Simultaneous determination of water- and fat-soluble vitamins in tablets and energy drinks by using a novel Vanquish Flex Duo system for Dual LC

Authors

Sylvia Grosse, Mauro De Pra, Frank Steiner
Thermo Fisher Scientific, Germering, Germany

Keywords

Water-soluble vitamins, fat-soluble vitamins, food quality, food safety, food, beverages, supplements, Acclaim PolarAdvantage II column, Vanquish Flex Duo system for Dual LC

Application benefits

• Workflow provides quantitative analysis of fat-soluble and water-soluble vitamins in drinks and food supplement tablets using the Thermo Scientific™ Vanquish™ Flex Duo UHPLC system for Dual LC.

• Workflow enables running two separate methods and columns in one instrument, simultaneously.

Goal

To describe a workflow for the simultaneous quantitative analysis of fat-soluble and water-soluble vitamins in drinks and food supplement tablets using the Vanquish Flex Duo system for Dual LC and the Thermo Scientific™ Acclaim™ PolarAdvantage II column.

Introduction

Vitamins are essential nutrients found in various natural food sources and food supplements. Vitamins can be classified as water-soluble vitamins (WSV) or fat-soluble vitamins (FSV), based on their hydrophobicity. Reversed-phase high-performance liquid chromatography is widely used to determine vitamins in food, supplements, and beverages. Because of the dramatically different hydrophobicity of WSV and FSV, simultaneous liquid chromatography analysis with the same method is difficult. While some WSV are poorly retained in

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reversed-phase even with very weak mobile phases, the FSV will elute only with very strong solvents, approaching 100% organic. A reversed-phase method, applicable for a wide range of polarities and at the same time providing an acceptable compromise in terms of resolution and runtime, is not easily implemented. A method for the separation of FSV and WSV was proposed where two columns were operated sequentially in one system.[1] For this approach, a conventional LC system had to be fitted with a pair of switching valves to connect the selected column to the injector and UV detector. The method was effective since the analysis of both vitamin classes could be run unattended. However, this solution required complex hardware setup, and even more complex chromatography data system programming. Additionally, since the columns were run after the other, throughput was low.

Other advanced applications are reported in the literature for the simultaneous analysis of both vitamin classes. For instance, one approach for the separation of FSV and WSV in the same column and with the same method made use of delayed injection of the sample expected to contain FSV.[2] This way, the highly hydrophobic FSV entered the column only when the strength of the mobile phase was sufficiently high. The method depended on the flexibility of the chromatography data system and of the UHPLC system. The complexity of programming and instrument setup made the implementation of this approach somewhat cumbersome.

In this work we introduce an effective workflow for the simultaneous analysis of WSV and FSV that overcomes challenges of all previously reported approaches. The workflow is based on a novel Vanquish Flex Duo system for Dual LC. The system consists of a Dual Pump F with two independent flow paths, a Dual Split Sampler FT with two separate injection ports and sample loops, one—or optionally two—Column Compartments H, and two detection systems. It enables the independent and simultaneous use of two different columns and methods. Thanks to these advanced features, two independent methods were developed and optimized for FSV and WSV and run simultaneously. The Dual LC workflow delivers much higher throughput compared to the column switching approach mentioned previously.[1] Moreover, potential issues such as incompatibility of the mobile phases in the shared flow path are eliminated—one example of such an issue is precipitation of buffer salts in high-organic solvent. Compared to the former solution,

the approach with the Vanquish Flex Duo system for Dual LC presented here is remarkably simple to implement, allows the use of better optimized methods, and increases throughput thanks to the simultaneous use of two columns with two methods and faster analysis cycles.

For both vitamin classes, the reversed-phase column chosen to run the Dual LC workflow is the Acclaim PolarAdvantage II (PA2) column. The column features a polar-embedded stationary phase that effectively operates over a wide range of mobile phase conditions and is highly suitable for the separation of components with very diverse hydrophobicity, such as vitamins.

Experimental
Chemicals
- Deionized (DI) water 18 MΩ·cm resistivity or higher

- Acetonitrile, Optima™ LC/MS grade (Fisher Scientific™ P/N 100001334)

- Methanol, Optima™ LC/MS grade (Fisher Scientific P/N 10767665)

- Acetic acid, Optima™ LC/MS grade (Fisher Scientific P/N 10860701)

- Ortho-phosphoric acid, HPLC grade (P/N 10644732)

- DMSO, HPLC grade, (P/N 10387791)

- Hexane, HPLC grade, (P/N 10703611)

- Ethanol, HPLC grade, (P/N 10542382)

- Acetone, HPLC grade, (P/N 10131560)

- Potassium dihydrogen phosphate, p.A. (purchased from a reputable vendor)

- Potassium hydroxide, p.A. (purchased from a reputable vendor)

- Potassium bicarbonate, p.A. (purchased from a reputable vendor)

- Water-soluble vitamins: ascorbic acid, cyanocobalamine, folic acid, nicotinamide, pantothenic acid, pyridoxal, pyridoxine, thiamine, riboflavin; analytical grade (purchased from a reputable vendor)

- Fat-soluble vitamins: cholecalciferol, ergocalciferol, phyllochinone, α-tocopherole, α-tocopherole acetate, δ-tocopherole, γ-tocopherole, retinol, retinol acetate, retinol palmitate; analytical grade (purchased from a reputable vendor)

Equipment

- pH meter, Thermo Scientific™ Orion™ 3-Star

- Ultrasonic bath with temperature regulation, Fisher Scientific

- Magnetic stirrer, Fisher Scientific

- Syringe filter, Minisart® cellulose acetate (CA) (Ø 26 mm; 0.45 µm pore size), (purchased from a reputable vendor)

- Conical tubes (50 mL), Fisher Scientific (P/N 10788561)

- Vials (amber, 2 mL), Fisher Scientific (P/N 15508760)

- Septa (Silicone/PTFE), Fisher Scientific (P/N 11548180)

Preparation of standards

Stock solutions of each water-soluble vitamin (thiamine, ascorbic acid, pyridoxal, pyridoxin, nicotinamide, pantothenic acid, and cyanocobalamin) at a concentration of 1 mg/mL were prepared using water. Folic acid was dissolved in 20 mM $KHCO_3$ and riboflavin in 5 mM KOH, as they are not stable in pure water.

Stock solutions of each fat-soluble vitamin (retinol, retinol acetate, retinol palmitate, ergocalciferol, cholecalciferol, δ-tocopherole, γ-tocopherole, α-tocopherole, and α-tocopherole acetate) at a concentration of 1 mg/mL were prepared using methanol. Menaquinone and phyllochinone were prepared using acetone instead of methanol due to better solubility.

Calibration standards were prepared at eight concentrations—100, 50, 25, 10, 5, 1, 0.5, and 0.1 µg/mL—using water for the water-soluble vitamins and methanol for the fat-soluble ones.

Thiamine was calibrated using the standard addition method in the concentration range of 5 µg/mL to 100 µg/mL by adding the appropriate amount of the 1 mg/mL stock solution to the extract of the prepared sample of WSV.

All standards and samples were filled into HPLC brown glass vials and sealed properly to protect the solutions from light and evaporation.

Sample preparation of a vitamin tablet and an energy drink

The vitamin tablet and the placebo tablet (used as a matrix blank and for the determination of recovery) were bought from a local pharmacy. The energy drink was purchased from a local supermarket.

A slightly different sample preparation protocol[3] is here described.

Water-soluble vitamins in the vitamin tablet

One vitamin tablet was weighed and ground with a mortar and pestle. The powder was transferred into a 250 mL bottle and the weight noted. Ten milliliters of DMSO were added to the bottle and then sonicated for 2 min. Next, 90 mL of 2% acetic acid in water were added to the solution and then stirred for 1 min. Afterwards, the sample was extracted by sonication; for this purpose, the sonicator was set at 40 °C for 5 min. The solution was then filtered through a CA syringe filter with 0.45 µm pore size. An aliquot was diluted 1:10 with water. The undiluted and diluted solutions were used for quantification, depending on the expected amount of analyte in the sample.

The same procedure was followed with a placebo tablet. The placebo tablet was analyzed without adding vitamin standard for matrix background subtraction. In addition, it was spiked with 10 µg/mL of a vitamin standard mixture to calculate the recovery rate of each analyte. For this purpose, 10 mL of a 100 µg/mL standard solution were added to the powdered tablet, shaken, and then incubated without shaking for 3 min. Afterwards, 10 mL of DMSO and 80 mL of 2% acetic acid solution were added so that the final sample volume was the same volume as the real samples. The sample preparation procedure was the same as described for the vitamin tablet.

All samples were prepared in three replicates and analyzed immediately after preparation.

Water-soluble vitamins in the beverage

The energy drink was sonicated for 15 min to degas. An aliquot was taken and 1:10 diluted with water. The solution was filtered through a CA syringe filter with 0.45 µm pore size directly into the HPLC vial for injection. Recovery rates were not assessed.

Fat-soluble vitamins in the vitamin tablet

One vitamin tablet was weighed and ground with a mortar and pestle. The powder was transferred into a 250 mL bottle and the weight noted. Eight milliliters of 80% ethanol solution were added, followed by 10 mL of hexane. The solution was shaken by hand for 10 s and extracted in a sonication bath set at 40 °C for 10 min. The solution was then transferred into a 50 mL conical tube for easier layer separation. The hexane layer was taken and filtered through a CA syringe filter with 0.45 µm pore size. One aliquot was used directly for injection and a second aliquot of the hexane layer was diluted 1:10 with hexane. The undiluted and diluted solutions were used for quantification, depending on the amount of analyte in the sample.

The same procedure was taken with a placebo tablet. The placebo tablet was analyzed without adding vitamin standard for matrix background subtraction. In addition, it was spiked with 18 µg/mL of a vitamin standard mixture to calculate the recovery rate of each analyte. For this purpose, 1.8 mL of 100 µg/mL standard solution were added and let stand for 3 min. Afterwards 6.2 mL of 80% ethanol solution and 10 mL of hexane were used to come up to the same volume as used for the real samples. The procedure was followed as described for the vitamin tablet.

All samples were prepared in three replicates and analyzed immediately after preparation.

Instrumentation

Vanquish Flex Duo system for Dual LC consisting of:

- System Base (P/N VF-S01-A-02)

- Dual Pump F (P/N VF-P32-A-01)

- Dual Split Sampler FT (P/N VF-A40-A-02)

- Column Compartment H (P/N VH-C10-A-02) with 2 passive pre-heaters, 1 µL (P/N 6732.0174)

- 2 Diode Array Detectors HL (P/N VH-D-10-A) each equipped with a 10 mm Thermo Scientific™ LightPipe™ Flow Cell (P/N 6083.0100)

Data acquisition and processing was done with Thermo Scientific™ Chromeleon™ 7.2.8 Chromatography Data System (CDS) software.

Chromatographic method

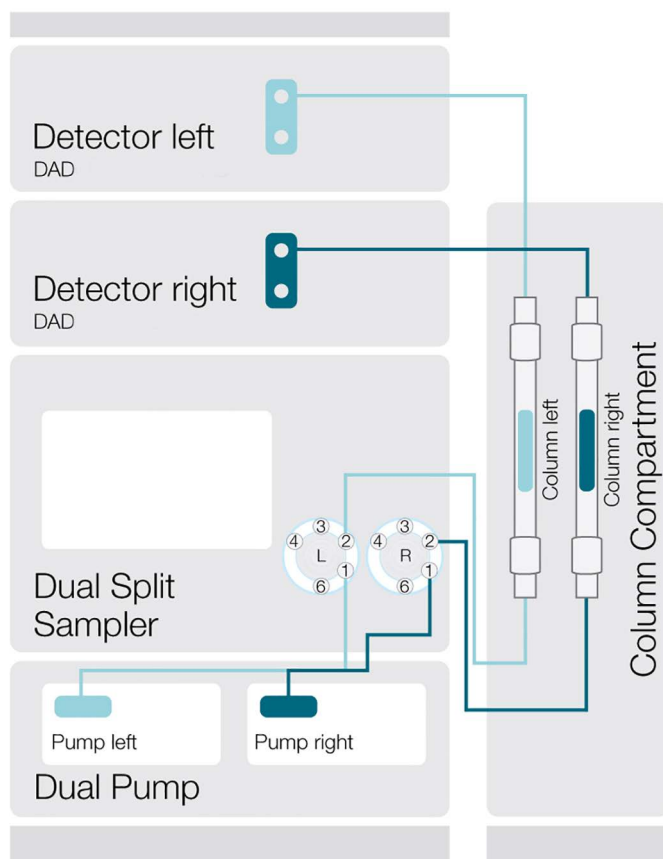Figure 1 illustrates the schematic fluidic setup of the Dual LC system used in this study.

Figure 1. Fluidic setup of Vanquish Flex Duo system for Dual LC.
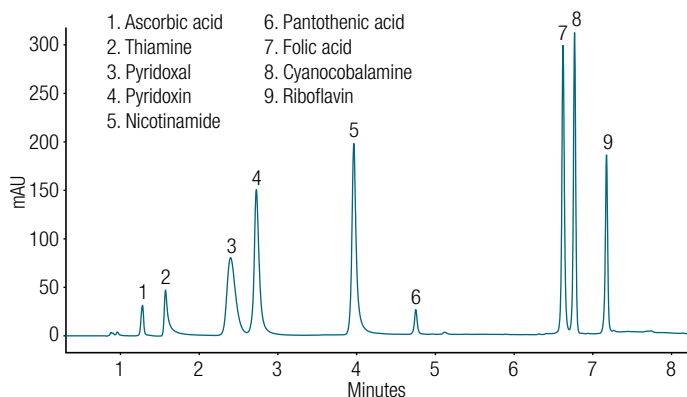
Table 1. Chromatographic conditions for WSV.

Column:	Acclaim RSLC PolarAdvantage II (150 × 2.1 mm, 2.2 μm, 120 Å) (P/N 071401)	
Mobile phase A:	25 mM potassium dihydrogen phosphate, pH 3.8 (adjusted with phosphoric acid)	
Mobile phase B:	70:30 acetonitrile/25 mM potassium dihydrogen phosphate, pH 3.8	
Gradient:	*Time (min)*	*B (%)*
	0	0
	5	36
	7	36
	10	100
	14	0
	25	0
Flow rate:	0.4 mL/min	
Temperature:	25 °C (with passive pre-heater)	

Detection Parameters

Channel	Wavelength [nm]
1	270
2	210
3	245
4	280
3D scan:	190–360 nm
Data collection rate:	10 Hz
Response time:	0.5 s

Other Parameters

Injection volume:	1 μL
Tray temperature:	5 °C
Needle wash solvent:	50% methanol
Needle wash mode:	Both (before and after draw)

Table 2. Chromatographic conditions for FSV.

Column:	Acclaim RSLC PolarAdvantage II (250 × 2.1 mm, 2.2 μm, 120 Å) (P/N 074814)	
Mobile phase A:	DI water	
Mobile phase B:	Methanol	
Gradient:	*Time (min)*	*B (%)*
	0	90
	2	96
	7.5	96
	8.6	100
	15	100
	15.1	90
	25	90
Flow rate:	0.4 mL/min	
Temperature:	25 °C (with passive pre-heater)	

Detection Parameters

Channel	Wavelength [nm]
1	325
2	265
3	290
4	280
3D scan:	240–350 nm
Data collection rate:	10 Hz
Response time:	0.5 s

Other Parameters

| Injection volume:	1 μL
Tray temperature:	5 °C
Needle wash solvent:	50% methanol
Needle wash mode:	Both (before and after draw)

Results and discussion

Figure 2 illustrates the separation of a WSV standard on an Acclaim PA2 column with detection at 210 nm. The polar analytes, ascorbic acid and thiamine (peak 1 and 2), are usually poorly retained and are not resolved with conventional reversed-phase columns, but the pair shows adequate retention and resolution with the Acclaim PA2 column. The other critical peak pair, folic acid and cyanocobalamin (peak 7 and 8), is also well separated with Rs > 2.0 (Table 5).

Some low-intensity unassigned peaks can be observed both in Figure 2 and Figure 3. The peaks were regarded as unknown impurities found in some of the standard vitamins.

As most of the vitamins show different UV absorption, the detection wavelength needs to be optimized to ensure high sensitivity quantitation of all components. The wavelengths used for quantification can be found in Table 3 and Table 4.

Figure 2. Separation of a water-soluble vitamins standard on an Acclaim PA2 column (150 × 2.1 mm, 2.2 µm) recorded at 210 nm.

Table 3. UV wavelength used for quantification of WSV.

Peak	Common Name	Chemical Name	UV [nm]
1	Vitamin C	Ascorbic acid	245
2	Vitamin B1	Thiamine	245
3	Vitamin B6-derivate	Pyridoxal	210
4	Vitamin B6	Pyridoxin	210
5	Vitamin B3	Nicotinamide	210
6	Vitamin B5	Pantothenic acid	210
7	Vitamin B9	Folic Acid	280
8	Vitamin B12	Cyanocobalamin	210
9	Vitamin B2	Riboflavin	270

The separation of a FSV standard is shown in Figure 3. The resolution was higher than 2 for most pairs. The only exceptions are the pair menaquinone – δ-tocopherole (peaks 5 and 6, Rs=1.4) and α-tocopherol acetate – γ-tocopherol (peaks 7 and 8, Rs=1).
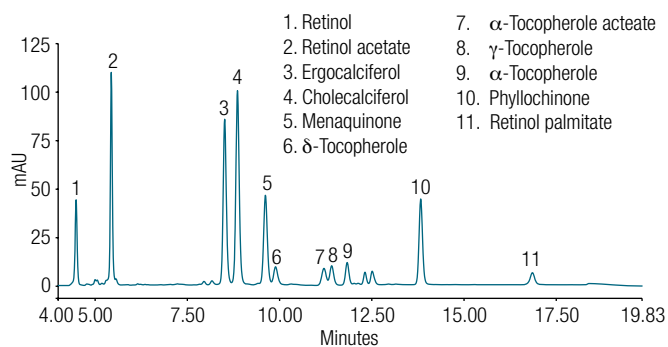
Figure 3. Separation of a fat-soluble vitamins standard on an Acclaim PA2 column (250 × 2.1 mm, 2.2 µm) recorded at 280 nm.

Table 4. UV wavelength used for quantification of FSV.

Peak	Common Name	Chemical Name	UV [nm]
1	Vitamin A	Retinol	325
2	Vitamin A-derivate	Retinol acetate	325
3	Vitamin D2	Ergocalciferol	265
4	Vitamin D	Cholecalciferol	265
5	Vitamin K2	Menaquinone	265
6	Vitamin E	δ-Tocopherole	290
7	Vitamin E-derivate	α-Tocopherole acetate	280
8	Vitamin E	γ-Tocopherole	290
9	Vitamin E	α-Tocopherole	290
10	Vitamin K1	Phyllochinone	265
11	Vitamin A-derivate	Retinol palmitate	325

Method precision

Repeatability tests were carried out by injecting 1 µL of 100 µg/mL standard mixtures of fat- and water-soluble vitamins ten times each. The results are summarized in Table 5 and Table 6.

Table 5. Chromatographic results of 10 consecutive injections of a water-soluble vitamins standard mixture with a concentration of 100 µg/mL on an Acclaim PA2 column.

WSV	RT Avg	RT RSD	Area RSD	Resolution
Ascorbic acid	1.27	0.00%	2.69%	3.2
Thiamine	1.50	0.05%	0.28%	6.1
Pyridoxal	2.39	0.04%	0.11%	2.2
Pyridoxin	2.74	0.03%	0.13%	12.2
Nicotinamide	3.95	0.03%	0.12%	10.7
Pantothenic acid	4.76	0.03%	0.12%	8.6
Folic acid	6.59	0.02%	0.13%	2.6
Cyano-cobalamin	6.73	0.01%	0.12%	8.0
Riboflavin	7.14	0.02%	0.11%	n.a.

n.a. = not available

Table 6. Chromatographic results of 10 consecutive injections of a fat-soluble vitamins standard mixture with a concentration of 100 µg/mL on an Acclaim PA2 column.

FSV	RT Avg	RT RSD	Area RSD	Resolution
Retinol	4.49	0.14%	0.57%	6.0
Retinol acetate	5.44	0.09%	0.37%	25.1
Ergocalciferol	8.52	0.10%	0.34%	2.2
Cholecalciferol	8.87	0.11%	0.34%	4.4
Menaquinone	9.63	0.11%	0.31%	1.4
δ-Tocopherole	9.90	0.13%	0.35%	6.1
α-Tocopherole acetate	11.22	0.13%	0.28%	1.0
γ-Tocopherole	11.43	0.12%	0.31%	2.3
α-Tocopherole	11.85	0.10%	0.28%	3.1
Phyllochinone	13.84	0.07%	0.31%	15.1
Retinol palmitate	16.86	0.05%	0.81%	n.a.

n.a. = not available

Both methods show good repeatability. The retention time relative standard deviation (RT RSD) was extremely low for the WSV runs, with values below 0.05%. The retention time of FSVs was slightly less precise, due to the shallow gradient required to achieve good resolution, but still satisfactory. For FSV, the retention time RSD was on the order of 0.1% for most of the peaks.

Peak area precision for the WSV was excellent. The RSD was below 0.3% for all peaks except ascorbic acid, which was 2.7%. The relatively low area precision of the ascorbic acid peak is related to the inherent chemical instability of the molecule, which is sensitive to both temperature and light. This is confirmed by the results of these 10 repetitive injections, where the peak area of ascorbic acid steadily decreased injection after injection. The area RSD of the FSV was in general below 0.5%, and slightly higher for retinol and retinol-palmitate.

Quantitative determination of water- and fat-soluble vitamins in a vitamin tablet and an energy drink

As mentioned in the experimental description above, the quantitation was performed with external standard calibration for all vitamins except thiamine. Thiamine required a different approach because its peak shape was strongly affected by the sample matrix. When thiamine was dissolved in water, the peak was broad and strongly tailed; when the sample was dissolved in the processed placebo, the peak was sharper and integration was easier (results not shown). Additionally, the peak area of a given amount of thiamine was larger when thiamine was dissolved in placebo solution rather than in water. An explanation of this behavior was not found; however, given the strong influence of the sample matrix on the peak shape, a calibration by standard addition was considered more reliable.

The calibration range of the analytes was adjusted based on the expected amounts of vitamins in the samples. Table 7 shows the results of the calibration of water-soluble vitamins. The regression coefficients were 0.999 or higher for most vitamins, indicating good linearity.

Table 7. Calibration parameters, sensitivity, and recovery rates for WSVs.

WSV	Calibration Range [µg/mL]	Regression	Recovery [%]	LOD [µg/mL]	LOQ [µg/mL]
Ascorbic acid	1–100	0.9955	63	0.019	0.063
Thiamine	5–100	0.9990	22	0.195	0.649
Pyridoxal	5–100	0.9996	101	0.043	0.143
Pyridoxin	1–100	0.9999	100	0.038	0.125
Nicotinamide	1–100	0.9999	101	0.019	0.063
Pantothenic acid	1–100	0.9999	100	0.052	0.172
Folic acid	0.1–100	0.9999	61	0.009	0.029
Cyanocobalamin	5–100	0.9999	105	0.021	0.071
Riboflavin	1–100	0.9929	63	0.008	0.025

The recovery was in general excellent for the vitamins known to be chemically stable. Recovery values were low for ascorbic acid, folic acid, and riboflavin. The low recovery for these molecules can be explained by the low chemical stability. In order to achieve higher recovery values for these unstable molecules, an optimized sample preparation strategy should be developed. For the purpose of this work, the low recovery of the instable molecules was considered acceptable, since the determined amounts were well in agreement with the amount described in the label of the analyzed tablet (Table 9). Thiamine showed low recovery as well. The molecule is considered stable at the conditions of the sample preparation, and the low recovery has a different root cause that could not be pinpointed in the present work. However, similarly to the rest of vitamins, the final calculated amount was in agreement with the labeled amount; therefore, the method was considered acceptable.
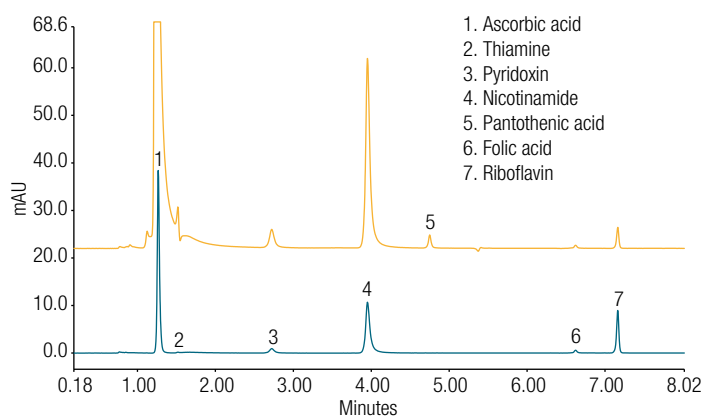
The FSVs show recovery rates between 81% and 121%, except for retinol, which was 33% (Table 8). Retinol was not detected in the food supplement. In general recovery rates were similar to those reported previously,[3] where a similar sample preparation method is used.

Table 8. Calibration parameters, sensitivity, and recovery rates of FSVs.

FSV	Calibration Range [µg/mL]	Regression	Recovery [%]	LOD [µg/mL]	LOQ [µg/mL]
Retinol	0.5–100	0.9999	33	0.011	0.036
Retinol acetate	0.1–100	0.9999	90	0.005	0.016
Ergocalciferol	0.1–100	1.0000	86	0.016	0.053
Cholecalciferol	0.1–100	0.9999	92	0.016	0.053
Menaquinone	0.5–100	0.9999	105	0.023	0.077
δ-Tocpherole	5–100	0.9999	84	0.150	0.500
α-Tocpherole acetate	25–100	0.9997	121	0.500	1.667
γ-Tocopherole	10–100	0.9985	110	0.300	1.000
α-Tocpherole	10–100	0.9999	102	0.150	0.500
Phyllochinone	1–100	0.9999	105	0.025	0.083
Retinol palmitate	1–100	0.9999	81	0.038	0.125

The whole vitamin tablet was ground and analyzed in triplicate. In a similar manner, a placebo tablet was prepared and its chromatogram used for sample baseline correction. By doing so, matrix and system peaks could be removed from the chromatogram. Figure 4 demonstrates the separation of water-soluble vitamins in the tablet at two different wavelengths (210 nm and 270 nm). Quantitative results can be seen in Table 9.

Figure 4. Separation of WSV in tablet. Orange trace: 210 nm; blue trace: 270 nm.

1. Ascorbic acid
2. Thiamine
3. Pyridoxin
4. Nicotinamide
5. Pantothenic acid
6. Folic acid
7. Riboflavin

Table 9. Quantitative results of WSV in tablet. The measured amount was corrected by recovery rate.

WSV	Stated Amount on Label [mg/tablet]	Measured Amount [mg/tablet]
Ascorbic acid	60	61.6
Thiamine	1.4	1.1
Pyridoxal	-	< 0.005*
Pyridoxin	2	2.5
Nicotinamide	18	19.9
Pantothenic acid	6	10.5
Folic acid	0.2	0.4
Cyanocobalamin	0.001	< 0.002*
Riboflavin	1.6	3.6

* Peak not observed: value estimated with the LOD corrected by recovery rate

Generally higher amounts were measured than those reported in the product label. This apparent mismatch can be explained by the common practice of nutraceutical producers to exceed the declared amount. In this way, the producer can guarantee the labeled amount of vitamin is in the product even as it degrades during the product lifetime. The amount of cyanocobalamin in the sample was less than the limit of detection and could not be determined with this method. The sensitivity of UV detectors is often not sufficient in the sub ng/mL range. To quantify at such a low level, alternative approaches such as sample enrichment are required.[1]

Results of the FSV show some discrepancies with the ingredient content declared by the vendor (Figure 5 and Table 10). For example, the ingredient leaflet indicated that the tablet contained 0.6 mg retinol; however, no retinol peak in the sample could be detected, which means the amount must be lower than 0.0002 mg/tablet. Retinol acetate instead was detected at a concentration of 0.9 mg/tablet, whereas in the ingredient leaflet this vitamin is not reported. On the leaflet, α-tocopherole acetate is reported with a concentration of 10 mg/tablet and α-tocopherole with 6.1 mg/tablet. The quantitative results of both analytes show a significantly higher value for α-tocopherole acetate and a significantly lower value for α-tocopherole. Phyllochinone is in good agreement with the stated amount on the label and the measured one.
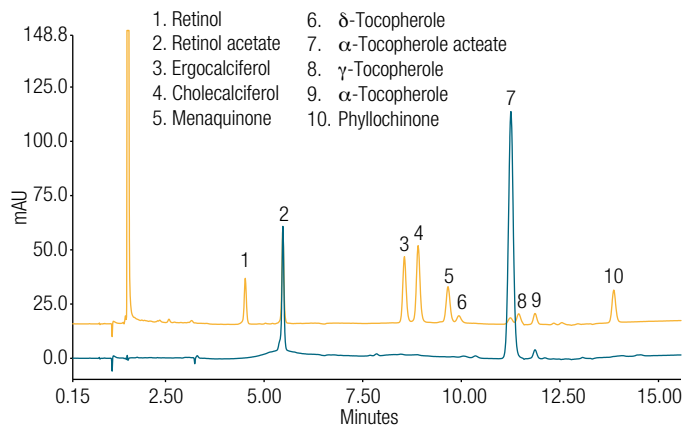
Figure 5. Comparison of the standard mixture (orange) with 100 μg/mL and the vitamin tablet (blue) at a wavelength of 280 nm.

Table 10. Quantitative results of FSV in a tablet. The measured amount was corrected by recovery rate.

FSV	Stated Amount on Label [mg/tablet]	Measured Amount [mg/tablet]
Retinol	0.6	< 0.0002*
Retinol acetate	-	0.9
Ergocalciferol	-	< 0.0002*
Cholecalciferol	-	< 0.0002*
Menaquinone	-	< 0.0002*
δ-Tocpherole	-	< 0.0017*
α-Tocopherole acetate	10	26.5
γ-Tocopherole	-	< 0.0027*
α-Tocopherole	6.1	0.4
Phyllochinone	0.03	0.01
Retinol palmitate	-	< 0.0005*

* Peak not observed: value estimated with the LOD corrected by recovery rate

The energy drink contains just four out of nine WSVs, namely pyridoxine, nicotinamide, pantothenic acid, and cyanocobalamin (Table 11). The labeled and the calculated amounts show excellent correspondence. Again, cyanocobalamin is added in a concentration below the limit of detection and can therefore not be quantified by this method.

Table 11. Quantitative results of WSV in an energy drink.

WSV	Stated Amount on Label [mg/100 mL]	Measured Amount [mg/100 mL]
Ascorbic acid	-	< 0.002*
Thiamine	-	< 0.020*
Pyridoxal	-	< 0.004*
Pyridoxin	2	3
Nicotinamide	8	8
Pantothenic acid	2	2
Folic acid	-	< 0.001*
Cyanocobalamin	0.002	< 0.002*
Riboflavin	-	< 0.001*

* Peak not observed: value estimated with the LOD

Conclusion

A method based on the Vanquish Flex Duo system for Dual LC and Acclaim PA2 columns for the quantitative analysis of FSV and WSV in drinks and food supplement tablets was described.

The presented workflow enables running two separate methods and columns in one instrument, simultaneously and without additional equipment. The approach provides substantial advantages in terms of throughput and eliminates issues such as solvent incompatibility. The workflow is remarkably easy to set up and operate, unlike previously reported solutions for simultaneous analysis of water- and fat-soluble vitamins. The Acclaim PA2 column proved to be a highly reliable and efficient workhorse for the separation of compounds with a wide range of hydrophobicity.

The analysis of the supplement tablet provided vitamin amounts generally higher than those reported in the ingredient leaflet. Additionally, retinol acetate was detected and quantified even though this component was not listed as ingredient by the manufacturer.

For the energy drink, the estimated and declared amounts were in excellent agreement.

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