC

Stationary phase, XDB C25 column (5µm, 150×4.6 mm), methanol: phosphate buffer 'pH 4.0' in a ratio (35:65, v/v) with a flow rate was 1.0 ml.min^{-1} as 'degassed and filtered' mobile phase and fluorescence detection at excitation and emission wavelengths 291/342nm, were the chromatographic conditions adopted. Construction the calibration curve was performed by transferring aliquots of itopride hydrochloride stock standard solution into a series of 10 ml volumetric flasks and diluting with the mobile phase to the volume, having a concentration range of 1.0-6.0 µg.ml^{-1}. Under the previously mentioned chromatographic conditions, 20.0-µl volume from each solution was injected in triplicate, the average peak area obtained for each concentration was plotted versus concentration and the regression equation was then computed.

Assay of the pharmaceutical preparations

Twenty tablets of Ganaton® were individually weighed to get the average weight of the tablets and finely powdered, respectively. A sample of the powdered tablets, claimed to contain 0.005 gm of itopride hydrochloride was transferred to 250 ml volumetric flask, sonicated for one hour with 25 ml of methanol, then the volume was brought to 250 ml with water, filtered to prepare stock standard solution and then the procedures mentioned under (2.4.1 and 2.4.2) were adopted. The concentrations of itopride hydrochloride

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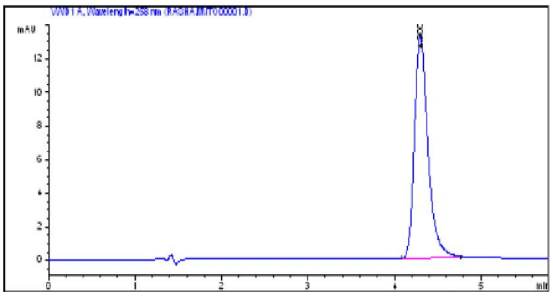

Figure 5a : HPLC chromatogram of itopride hydrochloride solution 4.00 µg.ml^{-1}, using UV-HPLC

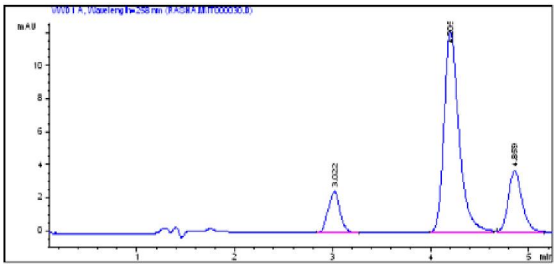

Figure 5b : HPLC chromatogram of mixture solution containing itopride hydrochloride 4.00µg.ml^{-1} with its acidic-degradates 4.00µg.ml^{-1}, using UV-HPLC

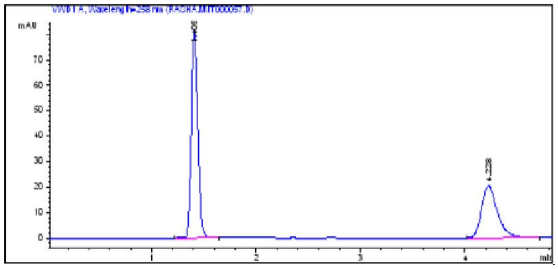

Figure 5c : HPLC chromatogram of mixture solution containing itopride hydrochloride 4.00µg.ml^{-1} with its oxidative-degradate 4.00 µg.ml^{-1}, using UV-HPLC

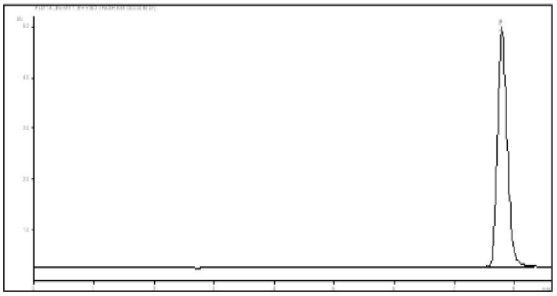

Figure 6a : HPLC chromatogram of itopride hydrochloride solution 5.00µg.ml^{-1}, using Fluorescence-HPLC

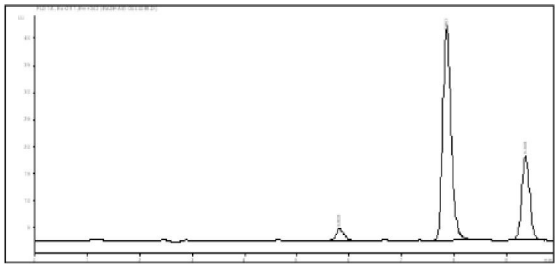

Figure 6b : HPLC chromatogram of mixture solution containing itopride hydrochloride 4.00µg.ml^{-1} with its acidic-degradates 4.00 µg.ml^{-1}, using Fluorescence-HPLC

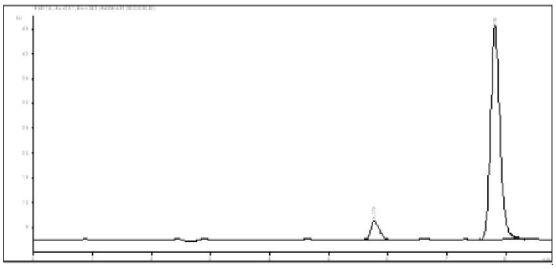

Figure 6c : HPLC chromatogram of mixture solution containing itopride hydrochloride 4.00µg.ml^{-1} with its oxidative-degradates 4.00µg.ml^{-1}, using Fluorescence-HPLC

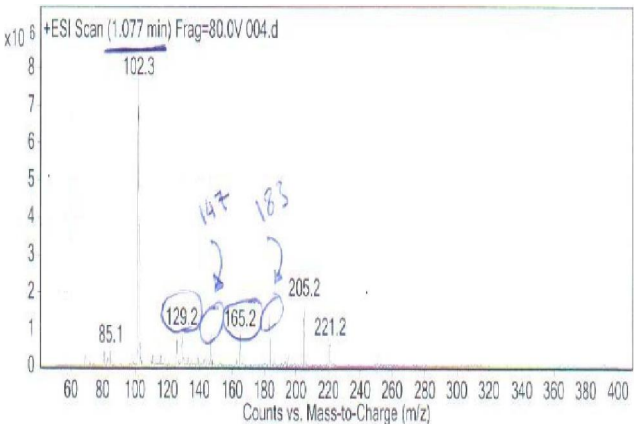

Figure 7 : LC-MS of acidic-degradates of itopride hydrochloride

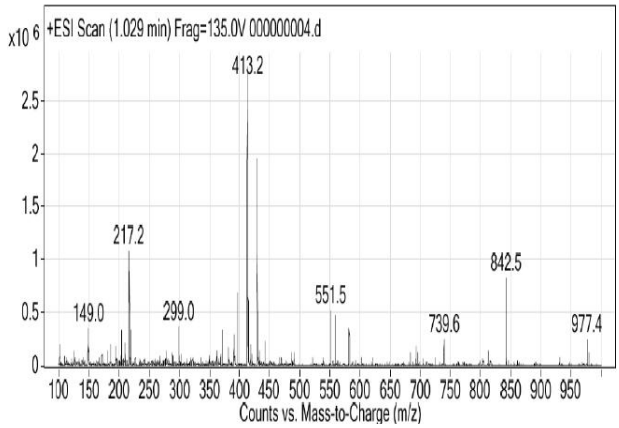

Figure 8 : LC-MS of oxidative-degradate of itopride hydrochloride

TABLE 1a : Validation report of the proposed derivative spectrophotometric methods for the determination of itopride hydrochloride

Parameters	Method	
	D^1	D^3
Linearity	1.0-12.0µg.ml^{-1}	3.0-12.0µg.ml^{-1}
Slope	0.105	0.016
Intercept	0.0223	-0.0017
Correlation coefficient (r)	0.9997	0.9999
Accuracy[a]	99.96 ±0.39	100.33± 0.593
Specificity[b]	98.22±0.250	98.87±0.618
Precision		
Repeatability[c] 'intra-day'	0.950	0.275
Intermediate precision[c] 'inter-day'	0.813	0.287

[a]Mean ±RSD (n = 6), [b]Mean ± RSD% (n = 6), [c]Mean ± RSD% (n = 9)

TABLE 1b : Validation report of the proposed derivative ratio spectrophotometric methods for the determination of itopride hydrochloride

Parameters	Method	
	DR^1	DR^3
Linearity	2.0-12.0µg.ml^{-1}	3.0-12.0µg.ml^{-1}
Slope	0.086	0.081
Intercept	0.02	-0.003
Correlation coefficient (r)	0.9999	0.9995
Accuracy[a]	100.29±0.760	99.26±0.809
Specificity[b]	99.43±1.272	98.87±1.310
Precision		
Repeatability[c] 'intra-day'	1.136	0.732
Intermediate precision[c] 'inter-day'	1.068	0.772

[a]Mean ± RSD (n = 6), [b]Mean ± RSD% (n = 6), [c]Mean ± RSD% (n = 9)

TABLE 1c : Validation report of the proposed UV-HPLC method for the determination of itopride hydrochloride

Parameters	Method in presence of	
	Acidic degradates	Oxidative degradates
Linearity	1.0-7.0µg.ml^{-1}	
Intercept	-15.43	
Slope	42.62	
Correlation coefficient (r)	0.9994	
Accuracy[a]	99.47 ± 1.287	
Precision		
Repeatability[a]	1.188	
Intermediate precision[a]	1.120	

[a]Mean ± R.S.D%

TABLE 1d : Validation report of the proposed fluorescence-HPLC method for the determination of itopride hydrochloride

Parameters	Method in presence of	
	Acidic degradates	Oxidative degradate
Linearity	1.0-6.0µg.ml^{-1}	
Intercept	-16.093	
Slope	80.74	
Correlation coefficient (r)	0.9994	
Accuracy[a]	100.01 ± 1.314	
Precision		
Repeatability[a]	0.963	
Intermediate precision[a]	0.802	

[a]Mean ± R.S.D%

TABLE 2 : Statistical comparison between the proposed [D^1, D^3, DR^1 DR^3, UV- HPLC and Fluorescence-HPLC] methods and the manufacturer's method* for determination of itopride hydrochloride

Parameters	Methods						
	D^1	D^3	DR^1	DR^3	U.V-HPLC	Fluo-HPLC	Manufacturer's Method*
Mean	98.60	98.66	98.74	98.79	98.73	98.72	98.75
S.D.	0.129	0.132	0.046	0.058	0.041	0.127	0.125
N	10	10	10	10	5	5	5
Variance	0.0168	0.0192	0.002	0.003	0.0017	0.0160	0.0155
t-test	-2.23	-1.37	-0.26	0.528	-0.375	-0.427	-
Cal. t-value	2.31	2.26	2.75	2.75	2.31	2.31	-
F-test	1.08	1.24	0.135	0.219	0.111	1.033	-
Cal. F-value	5.998	5.998	0.275	0.275	0.157	6.388	-

Values in parenthesis are the theoretical values of *t* and *F* at P = 0.05, *The manufacturer's method is the HPLC method; C8 column, acetonitrile: water (30: 70, v/v) at pH 3.0, as a mobile phase with a flow rate 1.0 ml.mint^{-1} and detection at 220 nm

TABLE 3a : Results of the laboratory prepared mixtures for itopride hydrochloride with its acidic and oxidative-degradates by the proposed spectrophotometric method

Sample no.	Itopride hydrochloride µg.ml^{-1}	Acidic &oxidative degradate µg.ml^{-1}	% Recovery*			
			In the presence of its acidic degradates		In the presence of its oxidative degradates	
			D^1 247.20 nm	DR^1 254.40 nm	D^3 298.20 nm	DR^3 301.40 nm
1	8.00	2.00	98.42	98.13	98.41	101.13
2	8.00	4.00	98.06	101.13	98.20	98.50
3	8.00	5.00	98.06	100.38	98.98	98.03
4	8.00	6.00	98.00	98.75	99.77	98.03
5	8.00	8.00	98.54	98.75	98.98	98.65
	Mean		98.22	99.43	98.87	98.87
	±R.S.D.%		±0.250	±1.272	±0.618	±1.310

*Mean of three determinations

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were calculated from the regression equation.

RESULTS AND DISCUSSION

Method development

(A) Spectrophotometric method

(1) Derivative spectrophotometric method

The UV-spectra of itopride hydrochloride and its acidic and oxidative-degradates showed overlapping as shown in figure 2a and 2b, which would not permit zero order determination of itopride hydrochloride in the presence of its degradates, so derivative spectrophotometric methods were adopted, where zero-crossing point for acidic and oxidative-degradate of itopride hydrochloride were indicated. The first (D^1) and the third (D^3) derivative spectrophotometric methods permitted a selective determination of itopride hydrochloride in the presence of its acidic and oxidative-degradates at 247.20 and 298.20 nm, respectively, as shown in figure 3a and 3b. The corresponding regression equations were found to be:

$$D^1_{247.20} = 0.105C + 0.0223 \quad r = 0.9997$$

$$D^3_{298.20} = 0.016C - 0.0017 \quad r = 0.9999$$

where, $D^1$247.20 and $D^3$298.2 are the peak amplitudes at 247.20nm and 298.20 nm, respectively, C are the concentration of itopride hydrochloride in $\mu g.ml^{-1}$ and r is the correlation coefficient.

(2) Derivative of ratio spectrophotometric method (DR^n)

The main advantage of derivative ratio spectra method (DR^n) might be the chance of taking measurement in correspondence to peaks and that the whole spectrum of interfering substance is cancelled, thus the wavelength selection for calibration is not critical. The main instrumental parameter conditions were optimized for a reliable determination of the compounds. Different divisor concentrations of acidic and oxidative-degradates were examined to select an appropriate concentration, which is very important factor in practice, where the best results were obtained by using 0.6 $\mu g.ml^{-1}$ and 7.0 $\mu g.ml^{-1}$ of acidic and oxidative-degradates, respectively as divisors. The first (DR^1) and the third (DR^3) derivative of the ratio spectra at 254.40 and 301.40 nm permitted a selective determination of itopride hydrochloride in the presence of its acidic and oxidative-degradates as shown in figure 4a and 4b, where no noises were observed from the selected divisors. The corresponding regression equations were found to be:

$$DR^1_{254.40} = 0.0858C + 0.0202 \quad r = 0.9999$$

$$DR^3_{301.40} = 0.0807C - 0.0029 \quad r = 0.9995$$

where, $DR^1_{254.40}$ and $DR^3_{301.40}$ are the peak ampli-

TABLE 3b : Results of the laboratory prepared mixtures for itopride hydrochloride with its oxidative-degradates by the proposed chromatographic method

Sample no.	Itopride hydrochloride $\mu g.ml^{-1}$	Acid& oxidative-degradate $\mu g.ml^{-1}$	% Recovery *			
			U.V-HPLC (acidic)	Fluori-HPLC (acidic)	U.V-HPLC (Oxidative)	Fluori-HPLC (Oxidative)
1	4.00	0.50	100.60	100.77	100.56	99.95
2	4.00	1.00	100.40	98.17	100.21	98.96
3	4.00	2.00	98.60	100.01	98.33	98.81
4	4.00	3.00	100.80	100.20	98.81	98.93
5	4.00	4.00	100.20	100.23	98.90	101.04
	Mean		100.12	99.88	99.36	99.54
	±R.S.D.%		±0.876	±0.995	±0.966	±0.961

*Mean of three determinations

TABLE 4a : Results from robustness testing of the proposed spectrophotometric and chromatographic methods for itopride hydrochloride

Methods		Robustness (mean ±RSD)
Spectrophotometric method	D^1	99.68±0.162
	D^3	100.31±0.324
	DR^1	99.75±1.41
	DR^3	98.85±0.730
UV-HPLC	UV detection	100.27±1.042
	Fluorescence detection	99.57±0.521

TABLE 4b : Results of system suitability of the proposed chromatographic methods for determination of Levetiracetam

Parameters	Itopride hydrochloride Hcl		
	UV detection	Fluorescence detection	Limit
Retention time (t_R)	4.22	7.858	-
Resolution (Rs)	4.49	7.41	Rs >2
Tailing factor (T)	1.125	1.25	T < 2
Capacity factor (K')	7.44	6.86	1< K' <10
Selectivity factor (α)	1.36	1.35	$\alpha > 1$
Column efficiency (N)	4163	12474	N > 2000
Retention time (t_R)	4.22	7.858	-

TABLE 5a : Determination of itopride hydrochloride in pharmaceutical preparations[a] by the proposed spectrophotometric methods and application of standard addition technique, in presence of its acidic and oxidative-degradates

Pharmaceutical preparations	Claimed	% Found ± R.S.D*				Standard addition technique				
							%Recovery[b]			
		D^1	D^3	DR^1	DR^3	Added ($\mu g.ml^{-1}$)	D^1	D^3	DR^1	DR^3
Ganaton® tablets B.N: 76583/3J/L [a]	50 mg	99.60± 0.131	99.66 ±0.132	98.74± 0.047	99.59± 0.298	3.00	101.17	100.73	101.00	99.80
						4.00	98.00	98.20	100.50	99.38
						5.00	98.13	98.14	100.20	100.90
		Mean ± RSD%					99.10 ±1.810	99.02 ±1.493	100.57 ±0.402	99.59 ±0.298

[a]Ganaton® tablets (Batch no: 76583/3J/L) (labeled to contain 50 mg itopride hydrochloride per tablet). [b]Mean of three determinations

tudes at 254.40nm and 301.40nm, respectively, C is the concentration of itopride hydrochloride in $\mu g.ml^{-1}$ and r is the correlation coefficient.

(B) Chromatographic method

(1) Chromatographic method using UV detector

The separation of itopride hydrochloride from its degradation-products has been performed on XDB C18 column. The proportion of the mobile phase components was optimized to reduce each of 'retention time and tailing' and to enable good resolution from its-degradates. Several trials were carried out to obtain good and optimum separation of itopride hydrochloride from its degradation products. Different composition mobile phases with different ratios were tried such as methanol: water (70: 30, v/v), and acetonitrile: water (10:90, v/v), but the best resolution was obtained upon using methanol: phosphate buffer pH 4.0 (35:65, v/v) adjusted by using O-phosphoric acid with a flow rate of 1.0 $mlmin^{-1}$ and a detection wavelength 258 nm, where the maximum sensitivity was observed. The average retention time was 4.30 ± 0.05 min as shown in figure 5a-5c. The regression equation was computed and found to be:

A = 42.621C – 15.426 r = 0.9994

where, A is the relative peak area; C is itopride hydrochloride concentration in $\mu g.ml^{-1}$ and r is the correlation coefficient.

(2) Chromatographic method using fluorescence detector

All the last procedure mentioned in Chromatographic method using UV detector, was adopted, where the separation of itopride hydrochloride from its degradation-products has been performed on XDB C25 column, and fluorescence detection at excitation and emission wavelengths 291/342nm, where the maximum sensitivity was observed. The average retention time was 7.789 ± 0.01 min as shown in figure 6a-6c. The regression equation was computed and found to be:

A = 80.74C – 16.093 r = 0.9994

where, A is the relative peak area; C is itopride hydrochloride concentration in $\mu g.ml^{-1}$ and r is the correlation coefficient.

(C) Method validation

ICH guidelines[9] for validation method were followed, where all validation parameters were shown in TABLE 1a-1d. All the obtained results were statistically compared to the manufacturer's method of itopride hydrochloride and no significant differences were found in TABLE 2.

Specificity

Degradation behavior of itopride hydrochloride was investigated by the proposed spectrophotometric and chromatographic methods, where the investigated drug was determined in solutions containing different amounts of its acidic and oxidative-degradates by spectrophotometric method using [(D^1 and D^3), and (DR^1 and DR^3)] techniques and by chromatographic method using [UV and fluorescence detection] techniques, respectively. Its Recovery % and R.S.D. % proved the high specificity of the adopted methods, where itopride hydrochloride could be determined in the presence of its acidic and oxidative-degradates (up to 100 %), as shown in TABLE 3a -3b.

Robustness and system suitability of the HPLC method

The robustness of an analytical procedure is a mea-

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TABLE 5b : Determination of itopride hydrochloride in pharmaceutical preparations[a] by the proposed chromatographic methods and application of standard addition technique, in presence of its oxidative-degradates

Pharmaceutical preparations	Claimed	% Found ± R.S.D*		Standard addition technique		
		U.V-HPLC	Fluori-HPLC	Added ($\mu g.ml^{-1}$)	%Recovery[b] U.V-HPLC	%Recovery[b] Fluori-HPLC
Ganaton® tablets B.N: 76583/3J/L[a]	50 mg	98.73± 0.042	98.72± 0.129	1.00	99.28	101.57
				2.00	101.75	101.66
				3.00	100.67	98.32
Mean ± RSD%					100.90± 1.391	100.74± 1.499

[a]Ganaton® tablets (Batch no: 76583/3J/L) (labeled to contain 50 mg itopride hydrochloride per tablet). [b]Mean of three determinations

sure of its capacity to remain unaffected after slit but deliberate changes in the analytical conditions. Separation of studied drug from its different degradates was performed under these conditions. In the propped spectrophotometric methods, the parameters of robustness were done in alterations of the used solvent and wavelengths, while in chromatographic methods; the alterations were done in wavelengths, flow rate, composition in mobile phase, and PH, (TABLE 4a). The system suitability parameters of HPLC method were evaluated[27] (TABLE 4b).

Standard addition technique

To check the validity of the proposed methods, the standard addition method was applied by adding each drug to the previously analyzed tablets. The recovery of it was calculated by comparing the concentration obtained from the spiked samples with that of the pure drug. The results of analysis of the commercial tablets and the standard addition method (recovery study) of itopride hydrochloride are shown in TABLE 5a-5b suggested that there is no interference from any excipients, which are normally present in tablets.

Identification of acidic and oxidative-degradates of itopride hydrochloride by structure elucidation

In this work, we were concerned with the acidic and oxidative-degradation of itopride hydrochloride, as it is completely degraded in a short time relative to alkaline-degradation, which is considered a new way for determination of itopride hydrochloride in presence of its acidic and oxidative- degradates by using spectrophotometric methods.

Itopride hydrochloride was influenced by refluxing with 5.0 M HCl for 3-hrs at 100°C, giving two acidic-degradates. Also, it was subjected to oxidative stress-testing by refluxing with30% H_2O_2 for 2 hours at 100°C, giving one oxidative-degradate.

The identity of the acidic and oxidative-degradates was confirmed by adopting LC-MS for each one, where the molecular ion peak of itopride hydrochloride at 394.9 m/z was completely disappeared and two new molecular ion peaks were delivered when it was subjected to acidic hydrolysis and resulting in N-dealkylation of itopride hydrochloride corresponding to 165 and 183 m/z, while on exposuring to oxidation resulting in formation of N-Oxide product, and a new molecular ion peak at 314 m/z was appeared as shown in figure 7 and 8.

CONCLUSION

The proposed methods are accurate, precise and specific ones, where itopride hydrochloride can be determined in bulk powder and in pharmaceutical preparations without interference from common excipients present, also it can be determined in the presence of its acidic and oxidative-degradates. ICH guidelines were followed throughout the study for method validation and stress testing, and the suggested methods can be applied for routine quality control analysis and stability studies.

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