Rapid Analysis of Hop Acids in Beer Using Strata™-X SPE and Kinetex® 2.6 µm Core-Shell Technology HPLC Column

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Iso-alpha acids, derived from hops, have a significant impact on the taste of all beers. These hydrophobic compounds are weakly acidic and are present in both cis and trans isomer form. The hop acids absorb ultra violet (UV) light strongly at a wavelength of 270 nm, which makes high performance liquid chromatography (HPLC) with UV detection a suitable analytical technique. Because of the impact these compounds have on flavor, monitoring the iso-alpha acid profile and content during beer production and in the final product with an analytical technique such as HPLC is very important.

Although the finished beer product is a fairly clean sample to inject onto an HPLC system, earlier in the production process the unfinished beer product may contain contaminants or particulates that can compromise column lifetime or performance. Therefore, sample cleanup such as Solid Phase Extraction (SPE) is a necessary step before performing HPLC analysis on an in-process beer sample.

Following SPE, the sample is analyzed by HPLC. Methods using fully porous HPLC media for analyzing these compounds can result in run times of 15-20 minutes or longer with undesirable peak shape. In contrast, superficially porous Kinetex HPLC columns utilize core-shell technology, which results in much higher efficiency while maintaining low backpressures. In conjunction with a quick and easy solid phase extraction cleanup method, an improved HPLC method for iso-alpha acids in beer is demonstrated using a Kinetex 2.6 µm core-shell column, resulting in improved peak shape, quantitation, and dramatically reduced analysis times.
Iso-alpha acid structures

Isohohumulone, isohumulone and isoahumulone are the acids that occur naturally in hops. These compounds are light sensitive. Once exposed to a significant amount of light these compounds give beer a “skunky” flavor. An example is Corona® Extra, which is bottled in a colorless glass bottle, permitting light penetration.

Tetrahydroisohumulone, tetrahydroisohumulone and tetrahydroisoahumulone are specially modified hop acids that are not light sensitive. Beer that utilizes these hop acids can be exposed to light without compromising flavor. An example is Miller® Genuine Draft which is bottled in a colorless glass bottle, which permits light penetration but does not develop a “skunky” off flavor.
2.6 µm Core-Shell Particle

Performance equivalent to or better than fully porous sub-2 µm particles

0.35 µm Porous Shell

1.9 µm Solid Core

Can be used on conventional HPLC systems and UHPLC/UPLC® systems

Kinetex Core-Shell Technology
Solid Phase Extraction

Each beer sample was degassed by stirring for approximately 30 min at room temperature

**Cartridge:** Strata-X 200 mg/6 mL  
**Part No.:** 8B-S100-FCH  
**Condition:** 4 mL acidified methanol (1-2 mL/min)  
**Equilibrate:** 4 mL water (1-2 mL/min)  
  - **Load:** 5 mL of beer degassed (1 mL/min)  
  - **Wash:** 4 mL of 40% methanol in water  
  - **Dry:** >10" Hg for 5 minutes to remove residual water  
  - **Elute:** 2 mL of acidified methanol (1 mL/min)  
**Drydown:** Nitrogen gas at 55 ºC  
**Reconstitute:** 500 µL of mobile phase
Table 1. SPE Recoveries of Iso-alpha Acids from Red Stripe® Jamaican Lager Beer Using Strata-X

<table>
<thead>
<tr>
<th>Iso-Alpha Acids</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isocohumulone</td>
<td>104</td>
</tr>
<tr>
<td>Isohumulone</td>
<td>104</td>
</tr>
<tr>
<td>Isoadhumulone</td>
<td>118</td>
</tr>
</tbody>
</table>
**Table 2. SPE Recoveries of Tetrahydro-Iso-alpha Acids from Miller® Genuine Draft Beer Using Strata-X**

<table>
<thead>
<tr>
<th>Tetrahydro-Iso-alpha Acids</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetrahydroisocohumulone</td>
<td>80</td>
</tr>
<tr>
<td>Tetrahydroisohumulone</td>
<td>82</td>
</tr>
<tr>
<td>Tetrahydroisoadhumulone</td>
<td>69</td>
</tr>
</tbody>
</table>
Figure 1. Iso-alpha acids on a conventional fully porous 5 µm C18 column

Column: Nucleosil® 5 µm C18 100 Å
Dimensions: 250 x 4.6 mm
Part No.: 00G-0323-E0
Mobile Phase: Methanol/Water/Phosphoric acid (75:24:1, v/v/v)
Flow Rate: 1.0 mL/min
Inj. Volume: 10 µL
Temperature: 50 °C
Detection: UV @ 270 nm

Sample:
1. Isocohumulone
2. Isohumulone
3. Isoadhumulone
4. Tetrahydroisocohumulone
5. Tetrahydroisohumulone
6. Tetrahydroisoadhumulone
Figure 2. Iso-alpha acids on a Kinetex 2.6 µm C18, 150 x 4.6 mm

Column: Kinetex 2.6 µm C18 100 Å
Dimensions: 150 x 4.6 mm
Part No.: 00F-4462-E0
Mobile Phase: Acetonitrile/0.1% Phosphoric acid (60:40)
Flow Rate: 1.8 mL/min
Inj. Volume: 10 µL
Temperature: 30 °C
Detection: UV @ 270 nm

Sample:
1. Isocohumulone
2. Isohumulone
3. Isoadhumulone
4. cis-Tetrahydroisocohumulone
5. trans-Tetrahydroisocohumulone
6. cis-Tetrahydroisohumulone
7. trans-Tetrahydroisohumulone
8. Tetrahydroisoadhumulone

Figure 3. Iso-alpha acids on a Kinetex 2.6 µm C18, 100 x 4.6 mm

Column: Kinetex 2.6 µm C18 100 Å
Dimensions: 100 x 4.6 mm
Part No.: 00D-4462-E0
Mobile Phase: Acetonitrile/0.1% Phosphoric acid (60:40)
Flow Rate: 2.0 mL/min
Inj. Volume: 10 µL
Temperature: 30 °C
Detection: UV @ 270 nm
Sample: Same as Figure 2

Figure 4. Iso-alpha acids on a Kinetex 2.6 µm C18, 50 x 4.6 mm

Column: Kinetex 2.6 µm C18 100 Å
Dimensions: 50 x 4.6 mm
Part No.: 00B-4462-E0
Mobile Phase: Acetonitrile/0.1% Phosphoric acid (60:40)
Flow Rate: 2.0 mL/min
Inj. Volume: 10 µL
Temperature: 30 °C
Detection: UV @ 270 nm
Sample: Same as Figure 2
**Figure 5.** Iso-alpha acids on a Kinetex 2.6 µm C18, 50 x 4.6 mm at 50 ºC

- Column: Kinetex 2.6 µm C18 100 Å
- Dimensions: 50 x 4.6 mm
- Part No.: 00B-4462-E0
- Mobile Phase: Acetonitrile/0.1% Phosphoric acid (60:40)
- Flow Rate: 2.0 mL/min
- Inj. Volume: 10 µL
- Temperature: 50 ºC
- Detection: UV @ 270 nm
- Sample: 1. Isohumulone
  2. Isoadhumulone
  3. Isocohumulone
  4. cis-Tetrahydroisocohumulone
  5. trans-Tetrahydroisocohumulone
  6. cis-Tetrahydroisohumulone
  7. trans-Tetrahydroisohumulone
  8. Tetrahydroisoadhumulone

Separation is temperature dependent.

**Figure 6.** Iso-alpha acids on a Kinetex 2.6 µm C18, 50 x 4.6 mm at 40 ºC

- Column: Kinetex 2.6 µm C18 100 Å
- Dimensions: 50 x 4.6 mm
- Part No.: 00B-4462-E0
- Mobile Phase: Acetonitrile/0.1% Phosphoric acid (60:40)
- Flow Rate: 2.0 mL/min
- Inj. Volume: 10 µL
- Temperature: 40 ºC
- Detection: UV @ 270 nm
- Sample: Same as Figure 5
Figure 7. Iso-alpha acids on a Kinetex 2.6 µm C18, 50 x 4.6 mm at 30 °C

Max resolution obtained at 30 °C

Column: Kinetex 2.6 µm C18 100 Å
Dimensions: 50 x 4.6 mm
Part No.: 00B-4462-E0
Mobile Phase: Acetonitrile/0.1 % Phosphoric acid (60:40)
Flow Rate: 2.0 mL/min
Inj. Volume: 10 µL
Temperature: 30 °C
Detection: UV @ 270 nm
Sample: Same as Figure 5

Figure 8. Iso-alpha acids on a Kinetex 2.6 µm C18, 50 x 4.6 mm at 25 °C

Column: Kinetex 2.6 µm C18 100 Å
Dimensions: 50 x 4.6 mm
Part No.: 00B-4462-E0
Mobile Phase: Acetonitrile/0.1 % Phosphoric acid (60:40)
Flow Rate: 2.0 mL/min
Inj. Volume: 10 µL
Temperature: 25 °C
Detection: UV @ 270 nm
Sample: Same as Figure 5
Why Use Kinetex?

**Figure 9.** Iso-alpha acids on a conventional fully porous 5 µm C18 column

- **Column:** Nucleosil 5 µm C18 100 Å
- **Dimensions:** 250 x 4.6 mm
- **Part No.:** 00G-0326-E0
- **Mobile Phase:** Methanol/Water/Phosphoric acid (75:24:1, v/v/v)
- **Flow Rate:** 1.0 mL/min
- **Inj. Volume:** 10 µL
- **Temperature:** 50 °C
- **Detection:** UV @ 270 nm
- **Sample:**
  1. Isocohumulone
  2. Isohumulone
  3. Isoadhumulone
  4. Tetrahydroisocohumulone
  5. Tetrahydroisohumulone
  6. Tetrahydroisoadhumulone

Long ~20 minute run time

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Iso-alpha acids on a Kinetex 2.6 µm C18, 50 x 4.6 mm

- **Column:** Kinetex 2.6 µm C18 100 Å
- **Dimensions:** 50 x 4.6 mm
- **Part No.:** 00B-4462-E0
- **Mobile Phase:** Acetonitrile/0.1 % Phosphoric acid (60:40)
- **Flow Rate:** 2.0 mL/min
- **Inj. Volume:** 10 µL
- **Temperature:** 30 °C
- **Detection:** UV @ 270 nm
- **Sample:**
  1. Isocohumulone
  2. Isohumulone
  3. Isoadhumulone
  4. cis-Tetrahydroisocohumulone
  5. trans-Tetrahydroisocohumulone
  6. cis-Tetrahydroisohumulone
  7. trans-Tetrahydroisohumulone
  8. Tetrahydroisoadhumulone

Narrow peaks, good resolution

Short 4 minute run time
Figure 1 displays poor peak shape for each of the iso-alpha acids, as the run time increases the peaks become even broader. The broad peak shape means that sensitivity is decreased, the last peak to elute (Tetrahydroiso-alpha acid) in particular has very poor sensitivity and therefore will be difficult to quantitate at lower levels.

In addition to the poor peak shape, resolution between the first three iso-alpha acids is limited. The lack of resolution will decrease the accuracy of quantitation. The resolution also was a direct impact on run time. Since the resolution is so poor, it is necessary to use a long 250 mm column in order to obtain the resolution in Figure 1, resulting in an analysis time of ~20 minutes.

Figures 2-4 show the iso-alpha acid analysis run on Kinetex 2.6µm C18 columns progressively decreasing in length from 150 mm to 50 mm. In comparison to the fully porous method, each of the iso-alpha acids exhibit good peak shape with narrow peak widths. The improved peak shape, will result in more accurate quantitation throughout the lifetime of the column as well as improved sensitivity for low level analyses. In addition, the greater efficiency of the Kinetex columns has now completely resolved the cis and trans isomers for Tetrahydroisocohumulone and Tetraisohumulone. In Figures 2 and 3, peak 7 (trans-Tetrahydroisohumulone) is displaying partial separation of isomeric forms. The increased separation gives the analyst even more information about a sample’s iso-alpha acid profile.

As the column length decreases from 150 mm (Figure 2) to 50 mm (Figure 4), run time as well as resolution between each of the compounds decreases. However, even on the short 50 mm column there is greater resolution than on the fully porous packed column, and the run time is significantly shorter - <4 minutes, compared to ~20 minutes on the fully porous column. The greater resolution, improved peak shape and decreased run time present significant advantages in using Kinetex over a traditional fully porous packed HPLC column.

Column temperature affects both column backpressure and resolution. In order to determine the optimum column temperature for the iso-alpha acid analysis, column temperatures ranging from 50 ºC down to 25 ºC were tested on a Kinetex 2.6 µm C18, 50 x 4.6 mm column. Figure 5 displays a run at 50 ºC, although resolution is sufficient for the majority of peaks, peaks 2 and 3 partially co-elute. As the temperature is decreased peaks 2 and 3 begin to separate. However, the optimal temperature is reached in Figure 7 at 30 ºC as any further decrease in column temperature does not enhance peak separation (Figures 7 and 8).

Figure 9 compares the conventional hop acid analysis method - using fully porous media - against the optimized core-shell particle method. The core-shell particle method stands out due to the significantly shorter runtime (5X shorter) and narrow peak widths. In a manufacturing setting, both runtime and narrow peak widths are very advantageous because it means that production is delayed as little as possible and results are easily quantitated.
Conclusions

- Converting hop acid HPLC-analysis from a fully porous particle column to a Kinetex 2.6 µm core-shell particle column brings a number of significant advantages
- Run times can be decreased by as much as 12 minutes per sample
- Resolution is increased
- Sensitivity and accuracy is increased
- When using Kinetex to improve your hop acid analysis it is recommended that a sample cleanup step involving Strata-X is used to obtain a clean baseline without sacrificing sensitivity

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Strata-X is patented by Phenomenex. U.S. Patent No. 7,119,145

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