INTRODUCTION
The analysis of fat soluble vitamin (FSV) formulations, often from oil filled capsules, powder filled capsules, and pressed tablets, can be a challenging task. Most often, analysis of these formulations employs normal phase chromatographic methods using traditional normal phase solvents (hexane, toluene, hexyl alcohol, ethyl acetate, dichloromethane and others) which can be expensive to procure and dispose of. Other techniques include reversed phase liquid chromatography, gas chromatography, thin layer chromatography, and colorimetric techniques for these analyses. The use of UPLC technology as an alternative to these techniques provides a simple alternative that lowers the use of organic solvents, provides fast analysis times, and maintains chromatographic data quality. Using the ACQUITY UPLC system (Figure 1), a series of FSV formulations were analyzed. The formulations examined (Table 1) contained Vitamin A only, Vitamin A + D3, Vitamin E, Vitamin D3 only, Vitamin K1 only, and Vitamin K2 only. Results from these experiments show UPLC has the potential to replace many of the separation chromatography, and colorimetric techniques for the analysis of fat soluble vitamin (FSV) formulations.

EXPERIMENTAL
Sample Preparation
Oil filled capsules (A, A & D3, D3) - contents of 4 individual capsules were removed and dissolved in 10 ml of iso-octane. No further pretreatment was used. For the Vitamin E capsule, the contents of 3 individual capsules was removed and dissolved in 10 ml of iso-octane. No further pretreatment was used.

Table 1. Fat Soluble Vitamin Formulations

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Content Description</th>
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<tbody>
<tr>
<td>A</td>
<td>2176 IU A Soy oil, gelatin, glycerin, water, as inactive ingredients. Two primary forms of vitamin A were labeled to be from fish liver oil (Figure 5).</td>
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<tr>
<td>A + D3</td>
<td>5176 IU A + 2000 IU D3 Soy oil, gelatin, glycerin, water, as inactive ingredients.</td>
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<tr>
<td>E</td>
<td>400 IU E Soy oil, gelatin, glycerin, water, 150 µg of each of the isomers were not available at the time of this study.</td>
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| D3            | 2000 IU D3 Sunflower oil, gelatin, glycerin, water, 2176 psi at 50 °C. Using this separation method, a separation was accomplished using a 2.0 mL/min gradient of carbon dioxide and methanol (containing 0.2% formic acid) 97.5:99.5 over 10 minutes at 50 °C. Using this separation method, a separation was accomplished using a 1.0 mL/min gradient of carbon dioxide and methanol, 99:1 to 100:0 at 50 °C. Each of the FSV formulations were analyzed rapidly and with little or no sample pretreatment. A summary of the separation conditions is given in Table 1. For each of the isomers 2 columns were used to resolve each pair.

RESULTS AND DISCUSSION

Figure 2. UPC2 Separation of a Vitamin A Capsule

Vitamin A
This formulation of Vitamin A was labeled to be from fish liver oil and contained soy oil, gelatin, glycerin, and water as inactive ingredients. Two forms of Vitamin A palmitate (cis and trans isomers), 2.626 and 2.851 minutes respectively, were noted before the bulk of exipient peaks.

Figure 3. UPC2 Separation of Vitamin A acetate, Vitamin A palmitate, and Retinol

Vitamin A + D3
Similar to the previous example, this formulation of Vitamin A & D was also labeled to be from fish liver oil and contained soy oil, gelatin, glycerin, and water as inactive ingredients. Again, two forms of Vitamin A palmitate (cis and trans isomers), 2.626 and 2.851 minutes respectively, were noted before the bulk of exipient peaks.

Figure 4. UPC2 Separation of a Vitamin A&D3 Capsule

Vitamin D3
For fully resolve Vitamin D3 (cholecalciferol, 6.867 minutes) from the major exipient materials and a number of other compounds contained in the formulation (Figure 4), it was necessary to use a longer 150 mm column to provide enough separation efficiency to accomplish this goal. The system provided enough separation efficiency to detect the Vitamin D3 peak (Figure 4, inset). This separation was accomplished using a 1.5 mL/min gradient of carbon dioxide and methanol (containing 0.2% formic acid) 90:10 over 10 minutes at 50 °C with an ABPR setting of 2176 psi.

Figure 5. UPC2 Separation of a Vitamin D3 capsule.

Figure 6. UPC2 Separation of a Vitamin E Capsule

Vitamin E
The Vitamin K1 tablets generated 2 fully resolved peaks (Figure 7). A very rapid gradient analysis (~ 90 second run time) that easily provided baseline resolution of the 4 tocopherol isomers (d-alpha, d-beta, d-gamma, d-delta) 2 was developed for the Vitamin E capsule (Figure 6). This separation was accomplished using a 2.5 mL/min gradient of carbon dioxide and methanol, 96.2 to 99.5 over 1.5 minutes at 50 °C, with an ABPR setting of 1885 psi.

Figure 7. UPC2 Separation of a Vitamin E tablet.

Vitamin K1
The Vitamin K1 tablets generated 2 fully resolved peaks (Figure 7). A very rapid gradient analysis (~ 90 second run time) that easily provided baseline resolution of the 4 tocopherol isomers (d-alpha, d-beta, d-gamma, d-delta) 2 was developed for the Vitamin E capsule (Figure 6). This separation was accomplished using a 2.5 mL/min gradient of carbon dioxide and methanol, 96.2 to 99.5 over 1.5 minutes at 50 °C, with an ABPR setting of 1885 psi.

Figure 8. UPC2 Separation of a Vitamin K2 Capsule.

Vitamin K2
The Vitamin K2 consists of menaquinones (MKs) forms MK-3 through MK-14. The various forms of Vitamin K2 have different numbers of unsaturated isoprenoid units. This tablet formulation should have contained predominantly MK-7. No other MK forms were noted in the MS analysis.

CONCLUSIONS
Waters ACQUITY UPLC System was able to successfully analyze all 6 different formulations of fat soluble vitamins.
Each of the FSV formulations were analyzed rapidly with little or no sample pretreatment. A summary of the separation conditions is given in Table 1. For each of the isomers 2 columns were used to resolve each pair.

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