



HPLC and UV Spectrophotometric estimation of Itopride in Pharmaceutical Formulations

Authors & Affiliation:

Sk. Jabeer, P.Srinivasa Rao, U. Sambasiva Rao, Sk.Janbee & JUM Rao*

Department of Chemistry (PG), Hindu College, Guntur, Andhra Pradesh, India.

Corresponding Author:

JUM Rao

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Abstract:

Itopride belongs to the Benzamide group that inhibits dopamine and has a gastrokinetic effect and used for the treatment of dyspepsia. HPLC and UV method was employed for the estimation of Itopride in three different pharmaceutical formulations. A mixture of Water: Methanol in the ratio of (30:70) (v/v) at a pH of 4.8 in isocratic conditions and separation was achieved on Zodiac C18 column (250 X 4.6 mm, 5 μ) at ambient temperature. UV detection was monitored at a wavelength of 258nm. In these conditions, the drug elutes at a retention time of 4.2 min for sample and standard solutions. In UV method, the standard drug was diluted using methanol solvent and maximum absorbance was found to be 258nm and the analysis was carried in this wavelength. Beers law limit was found to be in the range of 50-150 μ g/ml and 5-30 μ g/ml for HPLC and UV methods respectively. Retune, Gimate and Itcan (50, 50 and 150mg) brands were used for formulation analysis and the % assay was found to be more than 98% for both the HPLC and UV methods in all the brands in the study.

Introduction:

Itopride belongs to the benzamide group, a prokinetic benzamide derivative, inhibit dopamine and have a gastro kinetic effect. It is indicated for the treatment of functional dyspepsia and in the treatment of GI symptoms caused by reduced GI motility: dyspepsia of a non-ulcer type (gastric "fullness", discomfort, and possible pain), anorexia, heartburn, regurgitation, bloating, nausea and vomiting, other possible gastric, prolactin, or dopamine related conditions. It inhibits the dopamine D2 receptor at the parasympathetic nerve ends and thereby increases the release of acetylcholine and decreases the metabolism of acetylcholine by inhibiting the enzyme acetyl cholinesterase (ACHE). Itopride increases the oesophageal and gastrointestinal peristalsis, increases the lower oesophageal sphincter pressure, stimulates gastric motility, accelerates gastric emptying and improves gastroduodenal coordination. Its chemical name is N-[[4-(2-Dimethylaminoethoxy) phenyl] methyl]-3, 4-imethoxybenzamide with molecular formula $C_{20}H_{26}N_2O_4$ and molecular mass of about 358.43 g/mol.

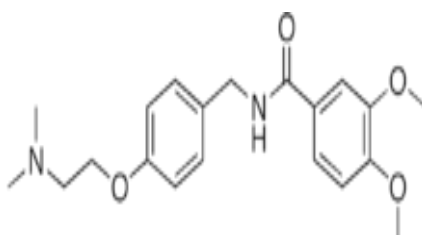


Figure 1A: Structure of Itopride

2. Materials and methods**2.1 Instrumentation**

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of Itopride an isocratic PEAKHPLC instrument with Zodiac C18 column (250 mm x 4.6 mm, 5 μ) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC – 7000 UV-detector. A 20 μ L Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software. Techcomp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Standard cuvettes of 10mm path length are used for analysis.

2.2 Chemicals and Solvents

All the solvents used for HPLC analysis were of HPLC grade and were purchased from Merck chemicals, Mumbai. The chemicals used in UV were of laboratory reagent grade and were purchased from Merck chemicals private limited, Mumbai.

2.3 Standard solution of the drug

For analysis Itopride accurately weighed 10mg of the standard and dissolved in 10ml of the diluents and sonicated for 5 min to dissolve the sample completely. Then it is filtered through 0.2micron meter ultipore filter paper to get a concentration of 1000 μ g/ml. Then take 1ml of the standard solution and make up to 10ml with diluents to get 100 μ g/ml standard solution and further required concentrations were prepared from 100 μ g/ml solution by proper dilution.

2.4 Preparation of mobile phase

A mixture of Methanol, Water and in the ratio of 70:30(v/v) was measured accurately. The solution was sonicated till the solvents mixed completely. Then it was filtered through 0.45 μ m nylon membrane filter paper using vacuum filtration. The final filtrate solution was used as a mobile phase for the estimation of Itopride.

2.5 Preparation of sample solution:

10 tablets from each of the brand selected for assay estimation of Itopride was grinded till to get a fine powder and homogenously mixed using a mortar and pestle. From the powder, an amount of the powder equivalent to 10mg of Itopride was weighed and was dissolved in 10ml of Methanol. The solution was sonicated for 10min to complete extraction of drugs in Methanol. The solution was centrifuged at 4000rpm for 10 min; the clear supernatant was collected and was filtered through 0.45 μ m nylon membrane filter paper. From this solution selected concentration of 40 μ g/ml was prepared by proper dilution. Similar procedure was followed for the preparation of remaining branded tablets separately. The prepared solutions were used for the assay of Itopride.

3.0 HPLC Estimation of Itopride:

For the estimation of Itopride in fixed dosage form, modified method of **Rajesh K. Patel et al** was followed. Modification in the reported method was performed as the steps given below.

Hplc method conditions: Rajesh K. Patel et al

PARAMETER	CONDITION
Mobile phase	Methanol: Water 70:30 (v/v/v)
Pump mode	Isocratic
PH	4.8
Diluents	Mobile phase
Column	Zodiac C18 column (250 X 4.6 mm, 5 μ)
Column Temp	Ambient
Wavelength	258nm
Injection Volume	20 μ l
Flow rate	1.0ml/min
Run time	10 minutes
Retention Time	4.3minutes

Table 3.1: Chromatographic conditions for the estimation of Itopride

3.1 Construction of calibration curve:

From the prepared stock solution, a series of calibration standards were prepared at concentrations of 25, 50, 75, 100, 125, & 150 μ g/ml using mobile phase as solvent. Each of these drug solutions (20 μ l) was injected into the column, the peak area and retention times were recorded. The calibration curve for Itopride was constructed by plotting the mean peak area against the drug concentration.

3.2 Assay sample preparation

From the prepared formulation solution, 20 μ l of the sample was injected in to HPLC system, peak area response of the prepared formulation solution was used for the assay of Olmesartan in the prepared solution. % assay of the method was calculated by considering peak area response of the formulation solution and substituting peak area value in the regression equation.

4. Estimation of Itopride using UV method:

4.1 Selection of Suitable wavelength

From the stock solution, working standard solution of drugs was prepared by appropriate dilution and was scanned from 400nm to 200nm using diluent as blank. The standard drug solution was scanned. The first derivative spectrum was calculated using software. In first derivative spectra Itopride shows specific high absorbance at 258nm.

4.2 Construction of calibration curve:

The calibration curves were plotted over a concentration range of 5-30µg/ml and the absorbance of the solutions was measured in the wavelength of 258nm. Linear regression equation was found to be $y = 0.019x + 0.254$ ($R^2 = 0.997$) for Itopride.

4.3 Formulation analysis:

The solution prepared from the formulation tablet was used for the application of the developed method for the analysis of Itopride in formulations. The prepared 20µg/ml of Itopride was analyzed in UV region. The obtained scanning spectrum was calculated for first derivative formula. The absorbance at 258nm (standard wavelength) was substituted in calibration curve equation and the % assay was calculated.

5. Results and Discussion

Calibration curve was obtained with in a concentration range of 50-100µg/ml. regression equation was found to be $y = 25245x - 572413$ with correlation of 0.9991. Linearity results were presented in Table 2 and calibration curve was shown in Figure 2. Chromatogram of standard was given in figure 3 and formulation was 4, 5 and 6 for Retune, Gimate and Itcan brands respectively.

Level	HPLC Method		UV Method	
	Concentration	Peak area	Concentration	Absorbance
Level – 1	25µg/ml	134719.4	5 µg/ml	0.339
Level – 2	50µg/ml	407836.7	10 µg/ml	0.451
Level – 3	75µg/ml	651578	15 µg/ml	0.542
Level – 4	100µg/ml	893484.1	20 µg/ml	0.647
Level – 5	125µg/ml	1149547	25 µg/ml	0.718
Level – 6	150µg/ml	1444392	30 µg/ml	0.822
	Slope :10303.31 Intercept :-121280 Correlation coefficient: 0.999		Slope :y = 0.019x Intercept :- 0.254 Correlation coefficient: 0.997	

Table 2: Linearity results for UV and HPLC methods.

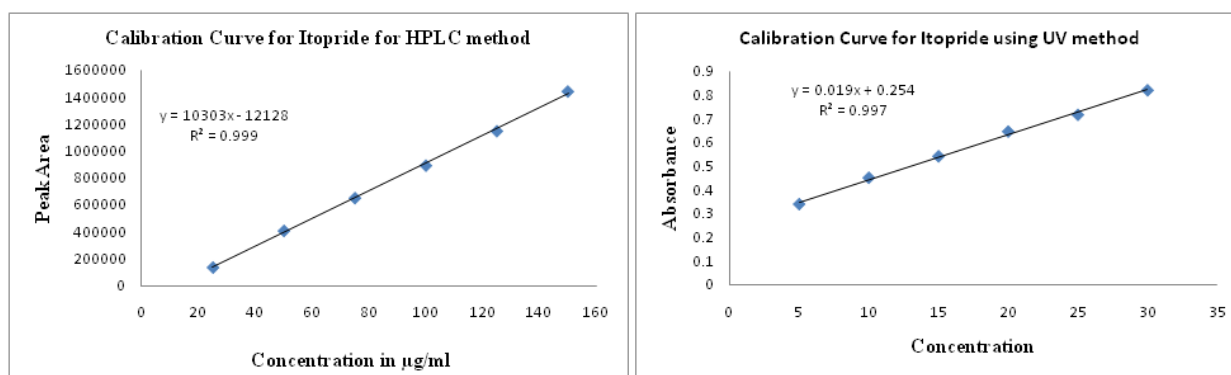


Figure 2: Calibration Curve for Itopride in HPLC and UV method

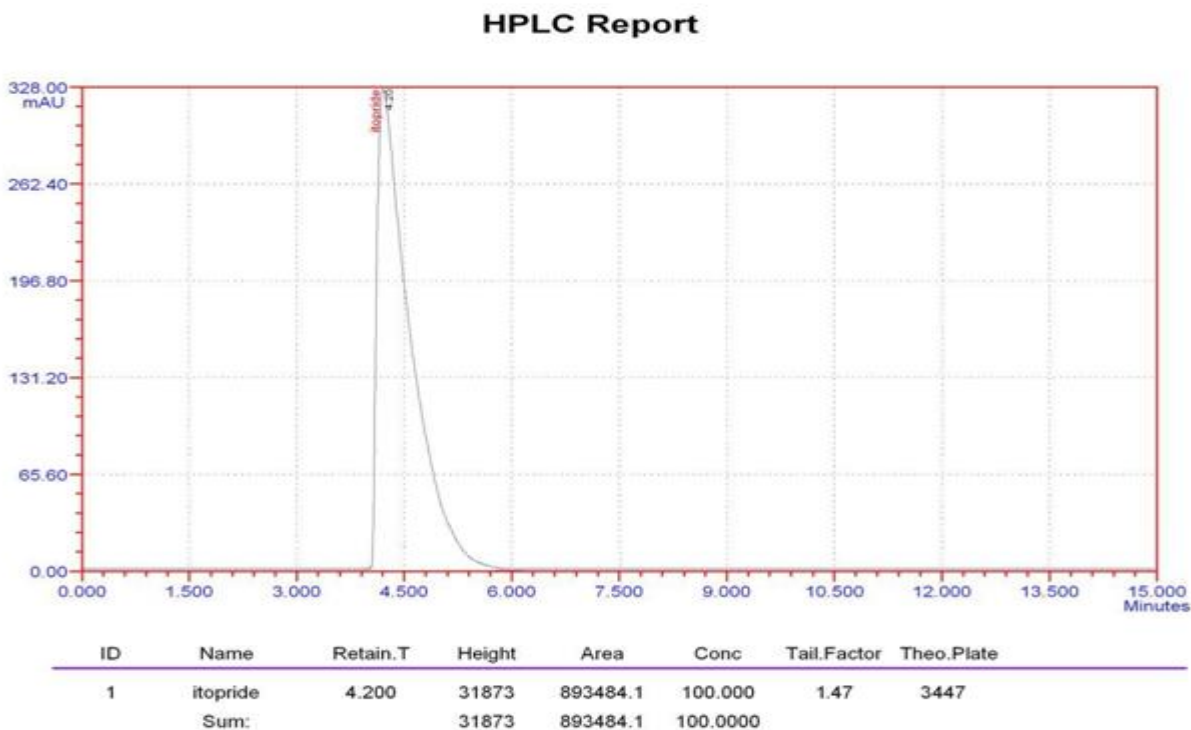


Figure 3: Standard chromatogram for Itopride

4.3 Formulation analysis:

The % assay was found to be more than 98% was found in all the brands under study by the given method. High amount of the drug was estimated in Itcan brand (i.e. 99.84% in HPLC 98.7% in UV method) and low amount of drug was estimated in Retune (i.e. 98.49% in HPLC; 97.8 in UV). Formulation results were shown in table 3.4. Chromatogram was shown in figure 3.9

Method	Drug	Brand	Dosage	Amount Prepared	% Assay
HPLC	Itopride	Retune	50mg	50µg/ml	98.49
	Itopride	Gimate	50mg	50µg/ml	98.94
	Itopride	Itcan	150mg	50µg/ml	99.84
UV	Itopride	Retune	50mg	50µg/ml	97.8
	Itopride	Gimate	50mg	50µg/ml	98.3
	Itopride	Itcan	150mg	50µg/ml	98.7

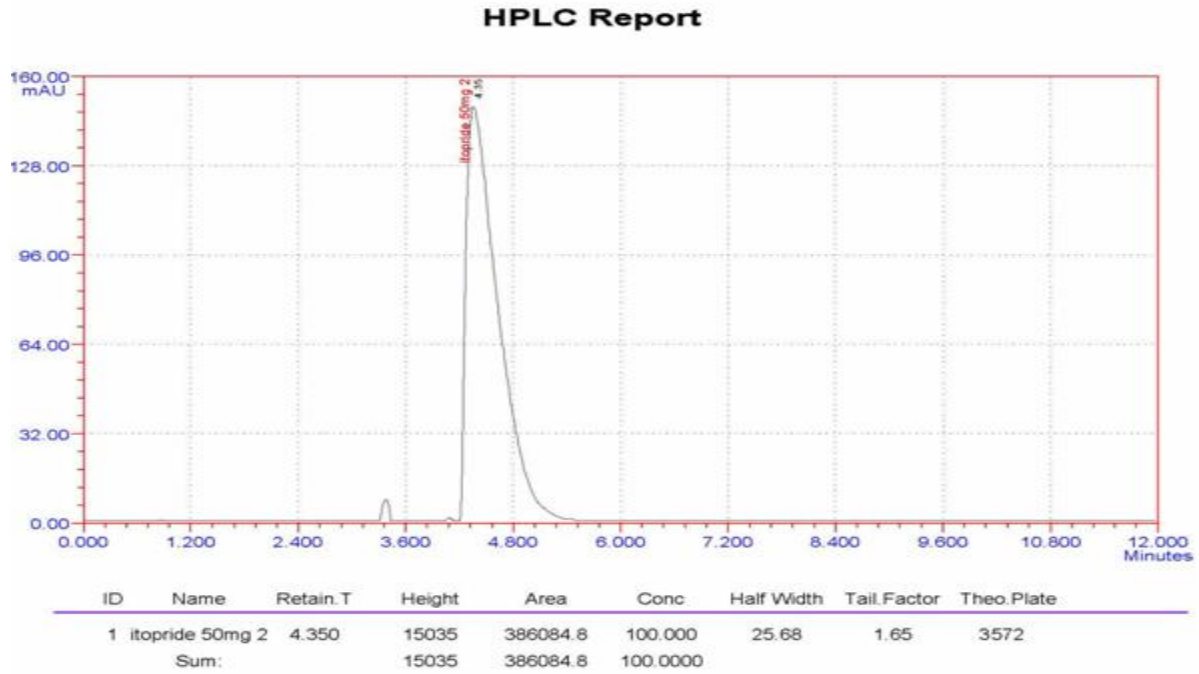


Figure 4: Formulation chromatogram of Retune Brand

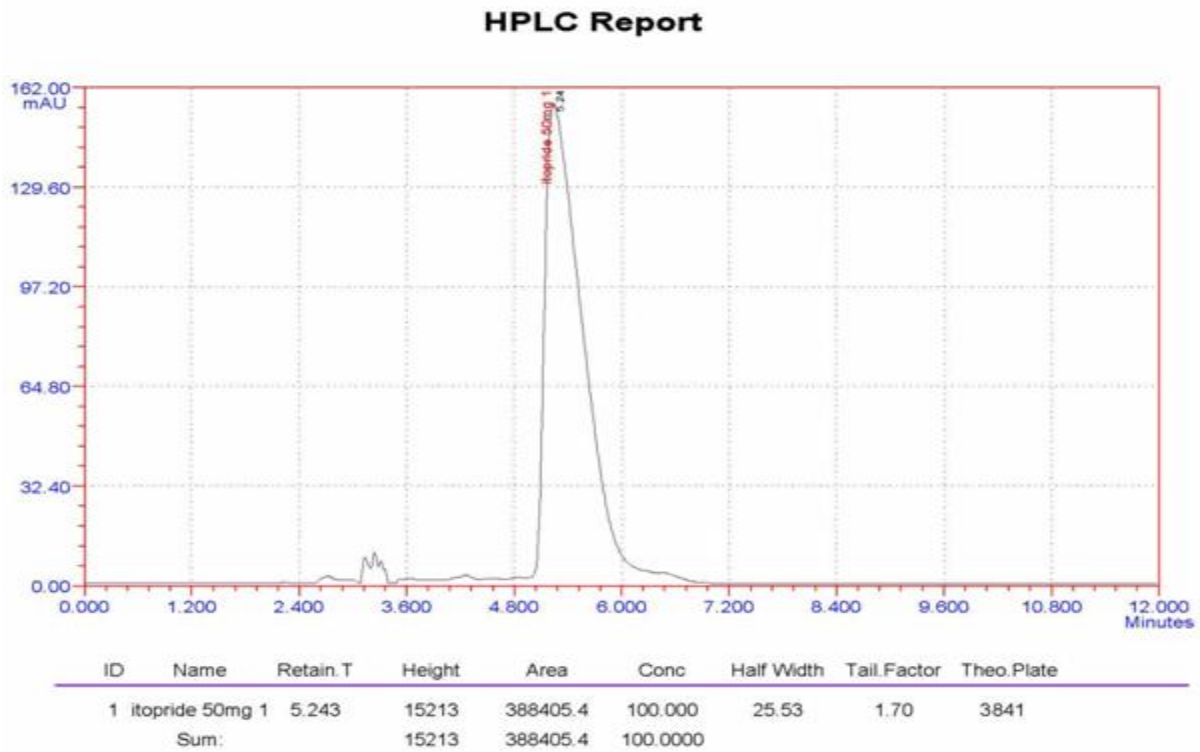


Figure 5: Formulation chromatogram of Gimate Brand

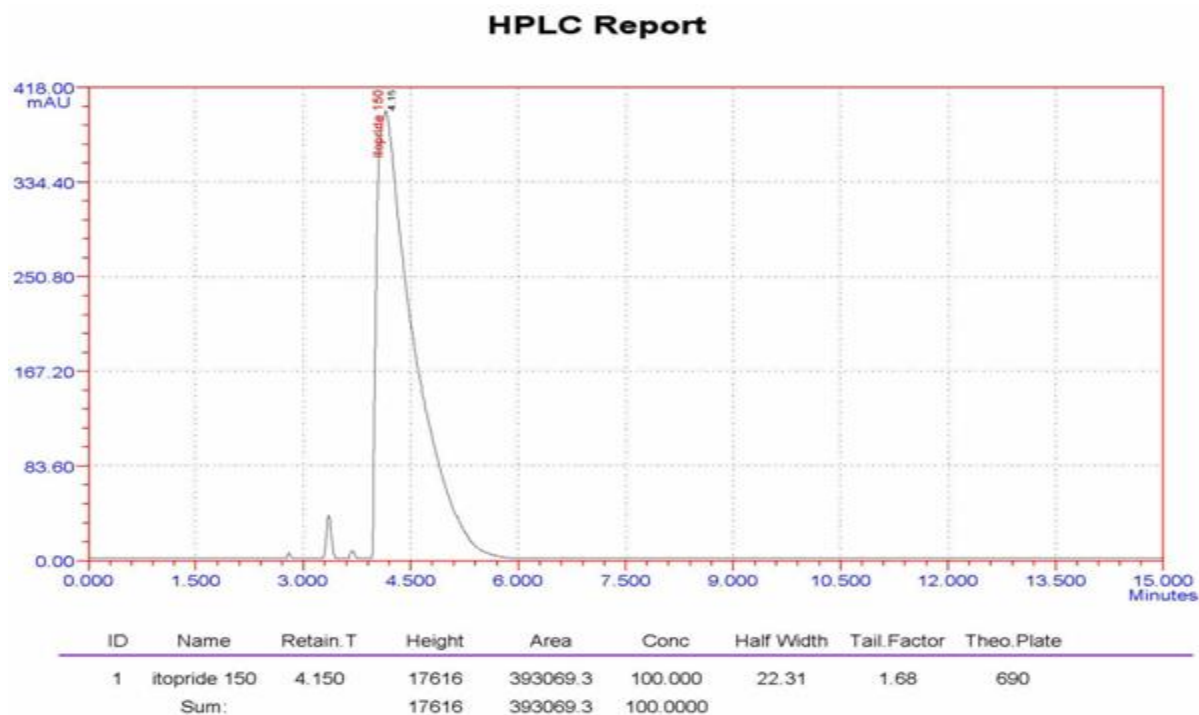


Figure 6: Formulation chromatogram of Itcan Brand

6 Conclusion:

All the three brands containing Itopride in the present study show more than 98% assay in selected HPLC and UV method. High amount of the drug was estimated in Itcan brand and low amount of drug was estimated in Retune. The method does not detect any pharmaceutical excipients used in the formulation, hence the no impurities or extra peaks detected in the formulation chromatogram. Hence Rajesh K. Patel et al HPLC method was successfully applied for the estimation of Itopride in pharmaceutical formulation. UV spectrophotometric method also found to estimate drug more than 98%.

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