Rapid and simultaneous determination of paracetamol, ibuprofen and related impurity of ibuprofen by UPLC/DAD

Ngoc Van Thi Nguyen¹, Tiet Nghia Thi Tran¹, Manh Quan Nguyen¹, Trung Kien Nguyen²

¹College of Pharmacy, Can Tho University of Medicine and Pharmacy
²Can Tho University of Medicine and Pharmacy, Can Tho, Viet Nam

Corresponding author: Trung Kien Nguyen
ntkien@ctump.edu.vn

KEYWORDS:
UPLC; Related impurity; Ibuprofen; Paracetamol

ABSTRACT
A reverse phase UPLC method was developed for simultaneous determination of paracetamol, ibuprofen and its related impurity. The UPLC column was BEH C₁₈ column, (2.1 × 100 mm, 1.7 µm). The mobile phase was composed of an aqueous solution of methanol-triethylamine in gradient mode. The UV detector wavelength was set at UV 230nm for paracetamol and ibuprofen for 254nm for the impurity. The calibration curves all showed good linear regression (r² >0.999) within the concentration ranges of 50 – 600 µg/mL for paracetamol, 50-600 µg/mL for ibuprofen and 0.05-0.5 µg/mL for the impurity. The RSDs of precision were < 2% for paracetamol, ibuprofen and < 2.5% for the impurity. The LOD and LOQ of ibuprofen impurity were 0.03 µg/mL and 0.05 µg/mL, respectively. The recovery of these three compounds was in the range of 98-102%. The proposed RP-UPLC method showed simple, time-saving (<10 min. for a run), but quite precise and accurate, and as such, applicable to the routine assessment of paracetamol, ibuprofen and the impurity of ibuprofen in pharmaceutical dosage forms.

1. INTRODUCTION
Paracetamol is widely used as an analgesic drug. It can be formulated in a variety of dosage forms, e.g. tablets, syrups, and suspensions both in the single-ingredient and multi-component ones. It is accepted as a very effective treatment for the relief of pain and fever in a variety of patients including children, pregnant women, and the elderly. Ibuprofen [RS-2-(4-isobutyl-phenyl) propionic acid], is one of the most potent orally active anti-inflammatory, analgesic and non-steroidal anti-inflammatory drug (NSAID) used extensively in the treatment of acute and chronic pain, osteoarthritis, rheumatoid arthritis and related conditions. Taken together these differing modes of action and related therapeutic effects suggest that ibuprofen and paracetamol may complement each other and improved analgesia may be obtained using a combination compared with individual administration¹⁻³.

The ibuprofen-related compound C (4-isobutylacetophenone) arises via radical-induced decarboxylation followed by benzylic oxidation, and it has shown adverse effects on the central nervous system⁴. It presents in ibuprofen products as a degradation of the ingredient and needs to be monitored and controlled over the shelf life of the product⁵. There are different quality control restrictions...
of ibuprofen impurities published in worldwide accepted pharmacopeias. The United States Pharmacopeia (USP) specifies an impurity C limit of not more than 0.1%, and the sum of all impurities should not exceed 1.0% in drug substances. For ibuprofen tablets, the limit test requires the amount of ibuprofen-related compound C to be not more than 0.25% per tablet.\textsuperscript{6,7}

Quality control of both drug substance and drug product is an essential procedure in the pharmaceutical industry. Ingredient (API) and related impurities in pharmaceutical products are the main focus of many pharmaceutical applications of High-performance liquid chromatography (HPLC). A number of HPLC methods have been already stated for determination of ibuprofen and paracetamol in their combination dosage form\textsuperscript{1-3}. Boyka et al. reported that the use of mobile phase containing phosphate buffer may be detrimental to HPLC column due to the precipitation of buffer salts\textsuperscript{3}. Samson et al. used a complex gradient mode and retention time of ibuprofen was too long (21 min). There are many published methods for the separation of ibuprofen and its impurities. Huidobro et al. achieved the separation of arginine, ibuprofen, and impurities of ibuprofen by liquid chromatography with tandem columns\textsuperscript{8}. Elzanfaly et al. used thin-layer chromatography (TLC) and HPLC to determine ibuprofen, famotidine in the presence of impurity C using phosphate buffer, but they did not develop a method for simultaneous determination of ingredient and related impurities\textsuperscript{9}.

All the reported methods did not determine paracetamol, ibuprofen and impurity C simultaneously. Ultra-performance (UP) LC is a recent technique in liquid chromatography, which enables significant reductions in separation time and solvent consumption\textsuperscript{10}. In order to establish an analytical method which may determine paracetamol, ibuprofen and impurity C (Figure 1) of both the drug substance and product, we developed a specific and sensitive UPLC method for simultaneous determination of paracetamol, ibuprofen and impurity C using detector gradient mode. This method was also validated according to the ICH guidelines\textsuperscript{11}.

2. MATERIALS AND METHODS

2.1. Chemical and materials

Paracetamol and ibuprofen (99%) were purchased from National Institute of Drug Quality Control of Viet Nam. Impurity C was purchased from Sigma-Aldrich. Paracetamol and Ibuprofen combined tablets (325 mg P and 200 mg I) were purchased over-the-counter from a local pharmacy. Ammonium acetate, triethylamine, orthophosphoric acid (analytical reagent), water (18 MΩ, Milli-Q), methanol and acetonitrile (HPLC grade) were used for the mobile phase and as the diluent for standard solution preparations.

![Figure 1. Structure of paracetamol (1), ibuprofen (2) and ibuprofen-related compound C (3)](image-url)
2.2. Instrumentation and chromatographic conditions

The experiment was carried out by the Waters UPLC Acquity H-CLASS, DAD detector (USA), the acquisition of chromatogram and integration used MassLynx SCN 85 software. The chromatographic separation was achieved using a UPLC BEH C18 column (2.1 x 100 mm, 1.7 µM) detected at 230 nm for paracetamol and ibuprofen (from 0 min to 7.20 min); 254 nm for impurity C at room temperature (from 7.21 min to 10 min), with an injection volume of 2 µL and flow rates of 0.2 mL/min. The mobile phase was a three-step linear solvent gradient system consisting of (A) 0.01% aqueous triethylamine (pH = 7) and (B) methanol. The elution profile was: 2.5 min 98% A; then the solvent B was increased first to 50% in 2 min and subsequently to 98% in 2.5 min. The mobile phases were prepared fresh each day, vacuum-filtered through a 0.22 µM and degassed for 15 min.

2.3. Preparation of stock solution

Stock solution of paracetamol and ibuprofen (3,250 µg/mL for paracetamol and 2,000 µg/mL for ibuprofen) was prepared by dissolving appropriate amount of drugs in solvent B. Working solutions of 162.5 µg/mL of paracetamol, and 100 µg/mL of ibuprofen were prepared from the previously described stock solution for related substance determination and assay determination. A stock solution of impurity C at 1 mg/mL was prepared in solvent B.

2.4. Preparation of sample solutions

Twenty tablets containing 325 mg of paracetamol and 200 mg of ibuprofen for each tablet were weighed and finely powdered. Tablet powder equivalent to 325 mg of paracetamol and 200 mg of ibuprofen was dissolved in solvent B with sonication for 30 min to give a solution containing 3,250 µg/mL of paracetamol and 2,000 µg/mL of ibuprofen, and 5 mL of this solution was diluted to 100 mL with mobile phase to give a solution containing 162.5 µg/mL of paracetamol and 100 µg/mL of ibuprofen. These solutions were filtered through a 0.45-µm pore size Nylon 66 membrane filter.

2.5. Method validation

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. The method was validated according to International Conference on Harmonization Q2 (R1) Guidelines for validation of analytical procedures in order to determine the specificity, linearity, LOD, LOQ, accuracy and precision.

2.5.1. Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. Stress studies were performed at an initial concentration of 3,250 µg/mL for paracetamol and 2,000 µg/mL for ibuprofen on tablets to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress condition of UV light (254 nm) and heat (105°C for 12 h) to evaluate the ability of the proposed method to separate paracetamol and ibuprofen from their degradation products. Additional, specificity was tested by applying the UPLC method to analyze standard, commercial product and spike standard to sample.

2.5.2. Linearity

Linearity test solutions for the assay method were prepared from paracetamol and ibuprofen stock solutions at six concentration levels (50 to 600 µg/mL for I and P). The impurity C solutions were prepared at five concentration levels from LOQ to 500% of the specification level 0.1% (LOQ to 0.5%). The peak area versus concentration was treated by least-squares linear regression analysis.

2.5.3. LOD and LOQ

The LOD and LOQ of impurity C were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentration. The precision study was also carried out at the LOQ level by injecting six individual preparations and calculated %RSD of the area.

2.5.4. Precision

The precision of the method was verified by repeatability and by intermediate precision. Repeatability was checked by injecting six individual preparations of paracetamol and ibuprofen on real sample spiked with 0.1% of impurity C. For this test, the impurity C at the concentration of 0.1% was spiked with respect to ibuprofen concentration 100 µg/mL. The %RSD of peak area for each compound was calculated.
The intermediate precision of the method was also evaluated using on a different day.

2.5.5. Accuracy

Standard addition and recovery experiments were conducted on the real sample to determine the accuracy of the method. The study was carried out in triplicate using three different concentration levels, i.e. 80, 100, and 120% of the assay solution (paracetamol and ibuprofen) and specification level (0.1%). The percent recovery for paracetamol, ibuprofen and the impurity C was calculated.

3. RESULTS AND DISCUSSION

3.1. Selection of chromatographic conditions

In this study, a UPLC method was developed for simultaneous determination of ingredients and impurity (ibuprofen-related compound C). To develop an optimal chromatographic method, several parameters were evaluated, including the pH of the buffer, detection wavelength, gradient, flow rates, and temperature. In addition, the method should be proved to be reproducible, specific, repeatable and accurate enough for routine analysis in the quality control laboratory.

In order to detect paracetamol, ibuprofen and impurity C at the same time, we scanned these compounds at 190–300 nm with a DAD detector. At 230 nm, impurity C could not detect at specification level (0.1%) while peak height of paracetamol and ibuprofen were good and the baseline of chromatogram was stable. In contrary, at 254 nm peak height of impurity C (0.1%) was good but ibuprofen peak was not detected in the chromatogram. Thus, detector gradient mode for simultaneous determination of ingredient and impurity (ibuprofen-related compound C) was chosen. The detection wavelength of 230 nm for paracetamol and ibuprofen (from 0 min to 7.20 min); 254nm for impurity C at room temperature (from 7.21 min to 10 min) was selected. The peak purity of three compounds in the sample was 0.999 obtained from spectrum overlaying graphs of three-point purity detection.

The mobile phase has a significant effect on peak shape, sensitivity, and resolution. The optimization of the chromatographic conditions was performed by using different compositions of mobile phases including (1) acetonitrile: 5 mM aqueous ammonium acetate; (2) acetonitrile – 25 mM aqueous acetic acid; (3) methanol: 100 mM orthophosphoric acid; 4) methanol: aqueous triethylamine with different ratio of solvent in isocratic mode. The result showed that peak shape of paracetamol and ibuprofen was not asymmetry (asymmetry > 1.7) (especially, impurity C retained too long (over 30 minutes) with the system (1) and (2). Early retain of impurity C (tR < 15 minutes) was obtained by using the system (3), however tailing peak always happen with paracetamol and ibuprofen. Good shape and resolution (1.5 < Rs < 2) were achieved with the system (4). The mobile phase was methanol: 0.01% aqueous triethylamine in the ratio of 50:50 (v/v) was chosen for the next investigation. As the pH of the mobile phase increased from 5.0 to 8.0, the resolution factor and asymmetry (As) was changed depending on compound behavior with the stationary phase. At pH 5.0 and pH 6.0 ibuprofen cannot elute to the column, at pH 8.0 ibuprofen resolution is poor. The separations of paracetamol, ibuprofen, and Impurity C (resolution, theoretical plates, and tailing factor) were significantly affected by the mobile phase pH, and the best separation was obtained with mobile phase at pH 7.0. Due to the limitation of Impurity C must be under 0.1% in raw material7, we increased resolution between major peak (ibuprofen) and minor peak (ibuprofen-related compound C) by using gradient mode to analyze them simultaneously. The representative chromatograms of the sample and standard were shown in Figure 2.

3.2. Method validation

3.2.1. System suitability

System suitability was tested by performing six replicate injections and determining resolution (Rs), and symmetry factor (As) and repeatability (RSD retention time and peak area) for the analyte of interest. As summarized in Table 1, the %RSD values of peak area and retention time were less than 1% indicating the precise analysis of paracetamol, ibuprofen and Impurity C by this system. All the results showed that the proposed method met the requirement.

3.2.2. Specificity

The method specificity was confirmed by peak purity and resolution. The analyte peaks were homogeneous, and none of the peaks co-eluted paracetamol, ibuprofen and Impurity C was well separated with resolution R > 1.5 between adjacent peaks and peak purity of 0.999.
Specificity was confirmed by comparing the retention time of each standard reference compound with that of the peaks obtained by analyzing the commercial sample, spike standard to sample and forced degradation samples (stress condition of UV light at 254 nm and heat at 105°C for 12 h). There was no interference with the peaks of paracetamol, ibuprofen and Impurity C in the sample (Figure 2). These results confirmed the high specificity of the method.
Table 1. System suitability parameters

<table>
<thead>
<tr>
<th></th>
<th>$t_R$ (min) (n=6)</th>
<th>Peak area (mAu.s)</th>
<th>Rs</th>
<th>As</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paracetamol</td>
<td>5.39 0.0</td>
<td>116314 0.18</td>
<td>2.25</td>
<td>1.04</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>7.02 0.0</td>
<td>59739 0.88</td>
<td>1.73</td>
<td>0.82</td>
</tr>
<tr>
<td>Impurity C</td>
<td>7.87 0.0</td>
<td>17448 0.48</td>
<td>6.72</td>
<td>1.42</td>
</tr>
</tbody>
</table>

$t_R$: retention time (min); $R_s$: Resolution; $A_s$: symmetry factor

Table 2. Linear regression data, precision of the UPLC method for determination of paracetamol, ibuprofen and impurity C

<table>
<thead>
<tr>
<th>Parameters</th>
<th>paracetamol</th>
<th>ibuprofen</th>
<th>impurity C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression equation</td>
<td>$y = 2615x-42370$</td>
<td>$y=1337x+8891$</td>
<td>$y=7968x-30$</td>
</tr>
<tr>
<td>Linearity range (µg/ml)</td>
<td>50-600</td>
<td>50-600</td>
<td>0.05-0.5</td>
</tr>
<tr>
<td>$r^2$</td>
<td>0.9998</td>
<td>0.9993</td>
<td>0.996</td>
</tr>
<tr>
<td>Repeatability (% RSD)</td>
<td>1.64</td>
<td>1.48</td>
<td>2.41</td>
</tr>
<tr>
<td>Intermediate precision (% RSD)</td>
<td>1.29</td>
<td>1.00</td>
<td>1.77</td>
</tr>
</tbody>
</table>

Regression curve data for six calibration points is $y = ax + b$, where $y$ is peak area of analytes, $x$ is concentration, $a$ is slope, $b$ is intercept, and $r^2$ is the squared correlation coefficient.

Table 3. Recoveries for the assay of the investigated paracetamol, ibuprofen and impurity C in tablets

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Sample</th>
<th>Concentration (µg/ml)</th>
<th>Recovery Mean recovery n=9</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Added</td>
<td>Found</td>
<td></td>
</tr>
<tr>
<td>Paracetamol</td>
<td>S₁ᵃ</td>
<td>15</td>
<td>15.25</td>
<td>101.69</td>
</tr>
<tr>
<td></td>
<td>S₂ᵇ</td>
<td>19</td>
<td>18.72</td>
<td>98.53</td>
</tr>
<tr>
<td></td>
<td>S₃ᶜ</td>
<td>22</td>
<td>21.70</td>
<td>98.64</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>S₁ᵃ</td>
<td>9.6</td>
<td>9.44</td>
<td>98.33</td>
</tr>
<tr>
<td></td>
<td>S₂ᵇ</td>
<td>12.0</td>
<td>11.74</td>
<td>97.83</td>
</tr>
<tr>
<td></td>
<td>S₃ᶜ</td>
<td>14.4</td>
<td>14.04</td>
<td>97.52</td>
</tr>
<tr>
<td>Impurity C</td>
<td>S₁ᵃ</td>
<td>0.08</td>
<td>0.079</td>
<td>99.17</td>
</tr>
<tr>
<td></td>
<td>S₂ᵇ</td>
<td>0.10</td>
<td>0.104</td>
<td>104.00</td>
</tr>
<tr>
<td></td>
<td>S₃ᶜ</td>
<td>0.12</td>
<td>0.123</td>
<td>102.22</td>
</tr>
</tbody>
</table>

Recovery (%) = (found/added) × 100.

a The samples added known amounts of standards at low level (80% of the known amounts).

b The samples added known amounts of standards at medium level (same as the known amounts).

c The samples added known amounts of standards at high level (120% of the known amounts)

3.2.3. Linearity, limits of detection and quantification

The result for the regression equation and coefficient of determination ($r^2$) are summarized in Table 2. The linearity calibration plot for the assay method was obtained over the calibration ranges tested and the $r^2$ obtained was greater than 0.999 for both paracetamol and ibuprofen. Linear calibration plot for impurities was obtained over the calibration ranges tested (i.e. LOQ to 0.50% for impurity). The $r^2$ obtained was greater than 0.996. The LOD and LOQ of impurity C were 0.03 µg/mL and 0.05 µg/mL, respectively.

3.2.4. Precision

Precision at requirement limit for impurity was reported in Table 2. The %RSD for the area of Impurity C was within 2%. The %RSD of assay of paracetamol and ibuprofen during the assay method repeatability study was 1.64, and 1.29% for paracetamol and ibuprofen, respectively. The %RSD of the assay results obtained in the intermediate precision study was within 2% for paracetamol and ibuprofen confirming good precision of the method. The %RSD values are presented in Table 2.
3.2.5. Accuracy

The percentage recovery of paracetamol and ibuprofen from tablets ranged from 98.53% to 101.69% for paracetamol and from 97.52 to 98.33% for ibuprofen. The percentage recovery of Impurity C in samples varied from 99.17% to 104.00%. The recovery values for paracetamol and ibuprofen and Impurity C are presented in Table 3. Considering the results of the recovery test, the method was deemed to be accurate. The LC chromatogram of the spiked sample at 0.10% level of Impurity C in the sample of paracetamol and ibuprofen tablet is shown in Figure 2.

3.3. Method application

The proposed method is applied to determine the content of paracetamol, ibuprofen and Impurity C in commercial tablets (Result in Table 4). A typical chromatography of commercial tablet is shown in Figure 3. The content of paracetamol, ibuprofen and Impurity C in the sample is calculated using the calibration curve method. The samples are prepared in the same manner as described for the repeatability test. The contents based on the average of three replicate measurements are found. The result deems to the reliable method, among 9 commercial tablets there is no sample exceed the limit of impurity (under 0.1%).

4. CONCLUSION

The rapid reproducible gradient RP-UPLC method developed for quantitative analysis of paracetamol, ibuprofen and ibuprofen related compound C (Impurity C) in pharmaceutical dosage forms is specific, linear, LOD, LOQ, precise and accurate. Satisfactory results were obtained from validation of the method. This is the first reported method for simultaneous quantitative analysis of paracetamol, ibuprofen and Impurity C. The method is stability-indicating and can be used for routine analysis of production samples.

![Chromatogram of commercial sample](image)

**Figure 3. Chromatogram of commercial sample**

**Table 4. Content of paracetamol, ibuprofen and impurity C in commercial tablets**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Paracetamol (%)</th>
<th>Ibuprofen (%)</th>
<th>Impurity C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>102.01±1.78</td>
<td>98.75±1.25</td>
<td>≤ 0.1%</td>
</tr>
<tr>
<td>Sample 2</td>
<td>97.91±1.83</td>
<td>98.225±1.60</td>
<td>≤ 0.1%</td>
</tr>
<tr>
<td>Sample 3</td>
<td>95.33±0.94</td>
<td>97.12±1.57</td>
<td></td>
</tr>
<tr>
<td>Sample 4</td>
<td>96.73±1.7</td>
<td>96.735±1.32</td>
<td></td>
</tr>
<tr>
<td>Sample 5</td>
<td>97.16±1.68</td>
<td>96.53±1.73</td>
<td>≤ 0.1%</td>
</tr>
<tr>
<td>Sample 6</td>
<td>98.39±1.37</td>
<td>97.91±1.79</td>
<td></td>
</tr>
<tr>
<td>Sample 7</td>
<td>98.84±1.26</td>
<td>96.02±1.77</td>
<td>-</td>
</tr>
<tr>
<td>Sample 8</td>
<td>95.84±1.82</td>
<td>97.71±1.07</td>
<td>-</td>
</tr>
<tr>
<td>Sample 9</td>
<td>97.60±1.30</td>
<td>99.52±1.28</td>
<td>-</td>
</tr>
</tbody>
</table>

*The sample solutions were prepared as described for the precision test.
*Each value is the mean response of 3 determinations, mean ±SD (n=3)
5. ACKNOWLEDGMENTS

This research was supported by Can Tho University of Medicine and Pharmacy, Viet Nam. The authors thank Warenteck Company for the use of analytical instruments. We also thank all of our colleagues for their excellent assistance.

Conflict of interest
None to declare

Funding
None to declare

Ethical approval
None to declare

Article info:
Received October 19, 2017
Received in revised form January 1, 2018
Accepted February 12, 2018

REFERENCES
7. United States Pharmacopoeia USP38, online version.
11. ICH guideline, Validation of analytical procedures: Text and Methodology Q2(R1), 2005.