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.

SAMPLE PREPARATION AND LC METHODS

Fat-Soluble Vitamins

Two dosage forms were analyzed, multivitamin tablets and a Vitamin E softgel. 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

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 50 mL centrifuge tube and diluted 1:1 with acetonitrile.

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

Additional sample dilutions were required. Additional sample cleanup may be required for more complex matrices[2].

alliance iS

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). System suitability results were comparable between the two systems (Table. 1).

Parameter	Acceptance Criteria	Alliance e2695	Alliance iS HPLC System
Peak Retention Time			
Vitamin B_{12} % RSD	≤2.0	0.064	0.022
Folic Acid % RSD	≤2.0	0.052	0.015
Peak Area			
Vitamin B_{12} % RSD	≤2.0	0.077	0.028
Folic Acid % RSD	≤2.0	0.298	0.026
USP Resolution Between Pyridoxal and Nicotinamide	≥2.0	4.531	5.243

Table 1. Water-Soluble Vitamin System Suitability Results for the Alliance e2695 System and the Alliance iS HPLC System.

Analysis of vitamins in supplements is critical to ensure product safety and accuracy of the product's label claim. A multivitamin sample containing both vitamin B_{12} and folic acid was analyzed on the Alliance e2695 and the Alliance iS HPLC System.

The analysis on each system used the same preparation of mobile phase, standard, and sample, as well as the same chromatographic column.

The results from both systems were compared to the label claim of the multivitamin supplement sample along with a comparison of the results between the two systems.

Example chromatograms of the vitamin standard and the multivitamin supplement sample for both systems are shown in Figures 1 and 2.

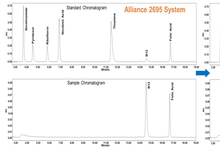

Figure 1. Alliance 2695 System Standard and Sample Chromatograms.

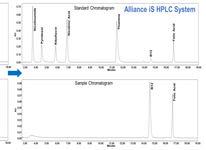

Figure 2. Alliance iS HPLC System Standard and Sample Chromatograms.

RESULTS AND DISCUSSION

Water-Soluble Vitamins

In addition, a 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 2).

The method was successfully migrated from the Alliance e2695 system to the Alliance iS HPLC system, as all the system suitability criteria were met without additional method adjustments.

The systems showed a slight shift in retention time. The Alliance e2695 system had retention times slightly earlier than the Alliance iS HPLC system, which can be seen in Figures 1 and 2. The delay volume differences between the two systems can contribute to this retention time shift.

Table 2. 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%

The % deviation from nominal concentration and R^2 results for both systems are shown in Table 3. The calibration curve R^2 values were 0.9999 and the % deviation from nominal concentration was within ± 1.75 for both systems.

Alliance e2695 System % Deviation at concentration level (µg/mL)							
Compound	R^2	1.57	3.13	6.25	12.5	25	50
Vitamin B_{12}	0.9999	0.49	-0.41	-0.50	0.07	0.58	-0.24
Folic Acid	0.9999	0.90	-1.47	0.05	0.27	0.51	-0.27

Alliance iS HPLC System % Deviation at concentration level (µg/mL)							
Compound	R^2	1.57	3.13	6.25	12.5	25	50
Vitamin B_{12}	0.9999	1.66	-0.11	-0.74	-0.74	-0.62	0.54
Folic Acid	0.9999	-1.72	-0.30	1.09	0.93	0.61	-0.60

Table 3. Vitamin B_{12} and Folic Acid Standards (1.57-50 µg/mL) R^2 and % deviation at concentration level linearity results on the Alliance e2695 System and Alliance iS HPLC System..

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 (Table 4 and Figure 3).

RESULTS AND DISCUSSION

Fat-Soluble Vitamins

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).

Parameter	Acceptance Criteria	Alliance e2695	System Y	Alliance iS HPLC System
α Peak Area %RSD	≤2.0 %	0.1	0.2	0.1
β Peak Area %RSD	≤2.0 %	0.1	0.2	0.1
γ Peak Area %RSD	≤2.0 %	0.1	0.2	0.1
δ Peak Area %RSD	≤2.0 %	0.0	0.1	0.0
USP Resolution (β - α)	≥5.0	7.0	6.7	7.8
USP Resolution (γ - β)	≥1.5	2.7	2.7	2.9
USP Resolution (δ - γ)	≥7.0	8.5	8.9	9.2

Table 4. Fat-Soluble Vitamin System Suitability Results for the Alliance e2695 System and the Alliance iS HPLC System.

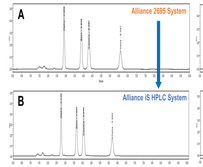

Figure 3. Standard Chromatograms From the Alliance 2695 System (**A**) and the Alliance iS HPLC System (**B**).

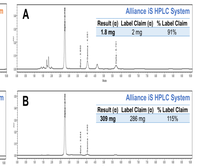

Figure 4. Multivitamin Tablets Samples (**A**) and Vitamin E Softgel Capsules (**B**) on the Alliance iS HPLC System and % Label Claims.

Comparable chromatography was obtained from the systems. System suitability criteria were also met on each of the systems.

Peak area precision was similar across the systems with the Alliance iS HPLC system and the legacy Alliance e2695 having equivalent precision for all tocopherols in the standard.

USP Resolution was slightly improved on the Alliance iS HPLC system. Since the results obtained met the specified acceptance criteria, the migration of the method to the Alliance iS HPLC system was defined as a success.

After the method was migrated to the Alliance iS HPLC system, it was used for the determination of tocopherols in two dietary supplement dosage forms - naturally sourced multivitamin tablets and in Vitamin E softgel capsules. This analysis involved a simple extraction and direct analysis of the extracts using a single point external standard for quantitation.

CONCLUSION

- Analysis of vitamin content in multivitamin supplements is critical to ensure accuracy of the product's label claim. While analysis of multivitamin supplements can pose certain challenges, the methods described here produced reproducible and reliable results on both the legacy Alliance e2695 and modern Alliance iS HPLC Systems.
- The close agreement of quantitative results obtained on the systems, within 1.2%, demonstrates successful migration of a HILIC method to a modern HPLC system.
- Quantification of the multivitamin supplement showed good agreement for water-soluble vitamins, within ± 9% of the label claim.
- 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 configured for normal phase chromatography by the easy replacement of check valves and waste 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

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