Replicating Water and Fat-Soluble Vitamins Analyses on a Modern HPLC System

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INTRODUCTION

With the increasing popularity of vitamin supplements, there is a need to ensure the products meet the content descriptions. High performance liquid chromatography (HPLC) is an essential analytical tool to measure the supplements, ensuring products meet label claims. However, the wide range of chemical characteristics make analysis of vitamins with a single mode of chromatography challenging. For highly non-polar, fat-soluble vitamins (e.g., vitamin E) analysis by normal phase is a preferred mode of chromatography, while water-soluble vitamins are often analyzed by hydrophilic interaction chromatography (HILIC) to address retention of highly polar vitamins[1]. In addition, vitamins can pose many analytical challenges as the samples can come in complex matrices and sample preparation can often be laborious.

In regulated labs, the ability to migrate these methods to different systems can be essential as systems are updated and replaced with newer systems. In addition, having a single system that can perform both types of analyses, with minimal changes, is crucial. In this work, we will document the ability move methods for both water-soluble vitamins under gradient HILIC conditions and fat-soluble vitamins under normal phase conditions across systems. This work will demonstrate the ability to achieve the same quantitative results on legacy HPLC systems and newer LC systems. In this work, the legacy Alliance™ 2695 System and the Alliance iS HPLC System were chosen for method migration.

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SAMPLE PREPARATION AND LC METHODS

Water-Soluble Vitamins

Ten (10) vitamin supplement tablets were placed into a 50 mL centrifuge tube with 25 mL of water and shaken for 30 mins. Sample were then centrifuged for 10 minutes at 3900 rpm. The supernatant was collected into a clean 50 mL centrifuge tube and diluted 1:1 with acetonitrile. Additional sample dilutions were required. Additional sample cleanup may be required for more complex matrices[2].

LC Systems	Alliance 2695 and Alliance iS HPLC Systems with TUV Detector
Column	XBridge™ Amide Column, 4.6 x 250 mm, 3.5 µm
Mobile Phase A	100 mM Ammonium Acetate, pH 5.5
Mobile Phase B	Acetonitrile
Mobile Phase C	Water
Wash Solvent	50:50 Water:Acetonitrile
Injection Volume	25.0 µL
Column Temp	30.0°C
Sample Temp	15.0°C
Detector	UV: λ = 265 nm; 2 Hz
Separation	Gradient, 30 min at 1 mL/min

Time	%A	%B	%C	Curve
0.00	15.0	80.0	5.0	Initial
4.00	15.0	80.0	5.0	6
19.00	15.0	55.0	30.0	6
19.10	15.0	80.0	5.0	6
30.00	15.0	80.0	5.0	6

Fat-Soluble Vitamins

Multivitamin tablets were ground into a fine powder using a mortar and pestle. Hexanes were added to the ground sample and the sample mixed by vortexing at 3000 rpm for 2 minutes. An aliquot of the mixed sample was centrifuged, and the supernatant drawn off and analyzed.

Vitamin E softgel capsules were cut in half and extracted into 100% hexanes by vortexing at 3000 rpm for 2 minutes. An aliquot of the extract was quantitatively diluted in 100% hexanes and injected.

LC Systems	Alliance 2695 and Alliance iS HPLC Systems with TUV Detector
Configuration	Alliance iS: Normal Phase kit (p/n 205002529)
Column	XBridge BEH™ HILIC Column, 4.6 x 150 mm, 5 µm
Separation	Isocratic, 10 min
Flow Rate	1.3 mL/min
Mobile Phase	95% Hexanes, 5% Ethyl Acetate, 0.05% Acetic Acid
Injection Volume	30.0 µL
Column Temp	40.0°C
Sample Temp	10.0°C
Detector	UV: λ = 295 nm; 10 Hz

RESULTS AND DISCUSSION

Water-Soluble Vitamins

As described previously, a HILIC gradient method was used for analysis of water-soluble vitamins. The method was first tested on the Alliance 2695 System to ensure separation of standards (Figure 1). The analysis was reproduced on the Alliance iS HPLC System (Figure 2). In addition, water-soluble vitamin supplement sample containing B_{12}, and folic acid was analyzed. The quantitative results were compared to the label claims of the water-soluble vitamin supplement sample, with both systems providing comparable results within expected label claims (Table 1).

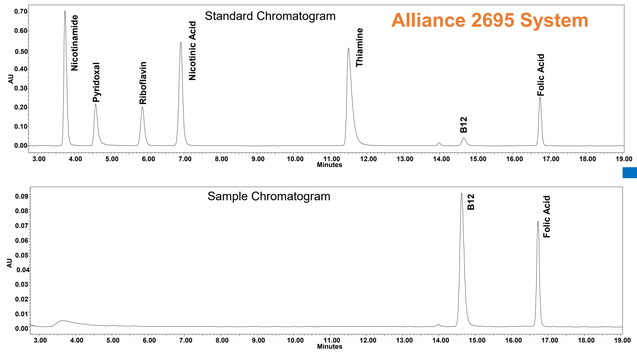

Figure 1. Alliance 2695 System Standard and Sample Chromatograms.

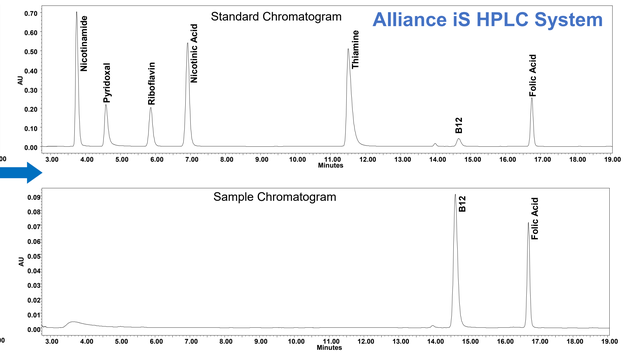

Figure 2. Alliance iS HPLC System Standard and Sample Chromatograms.

Table 1. Alliance 2695 System and Alliance iS HPLC System water-soluble sample results vs label claim.

	Alliance 2695 System			Alliance iS HPLC System			
Water Soluble Vitamins	Label Claim in Weighed Sample Amount (mg)	Results (mg)	**Difference between Label Claim vs. Results**	Label Claim in Weighed Sample Amount (mg)	Results (mg)	**Difference between Label Claim vs. Results**	**Result Differences between Systems**
B12	50.00	53.44	**6.9%**	50.00	53.28	**6.6%**	**0.30%**
Folic Acid	7.60	7.03	**-7.7%**	7.60	6.95	**-8.6%**	**1.20%**

Fat-Soluble Vitamins

A normal phase method was developed for the determination of four tocopherol isomers (α, ß, γ, δ). The analysis involved a simple extraction and direct analysis of the extracts by normal phase chromatography. This method was migrated from an Alliance 2695 system to an Alliance iS HPLC system and comparable system suitability results were obtained (Figure 3). The migrated method was then used for the determination of tocopherols in a naturally sourced multivitamin tablet and in vitamin E softgel supplements (Figure 4).

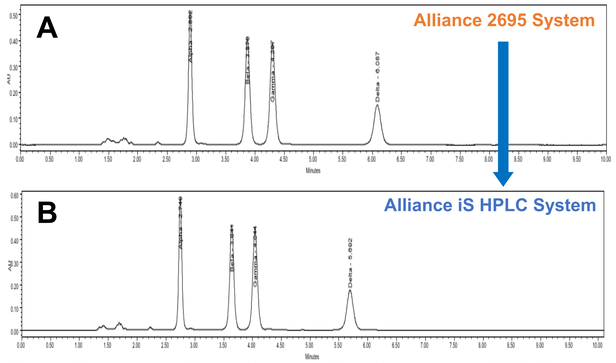

Figure 3. Standard Chromatograms From the Alliance 2695 System (**A**) and the Alliance iS HPLC System (**B**).

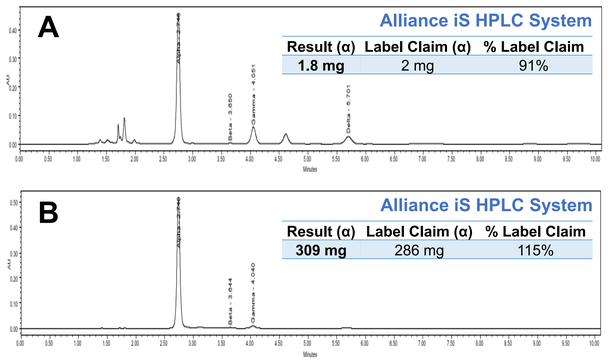

Figure 4. Multivitamin Tablets Samples (**A**) and Vitamin E Softgel Capsules (**B**) on the Alliance iS HPLC System and % Label Claims.

CONCLUSIONS

- **A HILIC method for water-soluble vitamins and a normal phase method for fat-soluble vitamins were successfully migrated from the Alliance 2695 System to the Alliance iS HPLC System.**
- **The Alliance iS HPLC System was easily configured for normal phase chromatography using check valves and outlet tubing.**
- **Water-soluble vitamin and fat-soluble vitamin samples were successfully analyzed on the Alliance iS HPLC System, providing good agreement with sample label claims for both methods.**

REFERENCES

1. **Eric S. Grumbach and Kenneth J. Fountain. Comprehensive Guide to HILIC Hydrophilic Interaction Chromatography. Waters Corporation, 2010.**
2. **Kim Tran and Peter Handcock. Analysis of Water-Soluble Vitamins and Caffeine in Beverage and Multivitamin Products by Arc HPLC System With PDA Detection. Waters Application Note, 720007357EN. 2021.**
3. **Waters Corporation Technology Brief (2010). Normal-Phase Separation of Tocopherols with the ACQUITY H-Class System featuring Auto Blend Technology, 720003690EN.**