HPLC Analysis of Acetaminophen Tablets with Waters Alliance and Agilent Supplies

Application Note

Small Molecule Pharmaceuticals

Authors

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Abstract

HPLC analysis and method validation for acetaminophen (paracetamol) tablets was performed using an Agilent Poroshell 120 column and Waters Alliance system, equipped with Agilent performance maintenance kits for pump, autosampler, and a UV lamp. The analysis was performed following the United States Pharmacopeia (USP) method [1]. The results showed expected linearity, precision, and sample recovery values, indicating that Agilent supplies for Alliance systems provide suitable alternatives to Waters parts.

Introduction

Acetaminophen, also known as paracetamol, is a common pharmaceutical widely used to relieve pain and reduce fever. The preparation and analysis of acetaminophen is clearly defined in the United States Pharmacopeia (USP) [1], and can be conducted on different HPLC systems using columns and sample preparation products from different manufacturers.

This application note focuses on the use of Agilent performance maintenance kits, including various frequently exchanged parts such as plungers, seals, injection needles, solvent filters, and so on. We also assessed Agilent lamps for a Waters Alliance system to evaluate compatibility. The method to analyze paracetamol tablets was validated. Detection limits, linearity, accuracy and precision, and sample recovery were determined. These parameters are important indicators of the functionality of the system, the column, and sample preparation (filtration) products.
Experimental

Chemicals and reagents
Acetaminophen USP-level standard and HPLC-grade methanol from Merck were purchased from Hind Associates (Delhi, India). Paracetamol tablets were purchased from a local pharmacy store in Manesar, India. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge.

Instrumentation
The HPLC analysis was performed with a Waters Alliance Separation Module (model no. 2695) with a Waters UV-VIS detector (model no. 2487). The system was equipped with an Agilent deuterium lamp (p/n 8005-0704), and parts from an Agilent performance maintenance kit (p/n 8005-0915), including plungers, plunger seals, diffuser, face seal, solvent filters, syringe, precolumn filter, check valve cartridges, PTFE washer, needle wash frit, needle assembly, and injector seals, among others. Spectral data were obtained with a Waters Acquity photodiode array (PDA) detector.

Conditions
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Agilent Poroshell 120 EC-C18, 4.6 x 75 mm, 2.7 µm (p/n 697975-902)</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>1:3 Methanol:water, isocratic</td>
</tr>
<tr>
<td>Sample dissolving solution/diluent (DDS)</td>
<td>1:3 Methanol:water</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.5 mL/min</td>
</tr>
<tr>
<td>Column temperature</td>
<td>25 °C</td>
</tr>
<tr>
<td>UV detection</td>
<td>243 nm</td>
</tr>
<tr>
<td>Injection volume</td>
<td>10 µL</td>
</tr>
</tbody>
</table>

Agilent supplies
Syringe filters: Agilent Captiva Premium syringe filter with regenerated cellulose membrane, 0.45 µm pore size (p/n 5190-5111)
Vials: Amber, write-on spot, 100/pk (p/n 5182-0716)
Vial caps: Blue, screw cap, 100/pk (p/n 5182-0717)

Preparation of standards
USP-level acetaminophen standards were accurately weighted and dissolved in sample dissolving solution (DDS) to obtain a stock solution of 20 µg/mL. Proper standards for method development and validation were prepared by subsequent dilution of the stock solution in DDS.

Preparation of drug tablet assay samples
Two different brands of paracetamol tablets, both labeled as 500 mg acetaminophen in each tablet, were tested. For each brand, 20 tablets, equivalent to 10 g acetaminophen, were dissolved in 1 L of DDS, and then 1 mL of the solution was diluted with DDS to 1 L, corresponding to an acetaminophen concentration of 10 µg/mL. The diluted solution was then filtered by syringe filter. The first 10 mL of filtrate was discarded, and the rest of the filtrate was used for HPLC analysis.

Results and Discussion
After installing Agilent parts and lamp in the Alliance system, the system was first tested by different qualification tests, including pump flow accuracy, wavelength accuracy, signal noise and drift, injection precision, carry-over, response linearity of a caffeine standard, and gradient composition accuracy. All tests were passed, demonstrating the full functionality of the system.

Acetaminophen standards and drug tablet samples were analyzed following the USP method [1], and method validation was done.

Identification
For the purpose of peak identification, a 10 µg/mL acetaminophen standard was analyzed and its chromatogram was overlaid with that of the drug assay sample, in Figure 1. The chromatograms matched perfectly, indicating that the major peak at 1.04 minutes was from acetaminophen.

![Figure 1. Overlay of 10 µg/mL acetaminophen standard and drug tablet assay.](image-url)
To assess the purity of the peak, UV absorption spectra of the standard and the drug assay were overlapped in Figure 2. Both spectra were identical, demonstrating that the peak was pure.

![Figure 2. UV absorption spectra of acetaminophen standard and drug tablet sample.](image)

**Limit of detection (LOD) and limit of quantitation (LOQ)**

The analyte concentration with a signal-to-noise ratio (S/N) above three was considered as LOD, and analyte concentration with S/N above 10 was considered as LOQ. The LOD and LOQ of acetaminophen were 10 ng/mL and 50 ng/mL, respectively (Figure 3).

**Calibration curve linearity**

The method gave a linear response to acetaminophen from 0.05 to 20 µg/mL. Six calibration standards with concentrations of 0.05, 1, 5, 7.5, 15, and 20 µg/mL were analyzed, and their peak areas were plotted against concentration, in Figure 4. The regression coefficient $R^2$ was 0.9999, indicating good linearity.

![Figure 3. Chromatograms of LOD and LOQ of acetaminophen.](image)

![Figure 4. Linear response of peak area against concentration of acetaminophen.](image)
Accuracy and precision

Acetaminophen QC standards at 0.5 µg/mL and 15 µg/mL were analyzed and calculated using the previously generated calibration curve to demonstrate method accuracy. To assess method precision, both QC standards were prepared three times, independently (three batches), and each batch was injected in a separate sequence with six replicates. The intra-batch precision was assessed by the standard deviation between the replicates in the same sequence, and the inter-batch precision was evaluated by the variation between different batches. The results of accuracy and precision studies are summarized in Table 1.

The accuracy for both concentrations and all batches was well within a low error range, below ±2%. The intra-batch and inter-batch RSD values were all below 0.7%, indicating good precision.

<table>
<thead>
<tr>
<th>QC sample concentration (µg/mL)</th>
<th>Batch no.</th>
<th>Calculated concentration (µg/mL)</th>
<th>Accuracy</th>
<th>Intra-batch RSD (%) n = 6</th>
<th>Inter-batch RSD (%) n = 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>1</td>
<td>0.51</td>
<td>101.08</td>
<td>0.23</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.51</td>
<td>101.41</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.50</td>
<td>100.24</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>1</td>
<td>15.06</td>
<td>100.41</td>
<td>0.15</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>14.85</td>
<td>98.98</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>14.93</td>
<td>99.52</td>
<td>0.07</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Accuracy and intra-batch and inter-batch precision of acetaminophen analysis.

Analysis of marketed drug tablets

Paracetamol tablets of two different brands, both labeled as 500 mg/tablet, were dissolved, filtered through an Agilent Captiva syringe filter, and analyzed. Regenerated cellulose was chosen as the filtration membrane since it showed excellent sample recovery for acetaminophen in a previous study [2].

Sample recoveries were 97.8% and 100.7%, both of which were well within the USP-specified range of 90 to 110%, showing the excellent filtration recovery of the syringe filter and reliability of the analysis method. RSDs between three different sample batches were 0.31% and 0.17%, indicating appropriate quantitation.

<table>
<thead>
<tr>
<th>Paracetamol</th>
<th>Labeled amount per tablet (mg)</th>
<th>Calculated amount (mg)</th>
<th>Accuracy (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brand A</td>
<td>500</td>
<td>488.8</td>
<td>97.8</td>
<td>0.31</td>
</tr>
<tr>
<td>Brand B</td>
<td>500</td>
<td>503.3</td>
<td>100.7</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Table 2. Accuracy and precision of paracetamol drug tablet assays.
Conclusions

Acetaminophen was analyzed according to the USP method, and specificity, linear range, accuracy, precision, and recovery studies were done to validate the method. A Waters Alliance system was used for HPLC analysis, equipped with comprehensive pump and autosampler parts contained in an Agilent performance maintenance kit, as well as an Agilent deuterium lamp for Alliance systems. Expected results were achieved, and no negative impact by using Agilent supplies could be identified compared to OEM parts, indicating that the Agilent supplies functioned as well as the OEM parts. This work is an example of how the Agilent product portfolio, including instrument parts, lamps, columns, and sample preparation products, can provide linear, accurate, precise, and reliable analysis of pharmaceuticals with Waters Alliance systems.

References


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