An HPLC Column for Multiresidue Determination of Fungicides in Environmental Samples

Michael S. Young and Kevin M. Jenkins, Waters Corp., Milford, Massachusetts, USA.

Benzimidazoles, such as thiabendazole, are compounds that are commonly used as fungicides for protection of fruit and vegetable crops. The LC analysis of these substances can be challenging. Thiabendazole, for example, shows significant tailing on most silica-based LC columns, particularly if the analysis is performed at acid pH. Thiabendazole and carbendazim show excellent retention and good peak shape when analysed at pH 10 on XTerra® MSC18,1 but there may be situations that require determination of basic fungicides along with other classes of fungicides or pesticides that are not stable at high pH.

In this application note, we demonstrate excellent retention and peak shape for fungicides analysed on a Waters SunFire™ C18 column with a pH 3.7 mobile phase buffer system. Included in this application are three classes of fungicides. Thiabendazole and carbendazim are benzimidazole fungicides and are weak bases. Procionazole and myclobutanil are carbamate fungicides and are very weakly basic. Thiophanate and thiophanate methyl are conizole fungicides and are very weakly acidic. A high pH LC analysis would not be suitable for this multiresidue analysis because the carbamate fungicides are not stable in a high pH mobile phase. Structures for these six fungicides are presented in Figure 1.

Experimental Conditions
Analytical separations were performed on a SunFire™ C18 column, 2.1 × 100 mm, 3.5 µM particle size. Mobile phases were A: water, B: acetonitrile and C: 500 mM ammonium formate buffer (pH 3.7). Gradient or isocratic conditions are given in the captions for Figures 2 and 3. All LC analysis was performed with column temperature of 30 °C. LC–MS analysis was performed using an Alliance 2695 separations module interfaced with a Waters ZQ mass-spectrometer. The MS conditions are given in Table 1 (selected ion recording, SIR).

Table 1: MS (SIR) Parameters for this study. Cone voltage was 25 V for each ion. The mass spectrometer was operated in ESI+ mode (source temperature 120 °C, desolvation temperature 350 °C).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Principle Ion [M+1]-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbendazim</td>
<td>192</td>
</tr>
<tr>
<td>Thiabendazole</td>
<td>202</td>
</tr>
<tr>
<td>Thiophanate methyl</td>
<td>343</td>
</tr>
<tr>
<td>Thiophanate</td>
<td>371</td>
</tr>
<tr>
<td>Myclobutanil</td>
<td>289</td>
</tr>
<tr>
<td>Propiconazole</td>
<td>342</td>
</tr>
</tbody>
</table>

Results
Figure 2 shows the gradient separation for six fungicides (two benzimidazoles, two conizoles and two carbamates) with good peak shape for all analytes. Figure 3 shows an isocratic separation of thiabendazole and carbendazim at pH 3.7. The observed tailing factor of 1.2 (measured at 10% peak height) compares well with the performance observed with the high pH separation on the XTerra® column and is superior to performance obtained under similar conditions on competitors’ silica-based columns.

Considerations for SPE Analysis
We have previously demonstrated a mixed-mode (cation-exchange) SPE method for determination of thiabendazole and benomyl (as carbendazim) in fruit juices.1 The Oasis MCX sorbent employed for that analysis can also be utilized for this multiresidue fungicide analysis. Briefly, the SPE method is as follows. An Oasis MCX cartridge is conditioned with 1 mL methanol and equilibrated with 1 mL water. The sample is adjusted to pH 3 with formic acid and is loaded at 5 mL/min. The cartridge is washed with 1 mL of 20:89:1 methanol/water/conc. ammonia. Elution is with 2 mL 2% ammonia in methanol. Since many of the compounds in this study are not bases, the loading step is accomplished in mixed-mode (load at pH 3) and the wash step is reversed-phase (pH 10). Since the carbamates are not stable in aqueous base, the eluent is evaporated and reconstituted in mobile phase. Typical recovery is 75% for the benzimidazoles and 95% for the other compounds. The LOQ for the SPE method (LC–MS conditions as given in Table 1) is...
Environmental

Figure 2: Gradient separations of six fungicides on SunFire™ C18 column (10 µL injection of 50 ng/mL standard). Gradient: 90:5:5 (A/B/C) to 0:95:5 (A/B/C) in 10 min. A: water, B: acetonitrile, C: 500 mM ammonium formate buffer pH 3.7.

Peaks: 1 = Carbendazim, 2 = Thiabendazole, 3 = Thiophanate methyl, 4 = Thiophanate, 5 = Myclobutanil, 6 = Propiconazole.

Figure 3: Isocratic separation of carbendazim and thiabendazole on SunFire™ C18 column (10 µL injection of 1 µg/mL standard). Mobile phase: 75:20:5 (A/B/C). A: water, B: acetonitrile, C: 500 mM ammonium formate buffer pH 3.7.

Peaks: 1 = Carbendazim, 2 = Thiabendazole.

Environmental is under 0.3 µg/L assuming a 50 mL untreated surface water sample and a 1 mL reconstitution volume.

Conclusions
The Sunfire C18 column is an excellent choice for the LC or LC–MS analysis of fungicides. Excellent peak shapes were obtained for three classes of fungicides analysed in a simple pH 3.7 mobile phase suitable for MS.

References