INTRODUCTION
The analysis of fat soluble vitamins (FSV) formulations, typically from capsules, can be a challenging task. Current methods employ normal phase and reverse phase liquid chromatography techniques as well as gas chromatography, thin layer chromatography, and colorimetric techniques for these analyses. The use of supercritical fluid chromatography (SFC) in fat-soluble vitamin analysis provides a viable alternative that lowers the use of organic solvents, provides faster analysis times, and maintains chromatographic data quality. SFC is generally considered a cost-effective, sustainable and green technology yet widespread adoption of analytical SFC has been hampered by instrumentation which does not perform to the standards established by modern HPLC systems. Using a newly designed analytical supercritical fluid chromatography system, the ACQUITY UPC2 system, a series of FSV formulations were analyzed. The formulations examined contained Vitamin A only, Vitamins A + D, Vitamin E, Vitamin K, Vitamins K + D, and Vitamin D only. Results from these experiments show SFC has the potential to replace many of the separation methods in use today.

METHODS

Samples
Fat soluble vitamin capsules were obtained from local stores. A total of 7 different formulations were examined and are listed below.
1. Vitamin A, 10,000 IU
2. Vitamin A+D3, 10,000, 400 IU
3. Vitamin E, 400 IU
4. Vitamin K1, 100 ug
5. Vitamin K2, 50 ug
6. Vitamin D3, 2000 IU
7. Vitamin D2+D3, 2500 IU, 90 ug

Sample Preparation
Oil filled capsules (sample 1, 2, 3, and 7) were opened and contents removed and dissolved in hexane. No further pretreatment was used. Tablet (sample 4) – crushed tablets were sonicated with hexane, following settling an aliquot of the extract was filtered directly into a sample vial through a 1.0 µm glass fiber filter.

System
ACQUITY UPC2 (figure 1) consisting of a UPC2 Binary Solvent Delivery System, the ACQUITY UPC2 system, a series of analytical supercritical fluid chromatography techniques as well as gas chromatography, thin layer chromatography, and colorimetric techniques for these analyses. The use of supercritical fluid chromatography (SFC) in fat-soluble vitamin analysis provides a viable alternative that lowers the use of organic solvents, provides faster analysis times, and maintains chromatographic data quality. SFC is generally considered a cost-effective, sustainable and green technology yet widespread adoption of analytical SFC has been hampered by instrumentation which does not perform to the standards established by modern HPLC systems. Using a newly designed analytical supercritical fluid chromatography system, the ACQUITY UPC2 system, a series of FSV formulations were analyzed. The formulations examined contained Vitamin A only, Vitamins A + D, Vitamin E, Vitamin K, Vitamins K + D, and Vitamin D only. Results from these experiments show SFC has the potential to replace many of the separation methods in use today.

RESULTS AND DISCUSSION

Data System, Empower III

Figure 1. Waters ACQUITY UPC2 System

Figure 2. UPC2 Separation of Vitamin A capsule

Figure 3. UPC2 Separation of Vitamin E capsule

Figure 4. UPC2 Separation of Vitamin K1 tablet

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 were noted and were well resolved from the excipient peaks (figure 2).

Vitamin E
A very rapid gradient analysis (~30 second run time) that easily separated the tocopherol isomers (α-, β-, γ-, δ-) was developed for the Vitamin E capsule.

Vitamin K1
The Vitamin K1 tablets generated 2 distinct peaks with a simple isocratic method. UV spectra (collected simultaneously along with the UV at 246 nm channel) of both peaks were similar indicating that the peaks were related. Although not confirmed (individual standards of each of the isomers were not available at time of analysis), it is likely that the 2 peaks are the cis and trans isomers of phylloquinone.

Vitamin K2
Vitamin K2 consists of menaquinone (MK) forms MK-3 through MK-9. The various forms of K2 have side chains lengths composed of a variable number of unsaturated isoprenoid units. This label formulation showed one predominant peak and several smaller ones (figure 6). UV spectra comparisons showed that the detected peaks were likely α related. UPC/MS analysis of this sample identified the major peak as MK-5 (m/z 511.4, ESI Neg. M-H). Interestingly, the label indicated that this formulation should have contained predominantly MK-7. It is unclear if the sample was mislabeled or degraded/damaged during processing.

Vitamin D3
Using the same separation method as A and A+D3, Vitamin D3 (cholecalciferol) was easily resolved from the capsule excipient material (primarily sunflower oil) in under 3 minutes (figure 7).

Vitamin D3 and K2
This formulation generated an unusual result. At least 8 K2 related compounds (based on UV spectral comparisons) were noted (figure 8) along with the D3 peak (2.3 minutes). UPC/MS analysis of this sample identified MK-5 (m/z 511.4, ESI Neg. M-H) but was unable to provide identifications of the other peaks in the sample. No effort was made to further resolve the peak groupings which would be helpful in determination of their identities.

CONCLUSION
- Waters ACQUITY UPC2 System was able to successfully analyze all 7 different formulation of fat soluble vitamins.
- Each of the FSV formulations were analyzed rapidly with components of interest resolved from excipient materials.
- This system can greatly streamline FSV analysis by allowing labs to use a single technique to analyze a wide range of FSV formulations.