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Full Length Research Paper

Simultaneous determination and method validation of ranitidine hydrochloride and itopride hydrochloride by UV-spectrophotometery

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An UV spectrophotometric method using simultaneous equation was developed for the simultaneous determination of ranitidine and itopride in a binary mixture. In the proposed method, the signals were measured at 315.0 and 258.0 nm corresponding to absorbance maxima of ranitidine and itopride hydrochloric acid (HCl) in double distilled water, respectively. Linearity range was observed in the concentration range of 2 to 20 µg/ml for both drugs. Concentration of each drug was obtained by using the absorptivity values calculated for both drugs at two wavelengths, 315.0 and 258.0 nm and solving the simultaneous equation. Developed method was applied to laboratory mixture. The method was validated statistically and recovery study was performed to confirm the accuracy of the method. The method was found to be rapid, simple, accurate and precise.

Key words: Itopride, simultaneous equation, spectrophotometry, ranitidine.

INTRODUCTION

Ranitidine hydrochloride (RanHCl) is a H_2-receptor antagonist which is used as anti-ulcer drug that reduces acid secretion by blocking the histamine receptor type. Ranitidine is a H_2-receptor antagonist, same as cimetidine. The only difference is that it contains furan ring in place of imidazole ring of cimetidine. This one is amino alkyl furan derivative. It is more potent in inhibiting the gastric acid secretion (Klaus, 2005). Itopride hydrochloride (ItoHCl) (Gupta et al., 2004) is a new gastro-prokinetic agent which is used in reflux oesophagaitis (Pillai and Singhvi, 2008). These two drugs are natural candidates for combination therapy in the treatment of reflux oesophagitis, dyspepsia, hyperacidity etc. Although no marketed product has yet been launched in India, however, those two drugs are logical candidates for combination formulation in hyperacidicty and reflux-oesophagitis. Therefore, characterization of formulations like tablets, capsules or microcapsules having this combination of drugs will require simultaneous determination of RanHCl and ItoHCl in aqueous buffer media. Because of this reason for determining the concentration of the drugs simultaneously in the acidic dissolution medium, a sensitive, validated, less time consuming and low cost analytical method by UV-spectrophotometry is required to be developed. The analytical method has been validated by different statistical parameter as calculated (Attimarad et al., 2012; Mohite et al., 2009). Therefore, in this project

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Table 1. Preparation of standard solutions of pure drugs.

S/No.	Volume of working stock solution (100 µg/ml)	Distilled water (ml)	Concentration of Drug (µg/ml)
1	2.0 ml	8.0	20
2	1.8 ml	8.2	18
3	1.5 ml	8.5	15
4	1.0 ml	9.0	10
5	0.5 ml	9.5	5
6	0.2 ml	9.8	2

Table 2. Preparation of standard solutions containing both the drugs.

S/No.	Volume of working stock solution of RanHCl (100 µg/ml)	Volume of working stock solution of ItoHCl (100 µg/ml)	Distilled Water (ml)	Concentration of RanHCl (µg/ml)	Concentration of ItoHCl (µg/ml)
1	2.0 ml	0.0 ml	8.0	20	0
2	1.8 ml	0.2 ml	8.0	18	2
3	1.5 ml	0.5 ml	8.0	15	5
4	1.0 ml	1.0 ml	8.0	10	10
5	0.5 ml	1.5 ml	8.0	5	15
6	0.2 ml	1.8 ml	8.0	2	18
7	0.0 ml	2.0 ml	8.0	0	20

work we have chosen to develop and validate a simultaneous UV-spectroscopy method to determine RanHCl and ItoHCl in aqueous medium. The two molecules were tried for separation by reversed-phase high-performance liquid chromatography (RP-HPLC) in the laboratory of this institute but by varying pH of the buffer and varying mobile phase composition, the two molecules were difficult to separate. In this context an assay method employing the simultaneous equation method was required to be developed for simultaneous determination of RanHCl and ItoHCl in the same solution, as first derivative spectrophotometry for simultaneous estimation of doxylamine succinate, pyridoxine HCl and folic acid in tablet formulations has been carried out at 270, 332.8 and 309.2 nm (Pathak and Rajput, 2008).

MATERIALS AND METHODS

Ranitidine hydrochloride was a kind gift from Strides Acrolab, Bangalore and Itopride hydrochloride was a kind gift from Themis Pharma Pvt Ltd, Thane, Mumbai. Distilled water was obtained from Barnstead type steel distillation apparatus. Double beam UV-Vis spectrophotometer, Pharmaspec 1700, Shimadzu was used for measuring the absorbance of the solutions. Volumetric flask 100 ml, graduated pipette 10 ml, beaker 100 ml, beaker 50 ml were procured from Borisil®. Single pan analytical balance (Dwijo, 200 G), wax paper, tissue paper were used.

Preparation of stock solutions of RanHCl and ItoHCl

RanHCl and ItoHCl were dried overnight in desiccator. RanHCl, 100 mg was dissolved in 100 ml double distilled water to prepare a stock solution of concentration 1000 µg/ml. Working standard of RanHCl was prepared by diluting the stock solution 100 times to obtain a standard concentration of 100 µg/ml. Similarly, working standard of ItoHCl of 100 µg/ml was also prepared.

Preparation of standard solutions

Standard RanHCl and standard ItoHCl solutions of both the drugs were prepared by using the working standard solutions of the two drugs according to Tables 1 and 2.

Preparation of standard solutions containing both RanHCl and ItOHCl

This is seen in Table 2.

Selection of two analytical wavelengths

UV spectra of pure drugs solutions and mixture solution of two drugs were constructed by taking the solutions in a 1 cm cuvette and scanned from 200 to 400 nm in a double beam UV-Spectrophotometer (Shimadzu Pharmaspec 1700). One wavelength was selected where one molecule will produce peak absorbance and the other some lower absorbance. Similarly, at the other wavelength the second molecule will produce maximum and the first molecule some lower absorbance value.

Construction of simultaneous equations

To determine the absorptivity of the pure component a standard curve was constructed by plotting absorbance (A) against standard

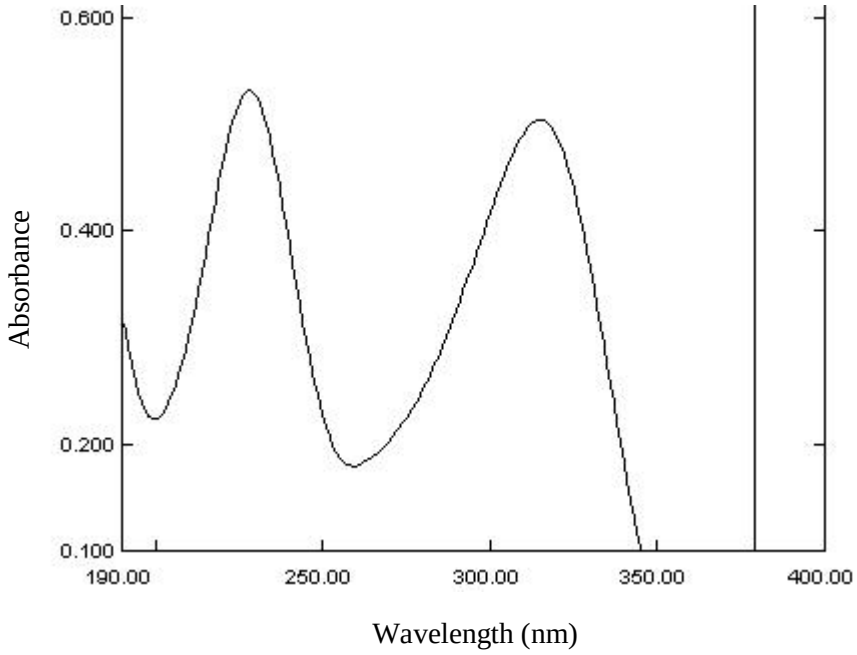

Figure 1. Pure RanHCl spectrum.

concentrations (C). The equation of the straight line was obtained from linear regression. The path-length of the light was 1 cm (1 cm cuvette), therefore the slope of the straight line was the absorptivity of the component. Four absorptivities were calculated for two wave lengths and two components. Two simultaneous equations were constructed as described. The equations were solved by using Cramer's rule (Beckett and Stenlake, 2002). For large number of calculations MS Excel and VB application were used. If the two simultaneous equations are as follows:

$$A1 = a_{x1} * C_X + a_{y1} * C \quad (1)$$

$$A2 = a_{x2} * C_X + a_{y2} * C_Y \quad (2)$$

Then the solution for C_X and C_Y were solved by the following equations:

$$C_x = \frac{\begin{vmatrix} A_1 & a_{y1} \\ A_2 & a_{y2} \end{vmatrix}}{\begin{vmatrix} a_{x1} & a_{y1} \\ a_{x2} & a_{y2} \end{vmatrix}} = \frac{A_1 a_{y2} - a_{y_1} A_2}{a_{x1} a_{y2} - a_{y1} a_{x2}}$$

$$C_y = \frac{\begin{vmatrix} a_{x1} & A_1 \\ a_{x2} & A_2 \end{vmatrix}}{\begin{vmatrix} a_{x1} & a_{y1} \\ a_{x2} & a_{y2} \end{vmatrix}} = \frac{a_{x1} A_2 - A_1 a_{x2}}{a_{x1} a_{y2} - a_{y1} a_{x2}}$$

RESULTS AND DISCUSSION

Determination of analytical wavelengths from UV-spectra

Three spetra were obtained from (i) standard solution of pure RanHCl (10 µg/ml), (ii) standard solution of pure ItoHCl (10 µg/ml) and (iii) standard solution containing 10 µg/ml of RanHCl and 10 µg/ml of ItoHCl. The spectra were provided as follows (Figures 1 to 4). RanHCl showed two peaks at 228.5 and 315 nm while ItoHCl showed a single maximum at 258 nm. Since there were two components in the mixture solution hence, two analytical wavelengths were selected at 258 and 315 nm.

Construction of simultaneous equations

Four standard straight lines were constructed by plotting the absorbances (Y-axis) of standard solutions of pure drugs, at two analytical wavelengths chosen in Equation (3) against the concentration (X-axis). The absorptivity values were calculated from the slopes of Figures 5 and 6. Therefore the simultaneous equations are:

$$A1 = 0.0388 * C_X + 0.0017 * C_Y \quad (3)$$

$$A2 = 0.0133 * C_X + 0.0306 * C_Y \quad (4)$$

Solving these two simultaneous equations by Cramer's rule yielded C_X and C_Y. These calculations were carried out using MS Excel. Two functions were generated with

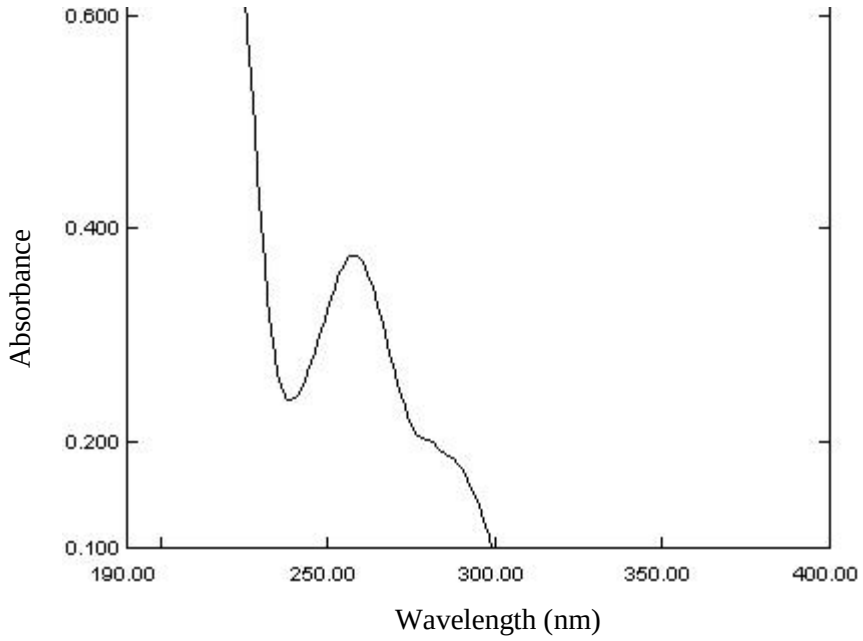

Figure 2. Pure ItoHCl spectrum.

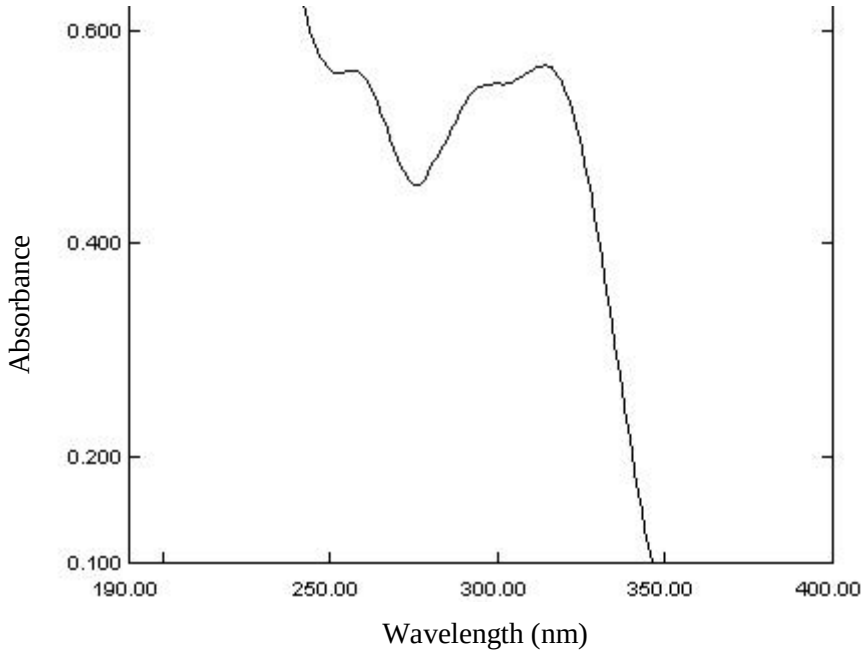

Figure 3. Mixture spectrum.

the help of VB Application programming. Now as per Table 3, seven standard solutions were prepared having mixture of two drugs RanHCl and ItoHCl in different proportion. Absorbances were taken against distilled water as blank at two wavelengths, 315 and 258 nm. The two absorbance values A1 (at 315 nm) and A2 (at 258 nm) were input in the function conc_X( ) and conc._Y( ) to obtain C_X and C_Y. The recovery results are given in Table 4.

The interim recovered values were far away from the actual values. Therefore, another operation was carried out where a linear regression equation was constructed in between Cx_actual (plotted in Y-axis) and Cx_recovered (plotted in X-axis). Similar operations were

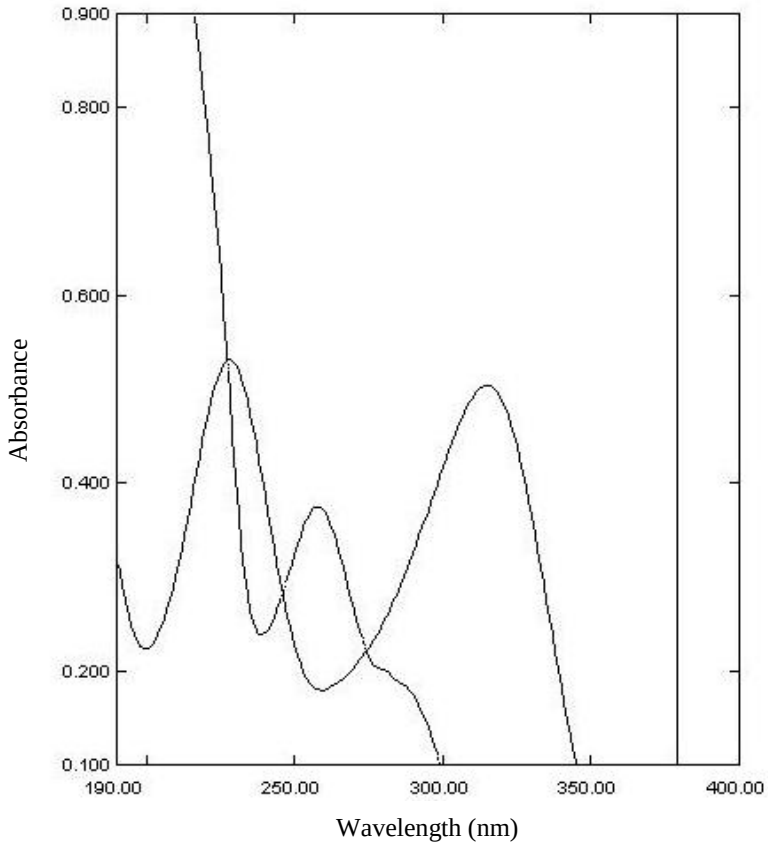

Figure 4. Superimposed spectra of pure RanHCl and ItoHCl.

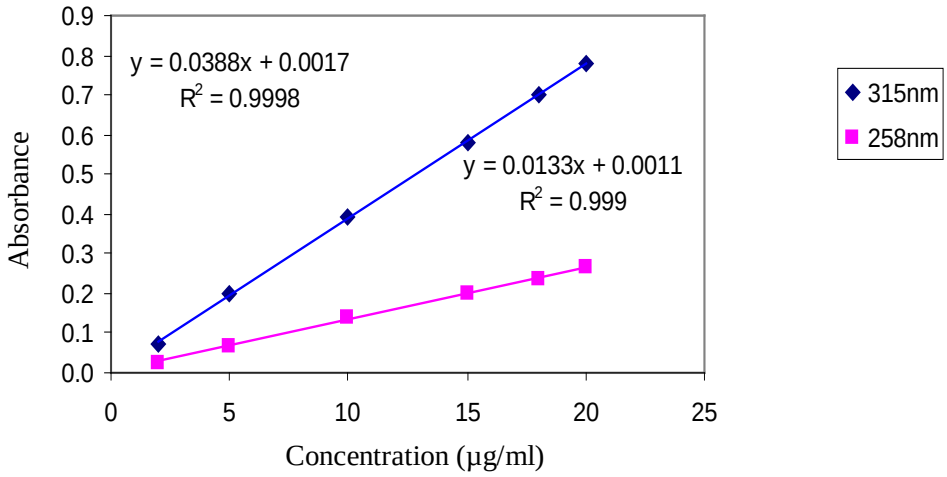

Figure 5. Standard straight line of RanHCl at two 315 and 258 nm.

carried out with data of C_Y also. The equations thus obtained from Figure 7 were applied for calculating the final values.

$Final\_C_X = 0.7558 \times Recovery\_X + 0.6393$
$Final\_C_Y = 0.8430 \times Recovery\_Y - 0.2135$

Results of determination of validation parameters

Specificity

Since this procedure determines the two drugs in a solution therefore this method alone was not sufficient to

Table 3. Absorbances of pure drug solutions at 315 and 258 nm.

Conc. of drug (µg/ml)	Ranitidine hydrochloride		Itopride hydrochloride	
	λ1 = 315 nm	λ2=258 nm	λ1 = 315 nm	λ2=258 nm
20	0.776	0.266	0.033	0.606
18	0.700	0.238	0.031	0.554
15	0.582	0.201	0.026	0.467
10	0.394	0.140	0.017	0.309
5	0.200	0.067	0.008	0.158
2	0.074	0.025	0.003	0.057
Slope	$a_{x1} = 0.0388$	$a_{x2} = 0.0133$	$a_{Y1} = 0.0017$	$a_{Y2} = 0.0306$
Intercept	$I_{x1} = 0.0017$	$I_{x1} = 0.0011$	$I_{x1} = -0.0004$	$I_{x1} = 0.0014$
R^2	0.9998	0.9990	0.9990	0.9994

Table 4. Recovery of C_X and C_Y.

A1	A2	Actual		Recovered values	
at 315 nm	at 258 nm	Cx	Cy	Cx	Cy
1.007	0.354	20	0	25.95	0.27
0.888	0.384	18	2	22.77	2.65
0.735	0.432	15	5	18.68	6.00
0.507	0.550	10	10	12.50	12.54
0.252	0.615	5	15	5.72	17.61
0.117	0.693	2	18	2.04	21.76
0.003	0.721	0	20	-0.98	23.99

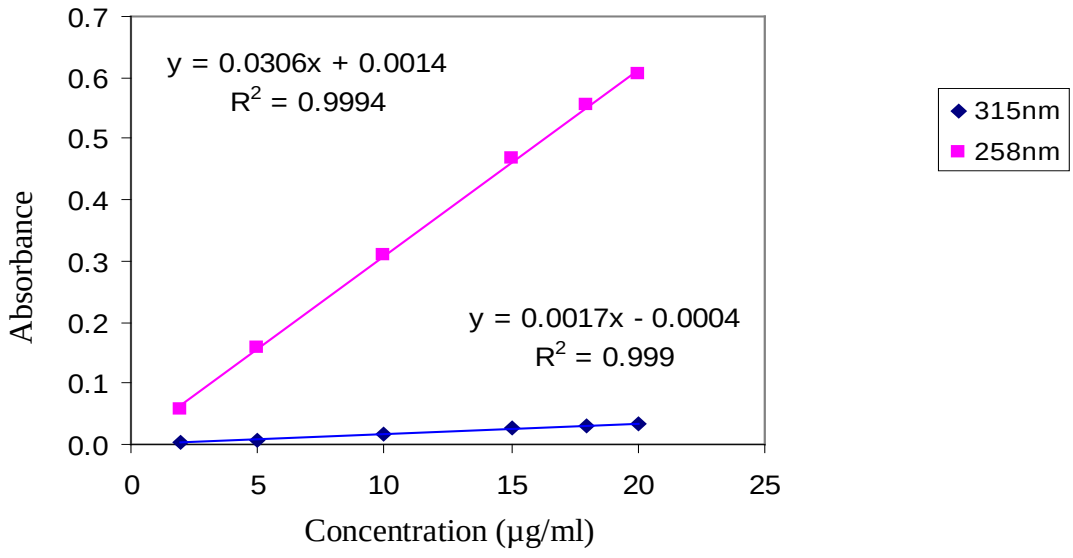

Figure 6. Standard straight line of ItoHCl at two 315 and 258 nm.

prove the specificity. For proving the specificity another method like high performance liquid chromatography (HPLC) is required in tandem with this procedure. However, no HPLC method is reported yet for separating

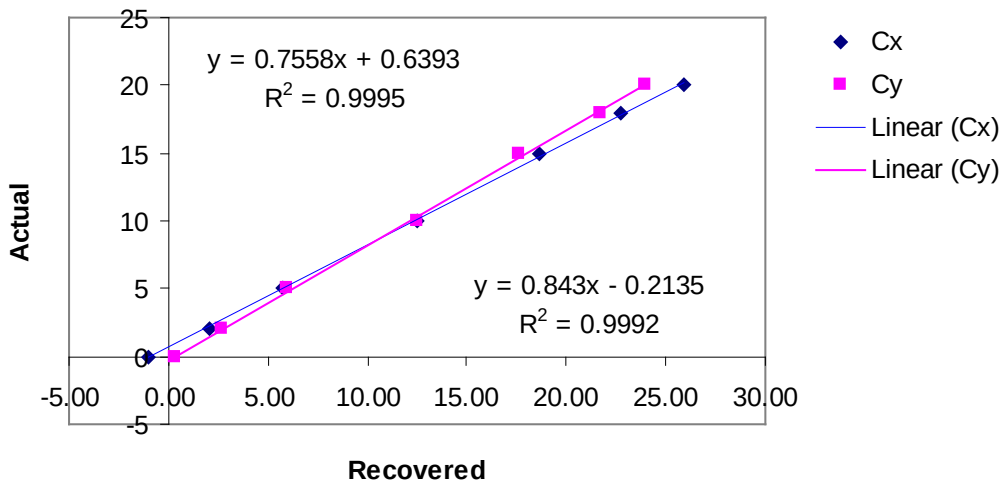

Figure 7. Plot of recovered values (in X-axis) and actual values (in Y-axis).

Table 5. Result for linearity.

A1 at 315nm	A2λ at 258nm	Actual conc. µg/ml)		Calculated conc. (µg/ml)	
		Cx	Cy	Cx	Cy
1.007	0.354	20	0	20.3	0.0
0.888	0.384	18	2	17.8	2.0
0.735	0.432	15	5	14.8	4.8
0.507	0.550	10	10	10.1	10.4
0.252	0.615	5	15	5.0	14.6
0.117	0.693	2	18	2.2	18.1
0.003	0.721	0	20	-0.1	20.0

these two drugs.

Linearity

Linearity calculation has been done as shown in Table 5.

Correlation coefficient (R) for C_X = 0.9997.
Correlation coefficient (R) for C_Y = 0.9996.

As per the acceptance criteria the correlation coefficients are higher than 0.9990. Therefore the method showed linearity within 2 to 20 µg/ml of each drug.

Limit of detection (LOD) and limit of quantitation (LOQ)

Determination of LOD and LOQ of RanHCl at 315 and 258 nm as shown in Table 6. Since it is a mixture solution therefore the LOD and LOQ will be the higher values that is 0.266 and 0.805 µg/ml, respectively for RanHCl (where ItoHCl is the background matrix). Determination of LOD and LOQ of ItoHCl at 315 and 258 nm has been done as mentioned in Table 7. Since it is a mixture solution therefore the LOD and LOQ will be the higher values i.e. 1.497 and 4.536 µg/ml, respectively for ItoHCl (where RanHCl is the background matrix).

Result of precision

As per Tables 8 and 9, the %RSD values are within 2.0 therefore, the method was found to be precise with respect to repeatability.

Accuracy

Acceptance criteria: Recovery should be within 98% to 102%. The recovery results indicating that the test method has an acceptable level of accuracy for the assay

Table 6. Determination of LOD and LOQ of RanHCl at 315 and 258 nm.

RanHCl conc. (µg/ml)	ItoHCl conc. (µg/ml)	Absorbance readings	
		Abs at 315 nm	Abs at 258 nm
0	10	0.017	0.309
0	10	0.016	0.306
0	10	0.017	0.307
0	10	0.015	0.306
0	10	0.017	0.307
0	10	0.016	0.307
2	10	0.090	0.332
5	10	0.216	0.374
10	10	0.410	0.447
15	10	0.598	0.508
Background matrix	δ =	0.00082	0.00110
	S =	0.039	0.0136
	LOD = 3.3 δ/S	0.069	0.266
	LOQ = 10 δ/S	0.209	0.805

Table 7. Determination of LOD and LOQ of ItoHCl at 315 and 258 nm.

RanHCl conc. (µg/ml)	ItoHCl conc. (µg/ml)	Absorbance readings	
		Abs at 315 nm	Abs at 258 nm
10	0	0.394	0.140
10	0	0.392	0.139
10	0	0.393	0.140
10	0	0.393	0.138
10	0	0.394	0.139
10	0	0.394	0.141
2	10	0.397	0.197
5	10	0.402	0.298
10	10	0.411	0.449
15	10	0.420	0.607
Background matrix	δ =	0.00082	0.00105
	S =	0.0018	0.0313
	LOD = 3.3δ/S	1.497	0.111
	LOQ = 10 δ/S	4.536	0.335

with respect to RanHCl (%RSD =1.43 < 2.0) but not with Itopride HCl (%RSD = 3.51 >2.0).

Range

From the linearity, precision and accuracy experiments the range of the analytical method was found to be between 5 to 20 µg/ml concentration for both RanHCl and ItoHCl.

Conclusion

In this context an easy and economical UV-spectrophotometeric simultaneous assay method was developed where a sample of solution containing a mixture of two drugs were subjected to UV-spectroscopy at two analytical wavelength of 315 and 258 nm. Two simultaneous equations were developed. The individual concentrations of two drugs were calculated from those equations. The method was validated and various

Table 8. Observations and results for System Precision

Concentration (µg/ml)	Measurement	Absorbance at 315 nm	Absorbance at 258 nm
10 µg/ml of RanHCl + 10 µg/ml of ItoHCl	Reading-1	0.507	0.550
	Reading -2	0.506	0.55
	Reading -3	0.507	0.551
	Reading -4	0.507	0.551
	Reading -5	0.506	0.550
	Reading -6	0.506	0.550
Statistical analysis	Mean	0.507	0.550
	SD	0.00055	0.00052
	%RSD	0.108	0.094

Acceptance Criteria : RSD should be not more than 2.0 %.

parameters were reported. Ruggedness parameter was not carried out because of limitation of multiple equipments.

Conflict of Interest

The authors have not declared any conflict of interest.

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