Stability indicating RP-HPLC method for simultaneous determination of pantoprazole sodium and itopride hydrochloride in bulk and capsule

Krishna R. Gupta*, Rajesh B. Chawla and Sudhir G. Wadodkar

Department of Pharmaceutical Chemistry, S. K. B. College of Pharmacy, New Kamptee 441002, Nagpur (MS) India

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ABSTRACT: A stability indicating reversed-phase HPLC method has been developed and subsequently validated for simultaneous estimation of pantoprazole present as pantoprazole sodium sesquihydrate (PSS), and itopride hydrochloride from their combination product. The proposed RP-HPLC method utilizes a Phenomenex® C18, 5 µm, 250 mm × 4.6 mm i.d. column, mobile phase consisting of phosphate buffer and acetonitrile in the proportion of 55:45 (v/v) with apparent pH adjusted to 5.0, and UV detection at 289.0 nm using a UV detector. PAN, ITH and their combination drug product were exposed to thermal, photolytic, hydrolytic and oxidative stress conditions, and the stressed samples were analyzed by the proposed method. The described method was linear over a range of 4–20 µg/mL for PAN and 15–75 µg/mL for ITH. The mean recoveries were 100.02 and 99.88 for PAN and ITH, respectively. Chromatographic peak purity data of PAN and ITH indicated no co-eluting peaks with the main peaks of drugs which demonstrated the specificity of assay method for their estimation in presence of degradation products. The proposed method can be useful in the quality control of combination drug products.

Keywords: stability indicating RP-HPLC; pantoprazole; itopride hydrochloride; UV detector

Introduction

Pantoprazole sodium sesquihydrate (PSS) [1] (Fig. 1) is sodium sesquihydrate salt of pantoprazole (PAN), which is benimidazole proton pump inhibitor. PAN suppresses gastric acid secretion by inhibiting the gastric H+ K+ ATPase at the secretory surface of
the gastric parietal cell and blocks the final step of gastric acid secretion. It is used as anti-ulcerative. Itopride hydrochloride (ITH) [2] (Fig. 2) is a gastroprokinetic agent. It increases the release of acetylcholine (Ach) through dopamine D2 receptor antagonistic action and inhibits decomposing of released Ach through its acetylcholinesterase inhibitory action, resulting in enhancement of gastrointestinal motility. It also exerts antiemetic action because of its dopamine D2 receptor antagonistic action at chemoreceptor trigger zone. Combination drug products of PAN and ITH are widely marketed and used in the treatment of acid related disorders eg peptic ulcer, reflux oesophagitis, as gastroprokinetic agent and antiemetic agent.

![Figure 1. Structure of pantoprazole sodium sesquihydrate](image1)

![Figure 2. Structure of itopride hydrochloride](image2)

Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, enabling recommendation of storage conditions, retests periods, and shelf lives to be established. The two main aspects of drug product that play an important role in shelf life determination are assay of active drug, and degradants generated, during the stability study. The assay of drug product in stability test sample needs to be determined using stability indicating method, as recommended by the International Conference on Harmonization (ICH) guidelines [3] and USP (United States Pharmacopoeia) 26 [4]. Although stability indicating methods have been reported for assay of various drugs in drug products, most of them describe assay procedures for drug products containing only one active drug substance. Only few stability indicating methods are reported for assay of combination drug products containing two or more active drug substances. The objective of this work was to develop an analytical LC procedure, which would serve as
stability indicating assay method for combination drug product of PAN and ITH.

Both the drugs PAN and ITH are not official with USP 30. Detailed survey of literature for PAN revealed several methods based on different techniques, viz. HPLC [5-6], UV spectrophotometry [7-10] for its determination from pharmaceuticals. Similarly, survey of literature for ITH revealed methods based on HPLC [11-13], HPTLC [14], UV spectrophotometry [15] for its determination from pharmaceuticals. Shirore et al. [16] reported an HPLC method for the estimation of PAN in combination with mosapride and Manoj and Anbazhagan [17] reported an HPLC method for the estimation of PAN in combination with domperidone. No stability indicating methods have been reported for determination of PAN and ITH. This manuscript describes the development and subsequent validation of a stability indicating isocratic reversed phase HPLC method for simultaneous determination of PAN and ITH in the presence of their degradants. To establish the stability indicating nature of the method, forced degradation of drug substances and drug product was performed under stress conditions (thermal, photolytic, UV-exposure, acid and basic hydrolytic and oxidative), and stressed samples were analyzed by the proposed method. The proposed LC method was able to separate both drugs from degradants generated during forced degradation studies.

Material and Methods

Chemicals and reagents

PAN and ITH working standards were generous gifts from Relief Lab Pvt. Ltd. (Nagpur, India) and ZIM Lab Ltd. (Nagpur, India), respectively. Combination product of PAN and ITH (Label claim: Pantoprazole 40 mg, as pantoprazole sodium sesquihydrate, and benazepril hydrochloride 150 mg), Itopan and Pantop-IT tablets (Alkem and Unichem Laboratories Ltd.) were purchased from the market. Acetonitrile, methanol, potassium dihydrogen phosphate, potassium hydroxide, sodium hydroxide, hydrochloric acid and hydrogen peroxide were from Qualigens Fine Chemicals (Glaxo Ltd.).

HPLC instrumentation and conditions

The Shimadzu Binary gradient HPLC system consisted of LC 10ATvp/ LC 10ADvp pump, Rheodyne injector and SPD 10 UV detector. The chromatographic separations were performed using Phenomenex C18, 5 μm, 250 mm × 4.6 mm i.d. column, maintained at ambient temperature, eluted with mobile phase at the flow rate of 1.0 mL/min. The mobile phase consisted of 50 mM potassium dihydrogen phosphate buffer–acetonitrile (55:45, v/v), apparent pH adjusted to 5.0 with 0.2 M potassium hydroxide solution, filtered through 0.45 μm nylon filter and degassed in ultrasonic bath prior to use. Measurements were made with injection volume 20 μL and ultraviolet (UV) detection at 289 nm. The output signal was integrated using Spinchrome CFR software (Spincotech
Standard and sample preparation

The mix standard stock solution containing 100 µg/mL and 375 µg/mL of PAN and ITH was prepared by dissolving working standards in sufficient proportion of mobile phase and later diluted to desired volume with mobile phase. Standard calibration solutions of PAN and ITH with concentration in the range of 4-20 µg/mL and 15-75 µg/mL, respectively, were prepared by diluting stock solution with mobile phase.

Analysis of dosage form

Twenty tablets were weighed, their mean weight determined, and crushed in mortar. An amount of powdered mass equivalent to 30 mg of ITH was transferred into a 100 mL volumetric flask containing 20 mL of mobile phase, mechanically shaken for 15 min, ultrasonicated for 5 min, and then diluted to volume with mobile phase (sample stock solution). The extract was filtered through Whatman filter paper No. 41 and residue was washed with mobile phase. About 5 mL of sample stock solution diluted to 50 mL with mobile phase (sample solution). A small portion of sample solution was filtered through 0.45 µm nylon filter and used for injection on HPLC.

Procedure for forced degradation study

Forced degradation of each drug substances and the drug product was carried out under thermolytic, photolytic, acid/base hydrolytic and oxidative stress conditions. Thermal and photo-degradation of drug substances and drug product was carried out in solid state. After the degradation these solutions were diluted with mobile phase to achieve a concentration of 20 µg/mL of PAN and 75 µg/mL of ITH (on label claim basis for marketed formulation).

Acid hydrolysis of drug substance and drug product in solution state was conducted by refluxing in 0.5 N hydrochloric acid for 6 h. Base hydrolysis of drug substance and drug product in solution state was conducted by refluxing in 0.5 N sodium hydroxide solution for 6 h. For oxidative stress, sample solutions of drug substance and drug product in 3% hydrogen peroxide were kept at 50 °C for 24 h.

For thermal stress, samples of drug substances and drug product were placed in a controlled-temperature oven at 60 °C for 24 h. For photolytic stress, samples of drug substances and drug product, both in solid state, were irradiated with UV radiation with peak intensities at 254 nm for 24 h. Photochemical exposure (direct sunlight) for 6 h was studied.

For humidity stress, samples drug substance and product were kept in 75% RH for 24 h.
Results and Discussion

Development of validated stability indicating method

To develop a precise, accurate, specific and suitable stability indicating RP-HPLC method for the simultaneous estimation of PAN and ITH, different mobile phases were employed and proposed chromatographic condition was found appropriate for the quantitative determination in the presence of degradation products. The optimal mobile phase consisted of acetonitrile and phosphate buffer (45:55, v/v), apparent pH adjusted to 5±0.1 with 0.2 M potassium hydroxide solution, which was selected because it was found to ideally resolve the peaks of ITH (tR_2.653 min) and PAN (tR_5.753 min), with clear line separation in presence of their degradation products at effluent flow rate of 1.0 mL/min. UV detection wavelength at 289 nm, injection volume of 20 μL, ambient temperature for column and HPLC system were found to be best for analysis.

HPLC studies on stressed solutions

Pantoprazole degraded to more than four degradation products in most of the stress conditions, except in UV and humidity (75% RH) studies while ITH showed slight degradation in most of the stress conditions. Table 1 indicates the percent degradation of PAN and ITH remained under various stress conditions. The retention times (Rt) and relative retention times (RRt) of the PAN and its degradation products as they appear on the chromatogram are given in Table 2 for various stress conditions. The degradation products and drugs carry the numerical notations in accordance with the sequence in which the peaks appeared from left to right on the HPLC chromatogram. HPLC studies of the stressed samples showed the following degradation behavior:

Table 1. Results of analysis of forced degradation samples by proposed method.

<table>
<thead>
<tr>
<th>Stress condition/duration/state</th>
<th>% Degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ITH</td>
</tr>
<tr>
<td><strong>Alkaline/ 0.5 N NaOH/ 6 h reflux/solution</strong></td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Acidic/ 0.5 N HCl/ 6 h reflux/ solution</strong></td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Oxide/ 3% H₂O₂/24 h/ solution</strong></td>
<td>2.54</td>
</tr>
<tr>
<td><strong>Thermal/60 °C / 24 h/ solid</strong></td>
<td>1.88</td>
</tr>
<tr>
<td><strong>Humidity/ 75% RH/ 24 h/ solid</strong></td>
<td>3.70</td>
</tr>
<tr>
<td><strong>UV/254 nm/24 h/solid</strong></td>
<td>5.84</td>
</tr>
<tr>
<td><strong>Hotochemical (sunlight)/6 h/solid</strong></td>
<td>9.71</td>
</tr>
</tbody>
</table>

Hydrolytic conditions

PAN showed very susceptible behavior towards acid and alkaline stress, showing more than 80% degradation in 0.5 N HCl or 0.5 N NaOH refluxed for 6 h. Pantoprazole
Gupta et al.


degraded to five or six degradation products while itopride was found to be slightly degraded in all the hydrolytic stress conditions as minute degradation peaks was seen.

Table 2. Retention times and relative retention times of various peaks of PAN.

<table>
<thead>
<tr>
<th>Stress condition/Peaks as on chromatogram</th>
<th>Retention time(Rt)</th>
<th>Relative retention time (RRt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alkali</td>
<td>Acid</td>
</tr>
<tr>
<td>1</td>
<td>----</td>
<td>2.853</td>
</tr>
<tr>
<td>2</td>
<td>----</td>
<td>3.307</td>
</tr>
<tr>
<td>3</td>
<td>2.790</td>
<td>4.687</td>
</tr>
<tr>
<td>4</td>
<td>3.327</td>
<td>5.040</td>
</tr>
<tr>
<td>5</td>
<td>4.567</td>
<td>5.437P</td>
</tr>
<tr>
<td>6</td>
<td>5.727P</td>
<td>10.227</td>
</tr>
<tr>
<td>7</td>
<td>7.347</td>
<td>11.360</td>
</tr>
<tr>
<td>8</td>
<td>8.683</td>
<td>14.113</td>
</tr>
</tbody>
</table>

*P indicates Retention time of PAN as in chromatograms

**Oxidative studies**

PAN was unstable under the stress and formed more than four degradation products. Not much change in ITH was observed even on exposure of the drug to 3% $H_2O_2$ for 24 h at 50 °C, showing that it was stable against oxidative stress.

**Photolytic studies**

Under light for 6 h, PAN showed more than 30% degradation, while ITH degraded to a certain extent without prominent degradation seen in the chromatogram.

**Solid state studies**

There was significant degradation of solid PAN on exposure to dry heat at 60 °C for 24 h, which indicated that the drug was not stable against thermal stress. Stable behavior of both drugs was observed on exposure of solid drug to the conditions of light (UV), humidity (75% RH) for 24 h.

Singh and Bakshi, in their article on stress testing [18], suggested a target degradation of 20-80% for the establishing stability indicating nature of the assay method, as even inter-mediate degradation products should not interfere with any stage of drug analysis. Though conditions used for forced degradation were attenuated to achieve degradation in the range of 20-80%, this could not be achieved in some cases even after exposure for prolonged duration. PAN showed extensive degradation in acidic and alkaline hydrolysis, oxide and thermal degradation conditions while ITH was found to be stable in all the stress conditions. Fig. 3a-c to 9a-c shows the chromatograms of
forced degraded samples and drug substances.

**Figure 3.** Chromatogram of alkali hydrolysis-degraded- a) PAN, b) capsule c) ITH.
Figure 4. Chromatogram of acid hydrolysis-degraded- a) PAN, b) capsule c) ITH.
**Figure 5.** Chromatogram of oxide hydrolysis-degraded- a) PAN, b) capsule c) ITH.
Figure 6. Chromatogram of humidity (75% RH)-degraded- a) PAN, b) capsule c) ITH.
Figure 7. Chromatogram of thermal-degraded- a) PAN, b) capsule c) ITH.
Figure 8. Chromatogram of UV-degraded- a) PAN, b) capsule c) ITH.
Method validation

The described method has been validated, apart from specificity, for response function, accuracy, and intermediate precision. The nominal concentrations of standard and test solutions for PAN and ITH were 8 and 30 µg/mL, respectively. Response function was determined by preparing standard solution at five different concentration levels ranging from 4.0 to 20.0 µg/mL for PAN and 15 to 75 µg/mL for ITH. The standard solutions for linearity were prepared five times and slope of regressed line was found to be 1.21 and 0.99% RSD for PAN and ITH, respectively. The correlation coefficients were found to be more than 0.9999 for both the drugs. Repeatability of measurements of peak area was carried out using seven replicates of same concentration (10 and 37.5 µg/mL for PAN and ITH, respectively).

Accuracy of the method was determined by performing the recovery experiment. This experiment was performed at four levels, in which sample stock solutions were spiked with standard drug solution Three replicate samples of each concentration level were prepared and the % recovery at each level and mean % recovery were determined (Table 3a). The mean recovery was 100.02 and 99.88% for PAN and ITH, respectively.
Precision of estimation of PAN and ITH by proposed method was ascertained by replicate analysis of homogeneous samples of capsule powder. Intermediate precision of the method was studied by intra- and inter-day variation of the method was carried out. The low %RSD values of within a day for PAN and ITH revealed that the proposed method is precise (Table 3b). Day to day variations studies showed that ITH was stable even on 15th day while PAN, on 4th day gave rise to prominent degradation product, which increased to a large extent on 15th day as can be seen on the chromatogram recorded on 15th day as shown in Fig. 10a-d.

![Chromatogram showing degradation product of PAN on a) day 1, b) day 4, c) day 7, d) day 15.](image)

**Figure 10.** Chromatogram showing degradation product of PAN on a) day 1, b) day 4, c) day 7, d) day 15.

A batch of tablets was analyzed by two different analysts on different days using proposed method, %RSD values were found to be less than 1% indicating ruggedness of the method.

**Conclusion**

Based on the results obtained from the analysis of forced degraded samples using
described method, it can be concluded that there is no other co-eluting peak with the main peaks and the method is specific for the estimation of PAN and ITH in presence of degradation products and impurities. The method has linear response in stated range and is accurate and precise. Though no attempt was made to identify the degradation products, described method can be used as stability indicating method for assay of PAN and ITH in their combination drug product. The proposed method can also be conveniently adopted for dissolution testing of tablets containing PAN and ITH.

**Table 3.** Method Validation data for PAN and ITH.

<table>
<thead>
<tr>
<th>Level</th>
<th>PAN Taken (mg)</th>
<th>PAN Recovered (mg)</th>
<th>(%) recovery</th>
<th>ITH Taken (mg)</th>
<th>ITH Recovered (mg)</th>
<th>(%) recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>3.8</td>
<td>3.775</td>
<td>99.34</td>
<td>15.0</td>
<td>15.142</td>
<td>100.94</td>
</tr>
<tr>
<td>Level 2</td>
<td>5.7</td>
<td>5.698</td>
<td>99.96</td>
<td>22.5</td>
<td>22.450</td>
<td>99.77</td>
</tr>
<tr>
<td>Level 3</td>
<td>7.6</td>
<td>7.641</td>
<td>100.54</td>
<td>30.0</td>
<td>29.862</td>
<td>99.54</td>
</tr>
<tr>
<td>Level 4</td>
<td>9.5</td>
<td>9.523</td>
<td>100.24</td>
<td>37.5</td>
<td>37.229</td>
<td>99.28</td>
</tr>
</tbody>
</table>

Mean % recovery: 100.02

% RSD: 0.73 99.88

**References and Notes**