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DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CINITAPRIDE AND PANTOPRAZOLE IN PHARMACEUTICAL DOSAGE FORM

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Abstract

A simple, selective, accurate high Performance Liquid Chromatographic (HPLC) method was developed and validated for the analysis of Cinitapride and Pantoprazole. Chromatographic separation achieved isocratically on a C18 column [Use Inertsil C18, 5µ , 250 mm x 4.6 mm] utilizing a mobile phase of acetonitrile: phosphate buffer (80:20 v/v, pH 6.8) at a flow rate of 1.0 ml/min with UV detection at 278 nm. The retention time of Cinitapride and Pantoprazole was 3.18 min and 4.725 min respectively. The method is accurate (98.22-101.66% and 98.5-101.40% for cinitapride and pantoprazole respectively), precise (method precision 0.44% and intermediate precision 0.78%) and linear within range 1.5-12µg/ml and 20-160 µg/ml for cinitapride and pantoprazole respectively. The correlation coefficient was found to be r^2=0.9991 for both the drugs. The detection limit of Cinitapride and Pantoprazole was 0.064 µg/ml and 0.78 µg/ml while quantification limit was 0.205 µg/ml and 2.38 µg/ml respectively. The proposed method is applicable to routine analysis for simultaneous estimation of Cinitapride and Pantoprazole in pharmaceutical dosage form.

Keywords: RP-HPLC, Validation, Cinitapride, Pantoprazole.

Introduction

Cinitapride, chemically 4-amino-*N*-[3-(Cyclohexan-1-yl-methyl)-4-piperidinyl]-2-ethoxy-5- Nitrobenzamide[1]. It has an empirical formula $C_{21}H_{30}N_4O_4$ and molecular weight 402.49 $g.mol^{-1}$. Cinitapride is a drug that has against action to the serotoninergic 5-HT2 and D2 dopaminergic receptors that has been indicated in the gastroesophageal reflux and in the functional disorders of gastrointestinal motility treatment. The use of cinitapride is efficient and safe in treatment of patients with disorders in the gastric emptiness related to gastroesophageal reflux and

functional dyspepsia as well as in individuals that present irritable bowel syndrome with constipation and abdominal pain[2,3]. Pantoprazole sodium is chemically Sodium 5-(difluoro methoxy)-2-[[(3,4-dimethoxy-2-pyridinyl) methyl] sulfinyl]-1H-benzimidazole sesquihydrate[4]. It has an empirical formula of $C_{16}H_{15}F_2N_3O_4S$ and molecular weight of 383.37 g mol^{-1}. The combination of Cinitapride (3mg) and pantoprazole (40mg) is widely used to treat the patients suffering from non-ulcer dyspepsia or gastroesophageal reflux disease. It is also used for treating ulcers of the stomach and duodenum, and the Zollinger-Ellison Syndrome.

The review of the literature revealed that no method is yet reported for the simultaneous estimation of both the drugs in combined dosage forms but few methods for individual determination of cinitapride and pantoprazole was reported[5,6,7]. These includes determination of free levels of drug in human plasma by Liquid Chromatography - Tendem Mass Spectrometry, Simple extractive colorimetric, RP-HPLC in human plasma[8,9,10] . To date, there have been no published reports about the simultaneous estimation of cinitapride and pantoprazole by HPLC pharmaceutical dosage forms. This present study reports for the first time simultaneous estimation of cinitapride and pantoprazole by HPLC in pharmaceutical dosage forms. The proposed method is validated as per ICH guidelines.

Materials and Methods

Material Used

- Cinitapride Hydrogen Tarterate Active Pharmaceutical Ingredient (API) – Cadila Pharmaceutical Limited, Ahmedabad.
- Pantoprazole Sesquihydrate, Active Pharmaceutical Ingredient (API) – Merril Pharmaceutical Ltd.
- Acetonitrile (HPLC grade) - Finar chemicals limited, Ahmedabad India.
- HPLC grade water- Purified with a Millipore system (Millipore Corp., Bangalore, India).
- KH_2PO_4 (HPLC grade): Finar chemicals limited, Ahmedabad India
- O- Phosphoric acid (H_3PO_4): Finar chemicals limited, Ahmedabad India
- Triethyl amine: Finar chemicals limited, Ahmedabad India
- Market preparation: Capsule of Cintodac (Alidac Corza) was procured from the local market, which contains Cinitapride 3 mg and Pantoprazole 40 mg.

Instrument

High Performance Liquid Chromatography (HPLC)

- Model: LC-2010 CHT
- Manufacturer: Shimadzu Corporation 2000, JAPAN.
- Column: Phenomanax C18
- Particle size: 5 µm, 12 A^o Pore size
- Length: 250 mm
- Diameter: 4.6 mm
- Detector: Deuterium
- Software : Class VP

Analytical Balance

- Model: Sartorius CP 124 s
- Capacity: 0.1- 100 gm.

Sonicator

- Model: FAST CLEAN
- Manufacturer: Toshniwal Process Instruments Pvt. Ltd., Ajmer.
- Ultrasonic Cleaner

pH Meter

- Model : Electroquip
- Manufacturer: EI Products, Parwanoo, H.P., India

Experimental

Preparation of mobile phase:

Mobile phase was prepared by mixing 800 ml of acetonitrile with 200 ml of phosphate buffer and its pH adjusted to 6.8. The mobile phase was sonicated for 15 min and then it was filtered through a 0.45 µ membrane filter paper.

Preparation of standard solutions:

The standard stock solution was prepared by transferring 3 mg of Cinitapride and 40 mg of Pantoprazole in 100 ml volumetric flask. Then working solution of 1.5, 3, 6, 9 and 12 µg/ml for cinitapride and 20, 40, 80, 120 and 160 µg/ml for pantoprazole were prepared and recorded.

Preparation of test solution

Accurately 20 intact capsules were weighed to determine average weight of capsule. Then capsule were finely crushed and powder equivalent to 3 mg Cinitapride and 40 mg Pantoprazole was transferred into 100 ml volumetric flask. Then 50 ml diluent was added to flask and sonicated for 40 minute with intermittent shaking.

Make up volume up to 100 ml. From that solution take 5 ml of Sample Solution was used and made up volume up to 50 ml using 50 ml volumetric flask using Diluent. Than solution was filtered through 0.45 µ PVDF millipore filter and the final concentration of test sample solution had concentration of Cinitapride and Pantoprazole 3 µg/ml and 40 µg/ml respectively. The spectra was shown in Figure 1.

Result and Discussion [11]

System suitability

The HPLC system was equilibrated with the initial mobile phase composition, followed by 10 injections of the same standard. These 10 consecutive injections were used to evaluate the system suitability on each day of method validation. The system suitability parameters including capacity factor >2, asymmetric factor<2, and Theoretical plates of the column were >3000.

Table-1: System Suitability Parameter.

Sr. No	Parameter(n= 5)	Cinitapride	Pantoprazole
1	Retention time (min)	3.18	4.71
2	Theoretical Plates	3171.35	3329.28
3	Asymmetry	1.06	1.2
4	Capacity Factor	8	10

Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to true value. In case of the assay of a drug in a formulated product, accuracy may be determined by application of the analytical method to the drug product components to which known amount of analyte has been added within the range of method. If it is not possible to obtain samples of all drug product components, it may be acceptable to add known quantities of the analyte to the drug product. In our studies, the later technique was adopted and cinitapride and pantoprazole was spiked in drug product. The result of % recovery was shown in (Table 2).

Precision

Precision is the degree of reproducibility or repeatability of the analytical method under normal operating conditions. The method passed the test for repeatability as determined by %RSD of the area of the peaks of six replicate injections at 100% test concentration. The results of intra-and inter-day variation are shown in (Table 2).

Table-2: Summary of Validation Parameter.

Sr. No	Parameter	Cinitapride	Pantoprazole
1	**λmax (nm)**	278	278
2	**Linearity range** **Correlation Coefficient**	1.5-12µg/ml $r^2 = 0.9991$	20-160µg/ml $r^2 = 0.9991$
3	**Regression equation**	Y = 51296x – 19985	Y = 0.42880x + 102918
4	**Accuracy**	98.22 –101.66 %	98 – 99.8%
5	**Precision**	0.05–0.40 %	0.10–0.20 %
6	**Specificity**	99.33 ± 0.11%	99.75 ± 0.35%
7	**LOD**	0.064 µg/ml	0.78 µg/ml
8	**LOQ**	0.205 µg/ml	2.38 µg/ml
9	**% Assay**	101.66 ± 0.81	101.15 ± 0.75

Range and Linearity

The linearity of an analytical method is its ability to elict test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. The linearity of the method was observed within the expected concentration range demonstrating its suitability for analysis are shown in (Table 2). The correlation coefficient (r2) was found to be 0.999.

Limits of Detection and Quantitation

The detection limit (LOD) is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. It may be expressed as a concentration that gives a signal-to-noise ratio > 2 or 3. Limit of Quantitation (LOQ) is the lowest amount analyte in a sample that can be determined

with acceptable precision and accuracy under the stated experimental conditions. A signal-to-noise ratio of > 10 can be taken as LOQ of the method are shown in (Table 2).

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present in the sample matrix. For demonstrating the specificity of the method for drug formulation the drug was spiked and the representative chromatogram (Figure-1). The excipiants used in different formulation products did not interfere with the drug peak and thus, the method is specific for cinitapride and pantoprazole.

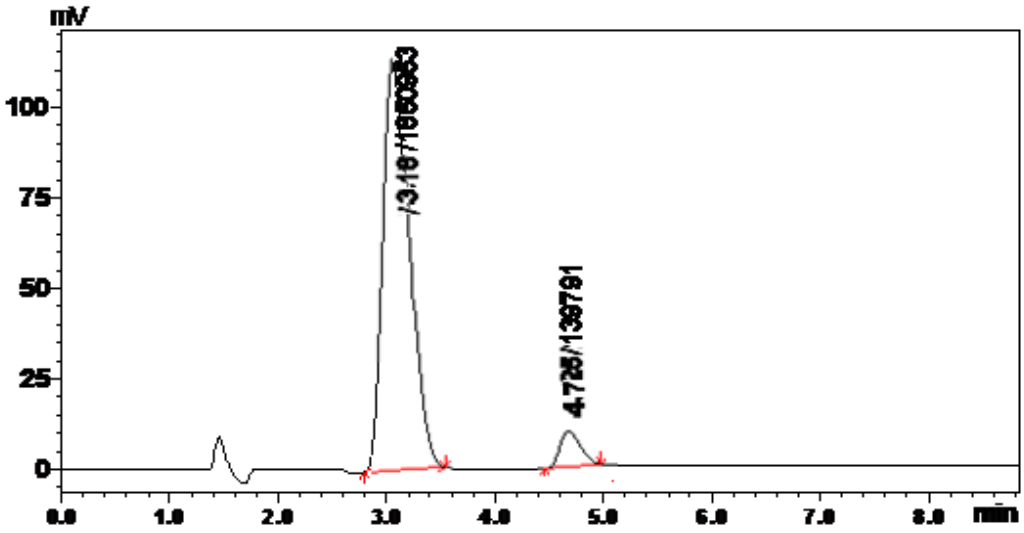

Figure 1: Spectra of marketed formulation.

Conclusion

The developed HPLC technique is precise, specific and accurate. Statistical analysis proves that the method is suitable for the analysis of cinitapride and pantoprazole in pharmaceutical formulation without any interference from the excipients. The proposed HPLC method is less expensive, simpler, rapid, and more flexible.

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