

# Use of a Novel UHPLC System for the Simultaneous UHPLC Analysis of Water-soluble and Fat-soluble Vitamins

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## ABSTRACT

A method based on dual LC for the quantitative analysis of fat-soluble vitamins (FSV) and water-soluble vitamins (WSV) in drinks and food supplements was developed. The method enabled the simultaneous quantitative analysis of two columns with optimized methods for both class of vitamins. The approach provides substantial advantages in terms of throughput and ease of use compared previously reported methods for simultaneous separation of WSV and FSV [1]. The Thermo Scientific™ Acclaim™ Vanquish™ PolarAdvantage II (PA2) column proved to be a highly reliable and efficient work horse for the separation of compounds covering a wide range of hydrophobicity. Analyzing food supplement tablets and supplemented soft drinks, the content of some vitamins was found to be slightly higher than reported in the ingredient list.

## 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, the simultaneous liquid chromatography analysis of WSV and FSV with the same method is difficult.

In this work we introduce an effective workflow for the simultaneous analysis of WSV and FSV. The workflow is based on a novel Thermo Scientific™ Vanquish™ Flex Duo UHPLC system. The system consists of a Thermo Scientific™ Vanquish™ Dual Pump F with two independent flow paths, a Thermo Scientific™ Vanquish™ Dual Split Sampler FT with two separate injection ports and sample loops, one or optionally two Thermo Scientific™ Vanquish™ Column Compartments H and two detection systems, and enables the independent and simultaneous run 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.

## MATERIALS AND METHODS

### Sample Preparation [2]

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

#### Water soluble vitamins in energy drink

- Sonicate 15 min to degas
- Filter through CA membrane with 0.45 µm pore size

#### Water soluble vitamins in tablets

- Add DMSO to powdered tablet and sonicate for 2 min
- Add 2% acetic acid in water, stir for 1 min and sonicate for 5 min
- Filter through CA membrane with 0.45 µm pore size and dilute if necessary

#### Fat soluble vitamins in tablets

- Add 80% ethanol to powdered tablet
- Add hexane, shake by hand for 10 sec and sonicate for 10 min
- Filter through CA membrane with 0.45 µm pore size and dilute if necessary

### Test Method(s)

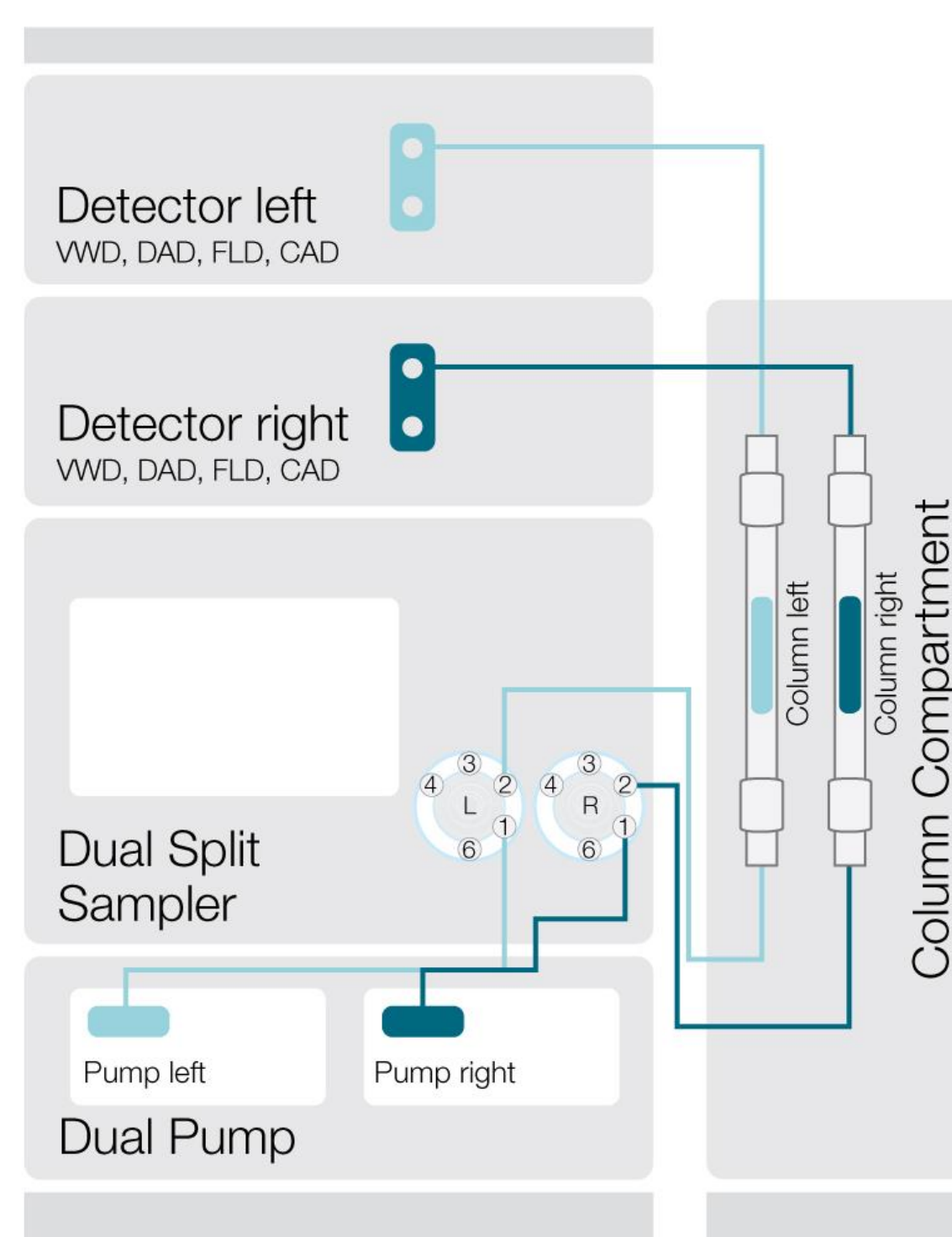
Calibration range:	0.1 µg/mL – 100 µg/mL
Recovery:	10 µg/mL spiked to powered placebo tablet (water soluble vitamins)
	18 µg/mL spiked to powered placebo tablet (fat soluble vitamins)

### Data Analysis

Data acquisition and processing was done by Thermo Scientific™ Chromeleon™ Chromatography Data System software 7.2.8.

## INSTRUMENTATION

Figure 1. Thermo Scientific™ Vanquish™ Flex Duo UHPLC system.



## METHOD

Table 1. Chromatographic conditions for WSV.

<b>Column:</b>	Acclaim RSLC PolarAdvantage II (150 x 2.1 mm, 2.2 µm, 120 Å)
<b>Mobile phase A:</b>	25 mM KH <sub>2</sub> PO <sub>4</sub> , pH 3.8
<b>Mobile phase B:</b>	30/70 (v/v) mobile phase A/ACN
<b>Gradient:</b>	
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)

<b>Detection Parameters</b>	
Channel	Wavelength [nm]
1	270
2	210
3	245
4	280

**3D Scan:** 190–360nm  
**Data collection rate:** 10 Hz  
**Response Time:** 0.5 s  
**Injection volume:** 1 µL

Table 2. Chromatographic conditions for FSV.

<b>Column:</b>	Acclaim RSLC PolarAdvantage II (250 x 2.1 mm, 2.2 µm, 120 Å)
<b>Mobile phase A:</b>	water
<b>Mobile phase B:</b>	methanol
<b>Gradient:</b>	
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)

<b>Detection Parameters</b>	
Channel	Wavelength [nm]
1	325
2	265
3	290
4	280

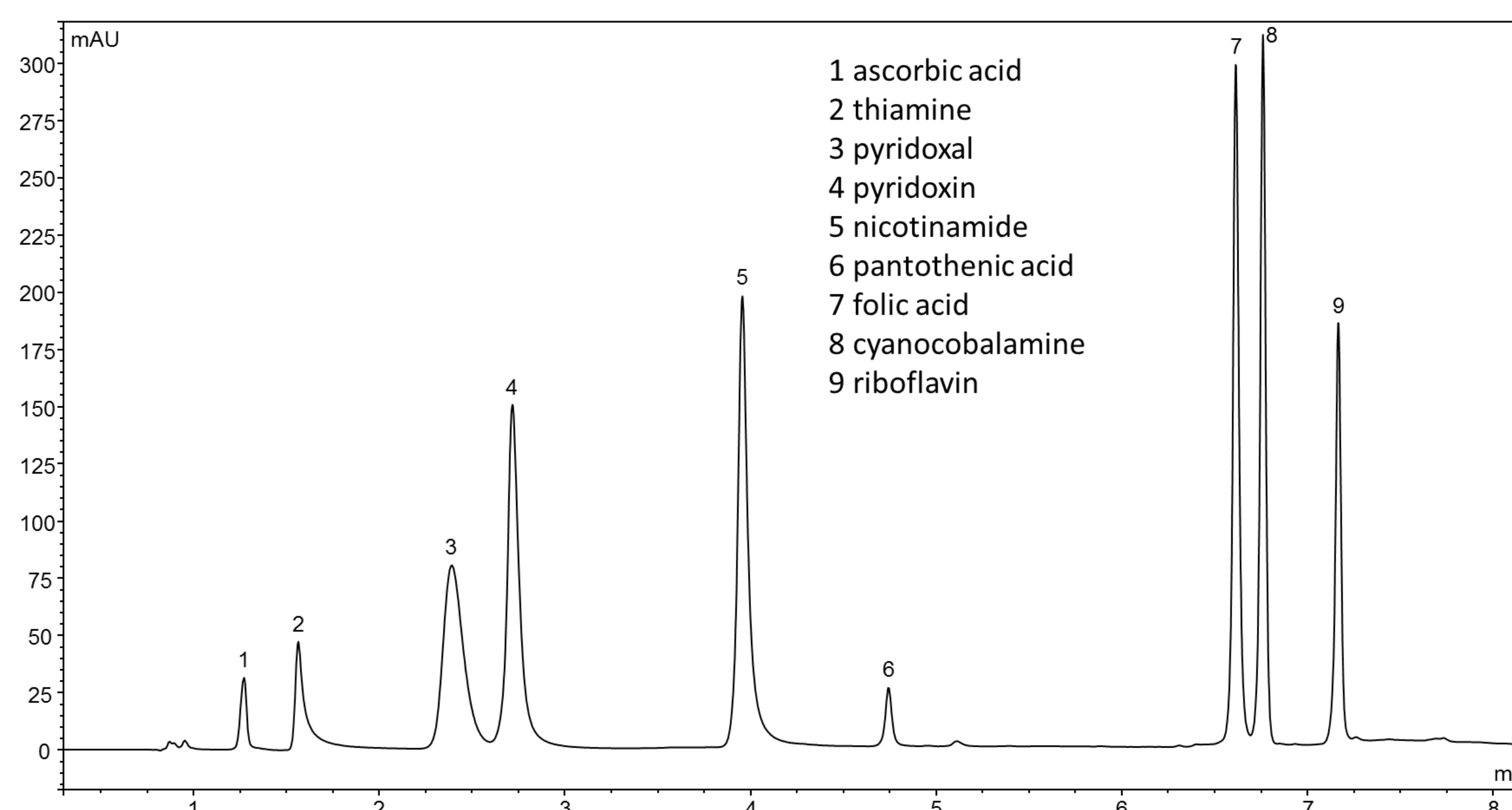
**3D Scan:** 240–350 nm  
**Data collection rate:** 10 Hz  
**Response Time:** 0.5 s  
**Injection volume:** 1 µL

## RESULTS

### Separation of WSV

Figure 2 illustrates the separation of WSV. The polar analytes ascorbic acid and thiamine, 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, is also well separated with  $R_s > 2.0$ .

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



### Separation of FSV

The separation of FSV is shown in Figure 3. Resolution was higher than 2 for most pairs. Only exceptions are the pair menaquinone – delta tocopherol ( $R_s=1.4$ ) and alpha tocopherol acetate – gamma tocopherol ( $R_s=1$ ).

Figure 3. Separation of fat soluble vitamins standard on an Acclaim PA2 column (250 x 2.1 mm, 2.2 µm) recorded at 280 nm.

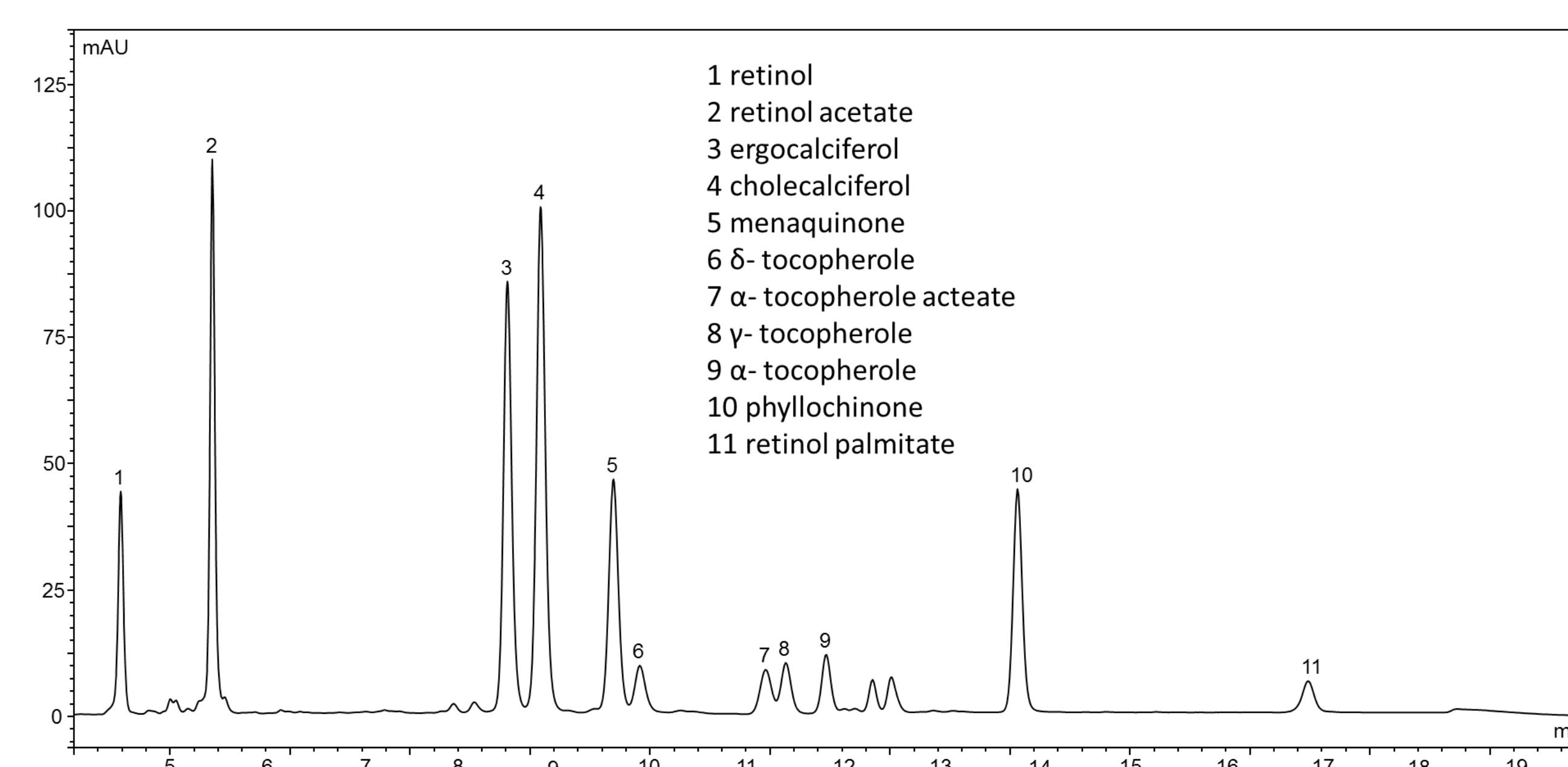


Table 3. Chromatographic results of 10 consecutive injections of a WSV standard mixture with a concentration of 100 µg/mL on an Acclaim PA2 column.

WSV	RT [min] average	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
Cyanocobalamin	6.73	0.01%	0.12%	8.0
Riboflavin	7.14	0.02%	0.11%	n.a.

n.a. = not available

Table 4. Chromatographic results of 10 consecutive injections of a FSV standard mixture with a concentration of 100 µg/mL on an Acclaim PA2 column.

FSV	RT [min] average	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
delta-Tocopherol	9.90	0.13%	0.35%	6.1
alpha-Tocopherol acetate	11.22	0.13%	0.28%	1.0
gamma-Tocopherol	11.43	0.12%	0.31%	2.3
alpha-Tocopherol	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

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

The energy drink contains four out of nine WSVs, namely pyridoxine, nicotinamide, pantothenic acid and cyanocobalamin (Table 5). The labeled and the calculated amounts show excellent correspondence. Cyanocobalamin is added in a concentration below the limit of detection and can therefore not be quantified by this method.

Table 5. Quantitative results of WSV in an energy drink.

WSV	Labeled amount [mg/100 mL]	Estimated amount [mg/100 mL]
Pyridoxin	2	3
Nicotinamide	8	8
Pantothenic acid	2	2
Cyanocobalamin	0.002	< 0.002*

\* Peak not observed: value estimated with the LOD

Generally higher amounts were measured than those reported in the product label. Results of the FSV show some discrepancies with the ingredient content declared by the vendor (Table 7). 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.

Figure 4. Separation of WSV in tablet. Blue trace: 210 nm; black trace: 270 nm.

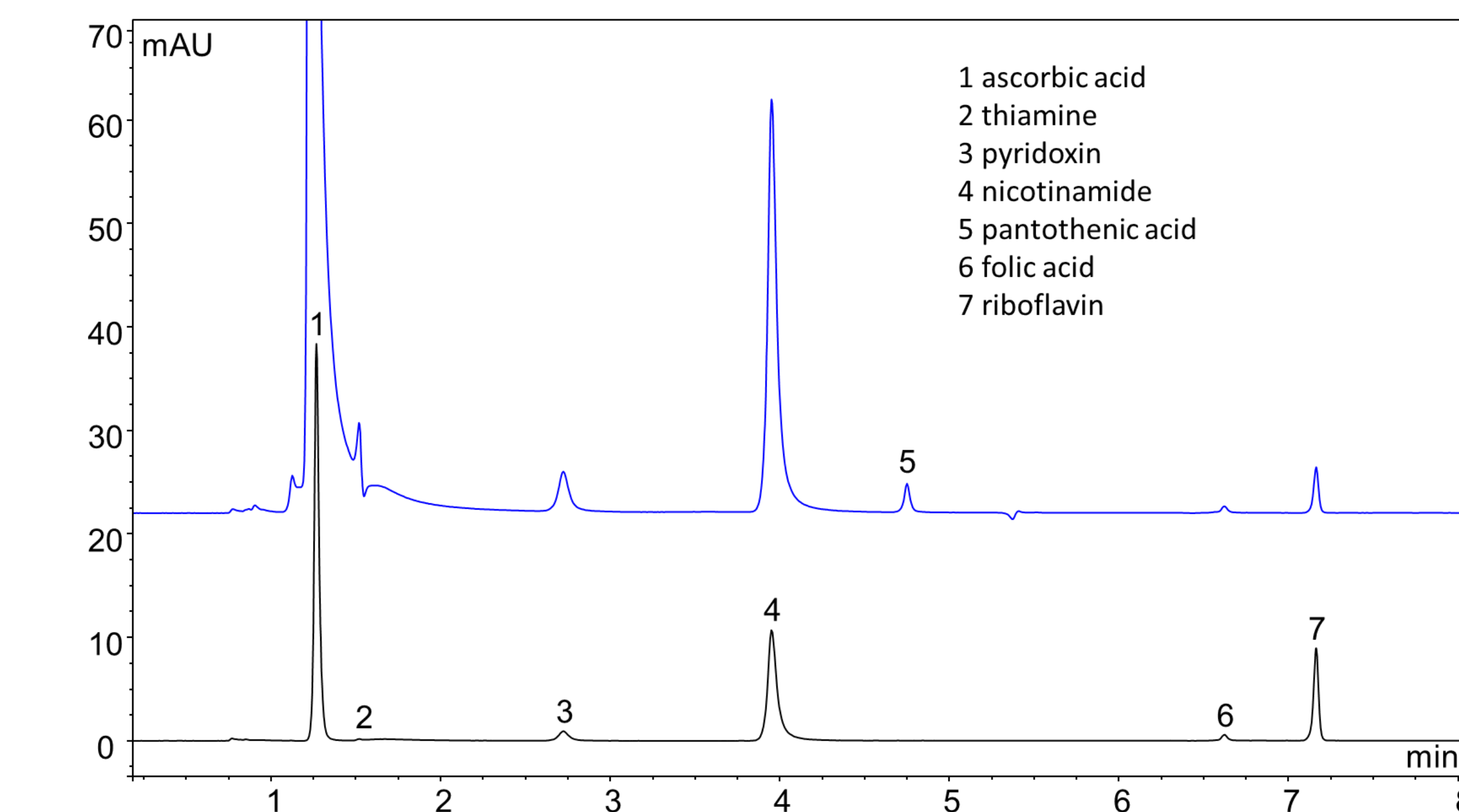


Figure 5. Comparison of the FSV standard mixture (black) with 100 µg/mL and the vitamin tablet (blue) at a wavelength of 280 nm.

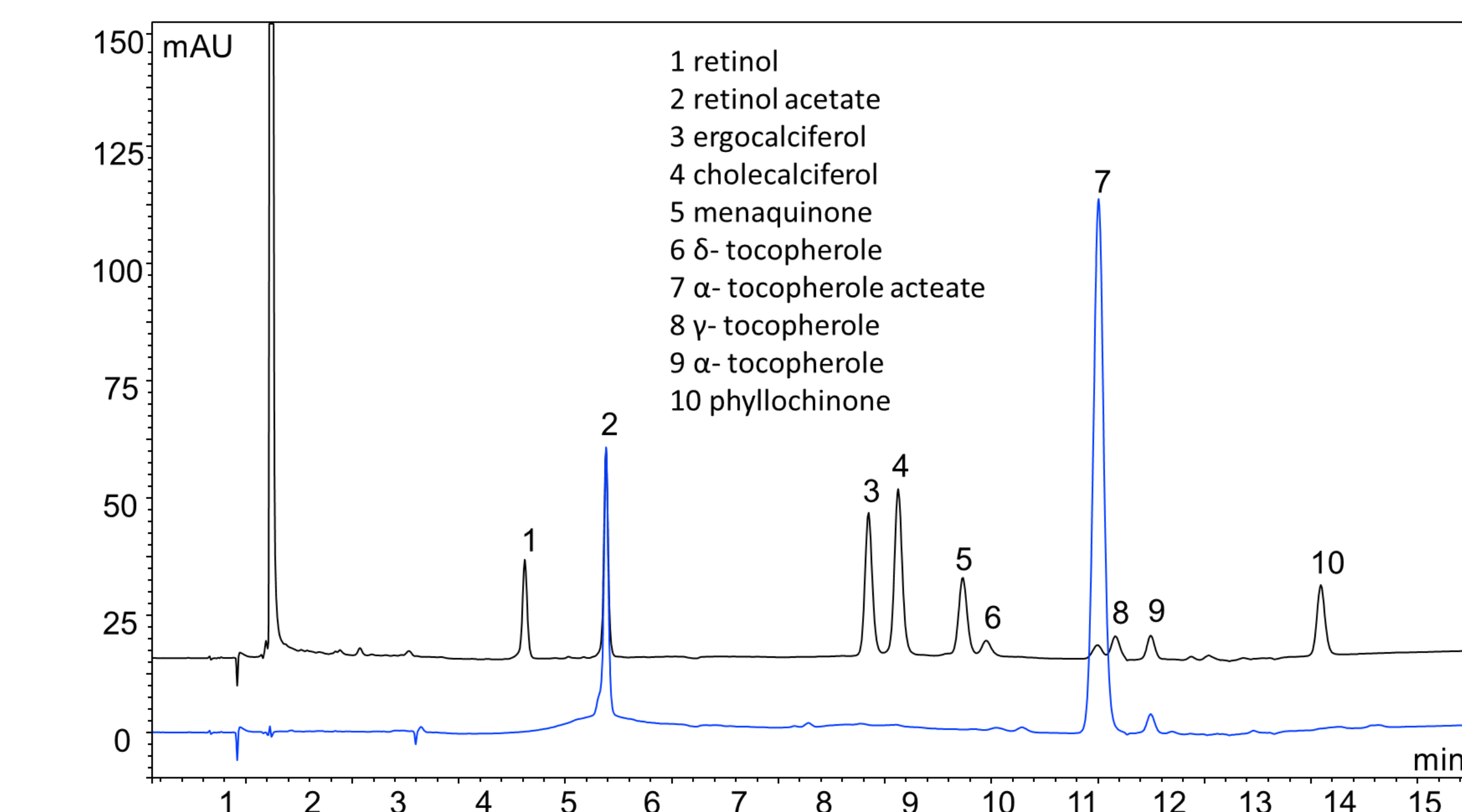


Table 6. Quantitative results of WSV in tablet. The measured amount was corrected by recovery rate of 100 µg/mL.

WSV	Labeled amount [mg/tablet]	Estimated amount [mg/tablet]
Ascorbic acid	60	61.6
Thiamine	1.4	1.1
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

Table 7. Quantitative results of FSV in a tablet. The measured amount was corrected by recovery rate.

FSV	Labeled amount [mg/tablet]	Estimated amount [mg/tablet]
Retinol	0.6	< 0.0002*
Retinol acetate	-	0.9
alpha-Tocopherol acetate	10	26.5
alpha-Tocopherol	6.1	0.4
Phyllochinone	0.03	0.01

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

## CONCLUSIONS

- Running two separate methods in one instrument simultaneously without additional equipment
- Substantial advantages in terms of throughput
- Easy to set up and operate workflow

## REFERENCES

1. Thermo Fisher Scientific Technical Note 72488: Determination of water- and fat soluble vitamins by HPLC.
2. Choi, C. K.; Dong, M. W. *Handbook of Pharmaceutical Analysis by HPLC*.
3. Thermo Fisher Application Note 72592: Simultaneous determination of water and fat-soluble vitamins in tablets and energy drinks by using a novel Thermo Scientific Vanquish Flex Duo UHPLC system.

## TRADEMARKS/LICENSING

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