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A NEW HPLC METHOD FOR THE QUANTIFICATION OF PANTOPRAZOLE IN PHARMACEUTICALS

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ABSTRACT

A new analytical method is developed and appropriately validated by means of a high performance liquid chromatography (HPLC) in reverse phase to quantify pantoprazole in pharmaceutical preparations. The HPLC determination was performed on a reversed phase column (Inertsil ODS 5µm; 150 x 4.6 mm i.d) using a mobile phase (0.6 mL min^{-1}) with UV-detection at 289 nm. A rectilinear relationship between mean peak area and concentration of PNT was observed in the range 25-200 µg mL^{-1} with a detection limit of 6.0 µg mL^{-1} and a quantitation limit of 20.0 µg mL^{-1}. Intra-day and inter-day precision, and accuracy of the methods have been established according to the current ICH guidelines. The results were statistically compared with those of the reference method by applying Student's t-test and F-test. Accuracy, evaluated by means of the spike recovery method was in the range 97.7-102.2%, with precision (RSD) better than 2%.

Key words: Pantoprazole, Quantification, HPLC, Pharmaceuticals

INTRODUCTION

Pantoprazole sodium sesquihydrate (PNT) is chemically known as sodium 5-(difluoromethoxy)-2-[(3,4-dimethoxy-2-p-methyl]sulfinyl]-1H-benzimidazole sesqui hydrate[1]. Pantoprazole inhibits H^+ K^+ AT Pase pump function; thereby, healing the acid related conditions. PNT is chemically more stable than omeprazole and lansoprazole in neutral to mildly acidic conditions, but under strongly acidic medium, active species is formed. PNT like omeprazole and lansoprazole also has a role in the eradication of Helicobacter Pylori[2].

The literature survey reveals that only few methods are available for the determination of PNT in dosage forms in HPLC.

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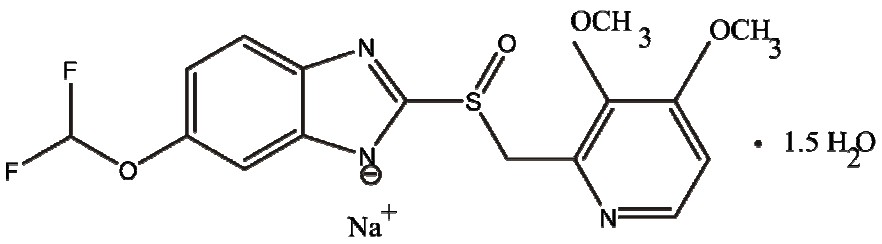

Structure of pantoprazole

Ding et al[3]. have reported chiral HPLC method for the determination of PNT. In their method PNT was determined on Chiral column (5 µm, 150 mm x 4.6 mm) with methanol : water (35 : 65, v/v) as mobile phase. The flow rate was 0.6 mL min^{-1}. Enantiomeric separation of PNT was also reported by Ding et al[4]. They have used hexane-isopropanol-acetic acid mixture (95 : 5 : 0.1, v/v) as the mobile phase at a flow rate of 2.0 mL min^{-1} at 25°C. Xue-Hui et al.[5] have reported determination of PNT in capsule by HPLC on a C18 column with mobile phase of MeCN-phosphate buffer (35 : 65), and detection at 288 nm. The reported linearity is in the range of 20-60 µg mL^{-1}. The other methods available includes HPTLC[6], UV spectrophotometry[7], chemometry[8] and visible spectrometry[9,10].

This paper deals with the development and validation of a sensitive method for the assay of PNT in pharmaceuticals, based on HPLC technique. The separation and determination were done on a reversed phase Inertsil ODS column with UV-detection at 289 nm. The method was demonstrated to be both; accurate and precise, which qualify it to be adopted for routine use in pharmaceutical quality control laboratories.

EXPERIMENTAL

Apparatus

The chromatographic system consisted of an Agilent 1100 series chromatograph equipped with an in built solvent degasser, quaternary pump, photo diode array detector with variable injector and auto sampler, and a reversed phase 5 µm Inertsil ODS column (150 x 4.6 mm, i.d.).

Reagents and standards

All chemicals used were of analytical reagent grade and HPLC grade acetonitrile (Merck. Ltd, Mumbai) was used. Distilled water filtered through 0.45 µm filter (Millipore) was used to prepare solutions.

The mobile phase A was prepared by dissolving 6.8 g of KH_2PO_4 and 1 g of 1-hexane sulphonic acid in 1000 mL distilled water and it was adjusted to pH 7.3 with 1M NaOH. Acetonitrile was used as mobile phase B.

The mobile phase used was prepared by mixing mobile phase A and mobile phase B in the ratio, 70 : 30. The mobile phase was used as a diluent for the sample preparations.

Pharmaceutical grade PNT, certified to be 99.8 % pure was procured from Cipla India Ltd, Mumbai, India, and was used as received. A stock standard containing 500 µg mL^{-1} PNT solution was prepared by dissolving accurately weighed 25 mg of pure drug in diluent and diluting to 50 mL in a calibrated flask with diluent.

Procedures

Chromatographic conditions

The separation was achieved at temperature of 35°C on the column using the mobile phase at a flow rate of 0.6 mL min^{-1} . The detector wavelength was set at 289 nm with a sensitivity of 0.2 a.u.f.s.

Calibration graph

Working standard solutions equivalent to 25 to 200 µg mL^{-1} PNT were prepared by appropriate dilution of stock standard solution (500 µg mL^{-1}) with the diluent solution. Ten µL aliquot of each solution was injected automatically on to the column in duplicate and the chromatograms were recorded. Calibration graph was prepared by plotting the mean peak area versus concentration of PNT.

The concentration of the unknown was read from the calibration graph or computed from the regression equation derived from the mean peak area-concentration data.

Assay in dosage forms

A quantity of tablet powder equivalent 50 mg of PNT was accurately weighed into a 100 mL calibrated flask, 60 mL of diluent solution added and contents were shaken for 20 min., Then the volume was diluted to the mark and mixed well. A small portion of the extract (say 10 mL) was withdrawn and filtered through 0.2 µm filter to ensure the absence

of particulate matter. The filtered solution was appropriately diluted with the diluent solution for analysis as described already.

RESULTS AND DISCUSSION

Method development

A solution of PNT was injected in duplicate on to the column and was monitored by UV-detection at 289 nm. Mobile phase consisting of mobile phase A : mobile phase B (70 : 30) was used after several preliminary experiments. At a flow rate of 0.6 mL min^{-1} , the retention time was 7.355 min (Fig. 1). Under the described experimental conditions, the peak was well-defined and free from tailing. PNT was determined by measuring the peak area. A plot of mean peak area against concentration (Fig. 2) gave a linear relationship (r = 0.99987, n = 5) over the concentration range 25-200 µg mL^{-1}. Using the regression analysis, the linear equation, Y = 31.3774 + 21.0704 X was obtained, where Y is the mean peak area and X is the concentration in µg mL^{-1}. The limits of detection and quantification calculated as per ICH guidelines[11] were 6.0 and 20.0 µg mL^{-1}, respectively.

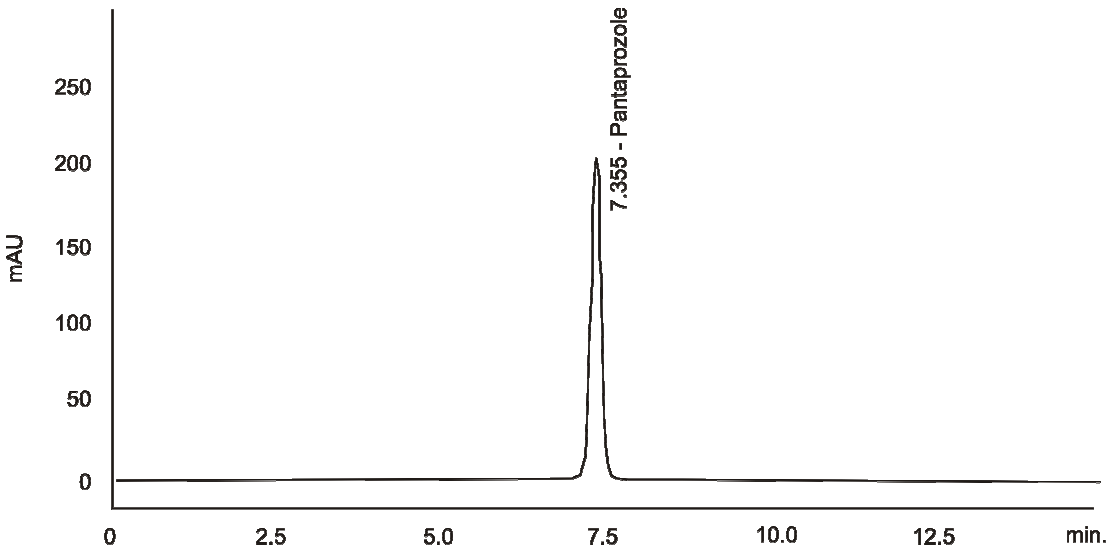

Fig. 1: Typical chromatogram

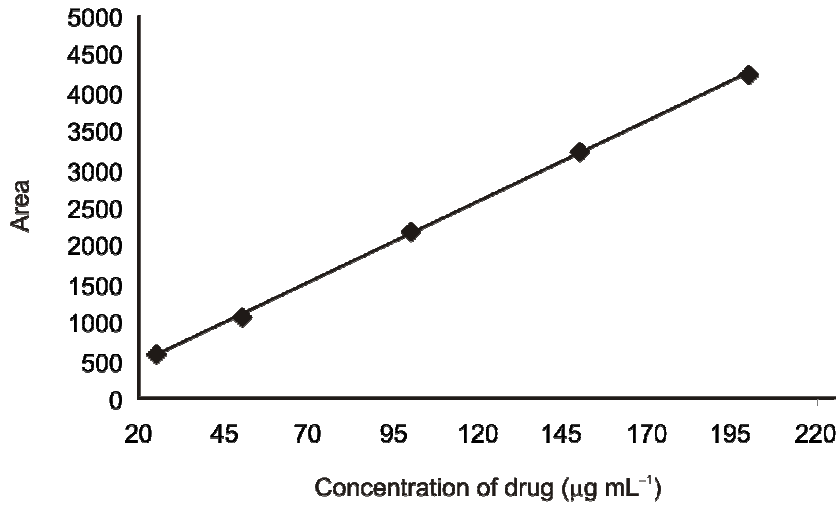

Fig. 2: Linearity curve

Method validation

Accuracy and precision

To determine the accuracy and intra-day precision, pure PNT solutions at three different concentrations were analyzed in seven replicates. The percent relative error, which is an index of accuracy, is < 1.0% and it is indicates good accuracy. The relative standard deviation (< 1%) at the 95% confidence level can be considered to be satisfactory.

Table 1. Accuracy and intra-day precision

PNT taken (µg mL^{-1})	PNT found* (µg mL^{-1})	Range (µg mL^{-1})	RE (%)	RSD* (%)	RSD** (%)
50	49.50	0.70	1.00	0.73	0.21
100	99.82	0.95	0.18	0.23	0.32
150	149.2	1.30	0.53	0.26	0.15

RE. relative error; RSD. Relative standard deviation;

Mean value of seven determinations; Based on peak area; ** Based on retention time

The inter-day precision was established by performing analyses over a period of five days on solutions prepared afresh each day. The peak-area based and retention-time-based RSD values were < 2.0 % and < 1 %, respectively.

Application

The results obtained are presented in Table 2 and compared well with label claim. The results were also compared statistically with those obtained by a reference method[3] by applying Student's t-test for accuracy and F-test for precision. At the 95% confidence level, the calculated t- and F-values did not exceed the tabulated values (t = 2.77 and F = 6.39) suggesting that the proposed methods are as accurate and precise as the reference method.

Table 2. Determination of pantoprazole in formulations and statistical compared with the reference method

Formulation Brand name*	Nominal amount (mg)	% Found* ± SD			
		Reference method	Proposed method	t-value	F-value
PAN[a]	20	101.2 ± 0.62	100.8 ± 1.29	0.66	4.33
PANTOCIP[b]	40	97.3 ± 1.05	98.2 ± 0.95	1.42	1.22
PANTOP[c]	40	101.5 ± 0.85	100.9 ± 1.2	0.92	1.99

*Mean value of seven determinations

**Marketed by: a. Alkem Ltd.; b. Cipla Ltd.; c. Aristo Ltd.

Tabulated t-value at 95% confidence level is 2.77

Tabulated F-value at 95% confidence level is 6.39.

The accuracy and validity of the proposed methods were further ascertained by performing recovery experiments. Preanalysed tablet powder was spiked with pure PNT at three different levels and the total was found by the proposed methods. Each determination was repeated three times. The recovery of pure drug added was quantitative (Table 3) and it revealed that coformulated substances such as talc, starch, gelatin, gum acacia, calcium carbonate, calcium gluconate, calcium dihydrogen orthophosphate, sodium alginate and magnesium stearate did not interfere in the determination.

Table 3. Recovery experiments

Formulation studied	PNT in formulation, (μg mL^{-1})	PNT added, (μg mL^{-1})	Total found (μg mL^{-1})	Pure PNT recovered* (%)
PAN-20	25.2	25	50.15	99.8
	25.2	75	101.85	102.2
	25.2	125	150.58	100.3
PANTOP-40	25.2	25	50.08	99.5
	25.2	75	98.48	97.7
	25.2	125	151.70	101.2

*Mean value of three determinations

CONCLUSIONS

The proposed method is simple, rapid and convenient and it is characterized by shorter retention time and a wide linear dynamic range of concentration, over which, it is applicable. There was no interference from matrix sources.

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