Simultaneous, Stability Indicating Method Development and Validation for Related Compounds of Ibuprofen and Paracetamol Tablets by RP-HPLC Method

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Abstract
A simple, precise, accurate, simultaneous and stability-indicating RPLC method developed with an effective resolution for active pharmaceutical ingredients and marketed drug products. This method effectively separate all the related substances of Ibuprofen and Paracetamol along with impurities. This method is using in the estimation assay of Ibuprofen and paracetamol in drug substance also. The method was developed using RP18 Embedded polar phase column. A mobile phase used in this method was a mixture of acetonitrile and 0.1% v/v orthophosphoric acid in 55:45 v/v ratio. At 230 nm compounds will eluted and monitored. Ibuprofen and Paracetamol was subjected to the stress conditions of acid, base, oxidative, thermal and photolytic degradation. The degradation products were well resolved from main peak and its impurities, proving the stability-indicating ability of the method. The developed method was validated as per USP and International Conference on Harmonization (ICH) guidelines. The current method has proven good linearity and accuracy over the range of all known impurities from LOQ to 150% of the target concentration. The degree of reproducibility as results obtained by deliberate changes in the method parameter and variety of condition has proven the method is robust and rugged.

Keywords: HPLC; Ibuprofen and Paracetamol; Validation; Reverse phase mode; Stability-indicating

Introduction
Ibuprofen is (±) - 2 - (p - isobutylphenyl) propionic acid [1] (Figure 1). It is a white powder and it is having a melting point of 74-77°C. It is very slightly soluble in < 1 mg/mL water and readily soluble in organic solvents like ethanol and acetone. It is under classification of nonsteroidal anti-inflammatory drug (NSAID) used for relief of symptoms of arthritis, fever as an analgesic (pain reliever), especially where there is an inflammatory component, and dysmenorrhea [2]. Nonsteroidal anti-inflammatory drugs such as ibuprofen work by inhibiting the enzyme cyclooxygenase (COX), which converts arachidonic acid to prostaglandin H2 (PGH2). PGH2, in turn, is converted by other enzymes to several other prostaglandins (which are mediators of pain, inflammation, and fever) and to thromboxane A2 (which stimulates platelet aggregation, leading to the formation of blood clots) [3].

Paracetamol or Acetaminophen [4] is used as pain reliever and antipyretic fever reducer. It is commonly used for the relief of headaches and other minor aches and pains and is a major ingredient in numerous cold and flu remedies [5] (Figure 2). The main mechanism proposed is the inhibition of cyclooxygenase (COX) and it is highly selective for COX-2. It is having Analgesic and antipyretic properties like NSAIDS. If inflammatory lesions have high level of peroxides that paracetamol shows limited anto inflammatory property [6].

There are plenty of validated simultaneous analytical methods available for combinational dosage form of Ibuprofen and paracetamol assay method. But there is not much literature available for simultaneous related substance for combinational dosage form. In USP, related substance method available for Ibuprofen, Paracetamol and its various formulations for single conventional dosage form not for combination [1,4]. RP-HPLC assay method for Paracetamol and Ibuprofen tablets [7]. RP-HPLC assay method for Paracetamol, Ibuprofen and chloroxazone tablets [8]. Ibuprofen and Paracetamol determination by HPLC in soft capsule [9]. Paracetamol and its process related substance determination and rapid separation by using RP-

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HPLC with PDA detector [10]. Ibuprofen degradants was studied by oxidative and thermal treatments [11]. There are many studies available but there is no literature available simultaneous stability indicating method for the combined formulation. So the current work presents simultaneous stability indicating reverse phase HPLC method with PDA detection. Validation of the method was performed according to the requirements of United States Pharmacopeia and ICH guidelines for related substance determination, which includes accuracy, precision (repeatability and intermediate precision, ruggedness), selectivity, robustness, linearity and range. Additionally, in order to meet the regulatory guidance of the Federal Drug Administration/Inter-national Conference on Harmonization (ICH) [12-15] stability of mobile phase was established, standard, resolution solution and sample solution was injected freshly prepared solution hence instability of impurity solution. Degradation study was performed for active pharmaceutical ingredient, placebo and drug products in acid, base, peroxide, thermal, humidity, and water including photo stability.

Experimental

Chemicals/Standards/Impurities

Acetonitrile HPLC grade was from Merck. Orthophosphoric acid, hydrochloric acid, sodium hydroxide, and hydrogen peroxide were from Merck. Standards and impurities include Ibuprofen, Ibuprofen Impurity-B, Paracetamol, 4-aminophenol and 4-chloroacetanilide. All impurities procured from Sigma-Aldrich grade.

Apparatus

HPLC system (Waters system, USA) with a detector (PDA-G06296), equipped with a quaternary pump, auto sampler (F06SM4854A), column compartment (H03SMH 052 M) and empower software was employed during this study. The analysis was performed with 55 volumes 0.1% orthophosphoric acid and 45 volumes of acetonitrile in isocratic mode at the flow rate of 1.0 mL/minutes through RP18 embedded polar stationary phase, 20 μL for about 60 minutes with the support of UV/PDA detection at 230 nm afforded the best separation of paracetamol, ibuprofen and its impurities.

Method development

After method development, validation of the current test method for ibuprofen and paracetamol tablets was performed in accordance with United States Pharmacopeia requirements/ICH guidelines for related substance method the parameter includes precision, accuracy, linearity, LOD and LOQ, precision and accuracy at LOQ level, specificity, selectivity includes blank, placebo, known impurity interference and interference of degradants by degradation study. Robustness and ruggedness was also performed.

System suitability

20 μL of standard solution six times injected into HPLC and recorded the chromatogram, % RSD of paracetamol, ibuprofen and its impurities peaks area was with in the limit of 5.0% and resolution between paracetamol and 4-aminophenol, ibuprofen and ibuprofen impurity-B was not more than 2.0 for the entire activity.

Precision

To evaluate precision 0.2% of impurity blend has been spiked in the sample preparation and analyzed and recorded the chromatogram, calculated percent RSD for the percent impurity of each individual impurity. The percentage impurity was found for 4-amino phenol, 4-chloro acetanilide, ibuprofen impurity-B and ibuprofen impurity-J is 0.195 to 0.207, 0.191 to 0.220, 0.190 to 0.215 and 0.198 to 0.219 and percent RSD of six replicate sample preparations was found 2.4, 4.9, 4.9 and 4.1 respectively. The results summarized in table 1 and blank, placebo, standard solution and impurity spiked sample preparation chromatogram shown in figures 3-6.

Intermediate precision

To evaluate intermediate precision, precision experiment was done for 6 replicates with a standard concentration of 20 μg/mL.

<table>
<thead>
<tr>
<th>Paracetamol</th>
<th>Ibuprofen</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Amino phenol</td>
<td>4-Chloro acetanilide</td>
</tr>
<tr>
<td>0.202</td>
<td>0.212</td>
</tr>
<tr>
<td>0.205</td>
<td>0.204</td>
</tr>
<tr>
<td>0.195</td>
<td>0.220</td>
</tr>
<tr>
<td>0.204</td>
<td>0.199</td>
</tr>
<tr>
<td>0.196</td>
<td>0.191</td>
</tr>
<tr>
<td>0.207</td>
<td>0.204</td>
</tr>
<tr>
<td>% RSD</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Table 1: Precision.

Results and Discussion

Method development

Preliminary studies involved trying C8, C18, C18 embedded phase and Zorbax reversed-phase columns and testing some mobile phase compositions were conducted for the separation of paracetamol and ibuprofen with good resolution of its impurities. A RP18 column (5 μm, 250 mm, 4.6 mm id.) as a stationary phase with a mobile phase of acetonitrile/0.1%/v/v orthophosphoric acid (45:55 v/v) at the flow of 1.0 mL/min and a detection wavelength of 230 nm afforded the best separation of paracetamol, ibuprofen and its impurities.

Figure 3: Typical chromatogram of Blank.
for ibuprofen, paracetamol and all the impurities. A plot of peak areas versus concentration was linear in the range from 0.05 to 0.75 μg/mL. The correlation coefficient (R) of 4-aminophenol, 4-chloroacetanilide, paracetamol, ibuprofen impurity – J, B, and ibuprofen was found 0.9986, 0.9983, 0.9991, 0.9963, 0.9991 and 0.9985 respectively. The results are summarized in table 3 and overall linearity graph for ibuprofen, paracetamol and its impurities was shown in figure 7.

**Accuracy**

Accuracy of the method was studied for three levels from 50% to 150% by spiking 0.1% for 50% level from the target concentration of paracetamol impurities and 0.2%, 0.3% for 100%, 150% level from the target concentration of ibuprofen impurities spiked in sample preparation and analyzed with unspiked sample preparation, recorded the chromatogram. Six preparation for 50%, 150% level and triplicate preparation of median level concentration were done. Recovery was found 90.2% to 108.2% and RSD was found 2.4% to 8.1% is lowest and highest value. Results are summarized in table 4.

**Precision and accuracy at LOQ level**

Precision and accuracy of the method was studied for the level by different analyst, day, HPLC system, and column and recorded the chromatogram, calculated percent RSD for the percent impurity of each individual impurity. The percentage impurity was found for 4-amino phenol, 4-chloro acetanilide, ibuprofen impurity-B and ibuprofen impurity-J is 0.190 to 0.211, 0.191 to 0.214, 0.185 to 0.211 and 0.199 to 0.214 and percent RSD of six replicate sample preparations was found 4.2, 4.4, 4.7 and 3.1 respectively. The results summarized in table 2.

**Linearity**

To evaluate linearity of the method, six levels calibration curve made includes LOQ level. Signal to noise ratio was observed about 10 for the concentration between 0.05 to 0.3 μg/mL for ibuprofen, paracetamol and its impurities. Hence linearity was established LOQ to 75 μg/mL.
Robustness

Robustness of the current method was investigated by analyzing the standard solution and established system suitability with the deliberate variation of mobile phase organic variation, flow rate and column temperature at 10 percentage level from the original value. RSD of five replicate injections of standard solution was found below 5.0% for all.

Selectivity (stability indicating evaluation)

Selectivity of the method was demonstrated by enhancing degradation of drug products under stress conditions acid, base, peroxide, 90% humidity, thermal, and UV light. Sample was analyzed and recorded the chromatogram up to 90 minutes. Degradation was found 0.5% to 5.3%, and the maximum degradation found in base degradation. Mass balance was satisfied from the degradation study report, the peak purity of ibuprofen and paracetamol was passing for all type of stressed samples. Detailed stress conditions and results are summarized in table 7 and chromatogram of stressed sample, peak purity angle and threshold of paracetamol and ibuprofen was shown in figures 8-10.

Table 4: Accuracy.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Peak Name</th>
<th>Result</th>
<th>Acceptance Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity</td>
<td>4-Amino phenol</td>
<td>0.9986</td>
<td>R ≥ 0.995</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>0.9991</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ibuprofen impurity-B</td>
<td>0.9963</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ibuprofen impurity-J</td>
<td>0.9991</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Precision and Accuracy at LOQ level.

| % RSD | 6.8 | 6.8 | 7.0 | 5.4 |

Table 6: Range.

<table>
<thead>
<tr>
<th>Stress condition</th>
<th>% Assay</th>
<th>% Net degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>6N Hydrochloric acid/Reflux 1 Hrs</td>
<td>95.4</td>
<td>4.6</td>
</tr>
<tr>
<td>6N Sodium hydroxide/Reflux 1 Hrs</td>
<td>94.7</td>
<td>5.3</td>
</tr>
<tr>
<td>6N Hydrogen peroxide/Reflux 1 Hrs</td>
<td>98.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Water/Reflux 1 Hrs</td>
<td>99.0</td>
<td>1.0</td>
</tr>
<tr>
<td>90% Humidity/7 Days</td>
<td>99.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Thermal 105°C/8 Hrs</td>
<td>99.3</td>
<td>0.7</td>
</tr>
<tr>
<td>UV light</td>
<td>99.2</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Peak purity was passed for ibuprofen and paracetamol peak for all type of stressed samples.

Table 7: Selectivity & Specificity.

| % RSD | 5.8 | 6.6 | 7.2 | 6.5 |

Figure 9: Typical chromatogram of peak purity for paracetamol.

Figure 10: Typical chromatogram of peak purity for ibuprofen.
the chromatographic condition and all peaks in standard solutions. Resolution between 4-aminophenol and paracetamol peak was found 6.2 to 7.9 and resolution between ibuprofen and Ibuprofen impurity B was found 1.6 to 2.0. The results are summarized in table 8.

### Conclusion

A simple, accurate, precise, simultaneous and stability-indicating RP-HPLC method was developed and validated for the routine analysis of related compounds of ibuprofen and paracetamol combined tablet formulation. The results of stress testing undertaken according to the International Conference on Harmonization guidelines reveal that the method is selective and stability-indicating.

### References