SUMMARY

A 5-minute reversed phase analysis of fat-soluble vitamins is described for the simultaneous detection of vitamins A, D, E and K using the Waters® ACQUITY UPLC® System with the PDA and SQ Detector.

The ACQUITY UPLC System is an advanced separation system that utilizes a 1.7 μm stationary phase particle size to improve resolution and peak shape in a shorter run-time.

The SQ Detector is compatible with both Waters Empower™ and MassLynx™ software. The MS set-up parameters are made easy with the new functionality of IntelliStart™, which has been incorporated into both software packages.

INTRODUCTION

Vitamins are essential nutrients that perform various roles to maintain good health. Eating a normal, well-balanced diet should provide sufficient levels of vitamins, however, modern-day lifestyles can lead to deficiencies. As a result, the practice of enriching foods with vitamins in order to provide the recommended daily allowance (RDA) has become commonplace.

Vitamin A (retinol) plays an important role in bone growth, tooth development, reproduction, cell division and gene expression. Sources of vitamin A are dairy products, carrots, pumpkin, dark green leafy vegetables and apricots.

Vitamin D helps increase the amount of calcium absorbed from the small intestine and helps form and maintain bones. Children need adequate amounts of vitamin D to help develop strong bones and healthy teeth. Common sources of vitamin D include a variety of enriched dairy products, most notably milk. Vitamin D can also be obtained through sunlight exposure of the skin.

Vitamin E is a fat-soluble anti-oxidant, and protects vitamins A and C, red blood cells and essential fatty acids from destruction. Sources of vitamin E are margarines and vegetable oils.

Vitamin K plays an essential role in normal blood clotting and helps promote bone health. Sources of vitamin K are green vegetables (spinach, broccoli), cauliflower, cabbage and certain vegetables oils (soybean oil, cottonseed oil, canola oil and olive oil).

METHOD

UPLC® Conditions
Solvent A: Water:Acetonitrile (90:10)
Solvent B: Acetonitrile:Methanol (50:50)
Flow rate: 0.7 mL/min
Column temp: 35 ºC
Column: ACQUITY BEH C18
        2.1 mm x 100 mm, 1.7 μm
RESULTS AND DISCUSSION

Published methods for the detection of fat-soluble vitamins can require 15 to 60 minutes. A standard solution of vitamins is shown in Figure 1 with an analysis time of 5 minutes by UPLC (Table 2 shows the values used for each vitamins analyzed).

### Table 1. UPLC solvent method.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% A</th>
<th>% B</th>
<th>Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>10</td>
<td>90</td>
<td>Initial</td>
</tr>
<tr>
<td>0.10</td>
<td>10</td>
<td>90</td>
<td>9</td>
</tr>
<tr>
<td>2.00</td>
<td>0</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>3.00</td>
<td>0</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>3.50</td>
<td>10</td>
<td>90</td>
<td>6</td>
</tr>
<tr>
<td>5.00</td>
<td>10</td>
<td>90</td>
<td>6</td>
</tr>
</tbody>
</table>

**ACQUITY UPLC PDA**
Wavelength range: 205 to 450 nm
Resolution: 1.2 nm
Sampling rate: 20 spectra/s

**SQ Detector Settings**
The single quadrupole MS was run in APCI mode.

- Corona current: 15.0 uA
- Cone voltage: 35.0 V
- Source Temperature: 145 °C
- Probe Temperature: 575 °C
- Cone Gas Flow: 200 L/Hr
- Desolvation Gas Flow: 600 L/Hr

(See Table 2 for the m/z values used for the SIR method)

**Software**
The total system was controlled with MassLynx Software (creation of methods and data-processing).

The SQ Detector parameters were set up via the IntelliStart software and using the on-line fluidics. IntelliStart monitors the performance of the mass spectrometer and will initiate corrective action if any problems are diagnosed. Processes such as tuning and calibration can be performed by the click of a button using the on-line fluidics.

![Figure 1. Standard mixture of fat-soluble vitamins.](image)

Vitamin A is the first to elute and Vitamin K is the last to elute on the ACQUITY BEH C18 column.

**Table 2. Retention times, UV maxima and m/z values (used for SIR) for the fat-soluble vitamins.**

<table>
<thead>
<tr>
<th>RT</th>
<th>Vitamin</th>
<th>UV&lt;sub&gt;max&lt;/sub&gt;</th>
<th>SIR - RT windows</th>
<th>SIR - m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.98</td>
<td>Vitamin A&lt;sub&gt;1&lt;/sub&gt;</td>
<td>325</td>
<td>0.00-1.40</td>
<td>269</td>
</tr>
<tr>
<td>2.25</td>
<td>Vitamin K&lt;sub&gt;2&lt;/sub&gt;</td>
<td>265, 245</td>
<td>1.20-2.70</td>
<td>445</td>
</tr>
<tr>
<td>2.37</td>
<td>Vitamin D&lt;sub&gt;2&lt;/sub&gt;</td>
<td>265&lt;sup&gt;2&lt;/sup&gt;</td>
<td>2.10-2.60</td>
<td>397</td>
</tr>
<tr>
<td>2.43</td>
<td>Vitamin D&lt;sub&gt;3&lt;/sub&gt;</td>
<td>265&lt;sup&gt;2&lt;/sup&gt;</td>
<td>2.20-2.80</td>
<td>385</td>
</tr>
<tr>
<td>2.77</td>
<td>Vitamin E</td>
<td>291</td>
<td>2.60-3.10</td>
<td>431</td>
</tr>
<tr>
<td>3.02</td>
<td>Vitamin E acetate</td>
<td>285</td>
<td>2.80-3.40</td>
<td>473</td>
</tr>
<tr>
<td>3.24</td>
<td>Vitamin K&lt;sub&gt;1&lt;/sub&gt;</td>
<td>265, 240</td>
<td>3.10-3.60</td>
<td>451</td>
</tr>
</tbody>
</table>

Figure 2 shows the presence of vitamin E (m/z 431) and vitamin E acetate (m/z 473) from the standard mixture (left) and from a butter sample (right) that has undergone liquid-liquid extraction. Selected ion recording (SIR) was used to increase the selectivity of the vitamins in the butter.
CONCLUSION

A 5-minute method utilizing UPLC has been developed to detect fat-soluble vitamins. Current published methods typically require at least 15 minutes to perform the same analysis. While identification of these vitamins is possible using a PDA, samples are often complex, requiring additional techniques to detect all components of interest. The PDA and SQ Detectors are complementary techniques that allow not only for the identification of vitamins but also for components that may not be amenable to PDA detection alone.

REFERENCES

