More speed, better resolution and lower LOD using liquid chromatography and fluorescence detection

Comparing the Agilent 1100 Series LC to the Agilent 1200 Series Rapid Resolution LC system

Application Note

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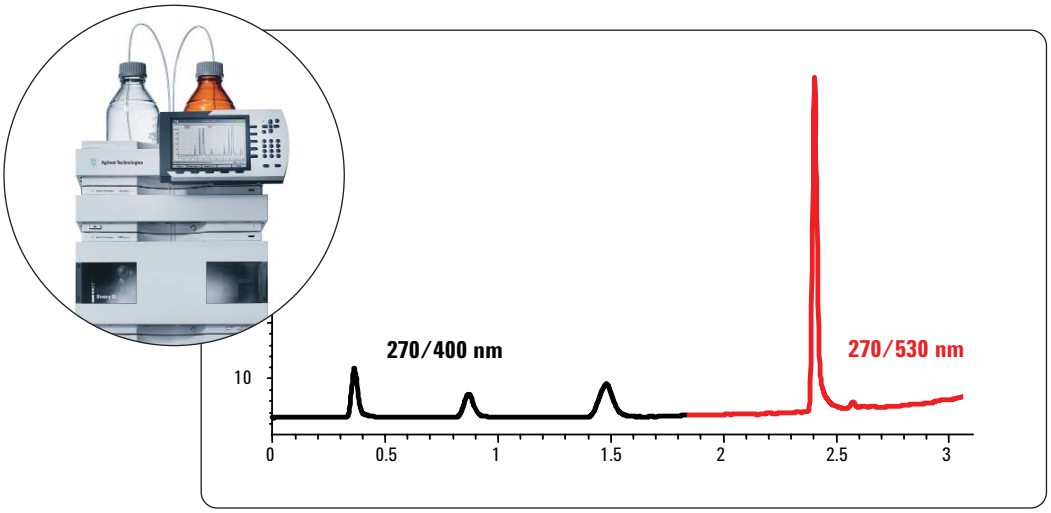

Abstract

Fluorescence detection is typically used to determine trace levels of vitamins B2 and B6 in biological samples. In the year 2000, these compounds were analyzed using the Agilent 1100 Series LC system with the Agilent 1100 Series fluorescence detector and a narrow-bore column packed with 5-µm particles. The performance is compared to the results now obtained on a sub-2-µm particle column with the new Agilent 1200 Series Rapid Resolution LC system. The increase in performance using the new column technology is significant:

- faster run time
- lower detection limit
- better resolution

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Introduction

Vitamin B2 and B6 are the only water soluble vitamins which show fluorescent properties. This property is of special importance for the analysis of these vitamins in serum or other matrices with low amounts of vitamin B2 and B6.[1] In 2000 we published an Agilent Application Note[2] showing the analysis of vitamin B2 and B6 using fluorescence detection (figure 1). The run time was about 10 min. The LOD for B2 was 20 pg and for B6 200 pg at a signal-to-noise ratio of 2. The resolution for the B6 vitamins was approximately 1.5.

In the Application Note here we show how state-of-the-art LC equipment in combination with new sub-2-µm particle columns can improve the performance of this application.

Equipment

An Agilent 1200 Series Rapid Resolution LC (RRLC) system was used with the following modules:

- Agilent 1200 Series binary pump SL and vacuum degasser for high-speed and high-resolution applications on short and long sub-2-µm particle columns
- Agilent 1200 Series high-performance autosampler SL for highest area precision
- Agilent 1200 Series thermostatted column compartment SL with new design for column temperatures up to 100 °C
- Agilent 1200 Series fluorescence detector (FLD) for operation at highest sensitivity and selectivity

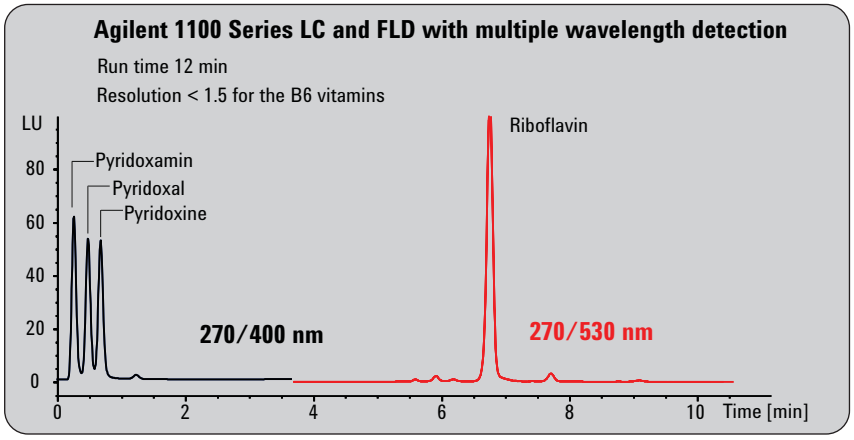

Figure 1
Analysis of vitamins, as published in the year 2000.

Chromatographic conditions

Column:	ZORBAX SB-C18, 5 µm, 50 x 2.1 mm
Concentration:	25 ng
Mobile phase:	H_2O (0.05mKH_2PO_4 pH 2.5)/ CH_3CN
Gradient:	0 to 25 % ACN in 10min
Injection volume:	5 µL
Column temperature:	35 °C
FLD data rate:	Response time 4 sec, EX = 270 nm, EM = 400 and 530 nm
Flow:	0.5 mL/min

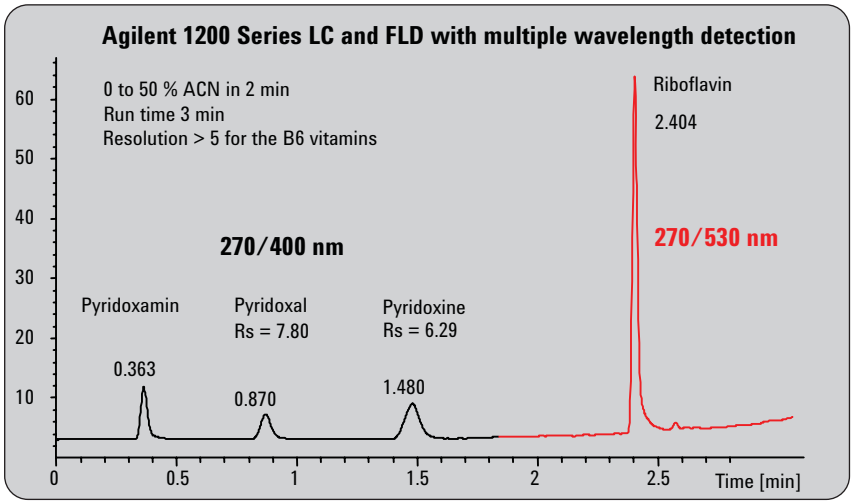

Figure 2
Analysis of vitamins in the year 2006., using the new column technology.

Chromatographic conditions

Column:	ZORBAX SB-C18, 1.8 µm, 50 x 2.1 mm
Concentration:	100 pg/µL Pyridoxamin, 90 pg/µL Pyridoxal, 180 pg/µL Pyridoxin, 210 pg/µL Riboflavin
Mobile phase:	H_2O / CH_3CN = 100/0
Gradient:	0 to 50 % ACN in 2min
Injection volume:	1 µL
Column temperature:	30 °C
FLD data rate:	9.75 Hz, 1 sec response time, 8 µL cell, multi signal detection at EX = 270 nm and EM = 400 and 30 nm
Flow:	0.7 mL/min

Results and discussion

The analysis in 2006, using the new column technology and the Agilent 1200 Series RRLC system, achieved shorter run times and improved the performance significantly (figure 2).

Using the sub-2 µm particle columns and the Agilent 1200 Series RRLC system, speed of analysis and performance with respect to resolution and limit of detection could be improved. The run time is only about 3 min. The LOD for vitamin B2 is 1.1 pg and for B6 < 25 pg with S/N = 2. The resolution for vitamin B6 is > 5.

The results confirm that the new ZORBAX SB C18 columns, with an internal diameter of 0.21 mm, can be used successfully in combination with the Agilent 1200 Series RRLC system and fluorescence detection, even though the 8 µl cell volume of the FLD might be considered to be too high for applications using 2.1 mm ID columns and sub-2-micron particles.

The dispersion volume and the delay volume of the system should be as low as possible when using small ID columns at low flow rates. Therefore, it is a wise choice to use the low delay volume configuration of the Agilent 1200 Series RRLC system for gradient analysis. The low delay volume configuration removes the mixer and the damper from the flow path. The capillary connec-

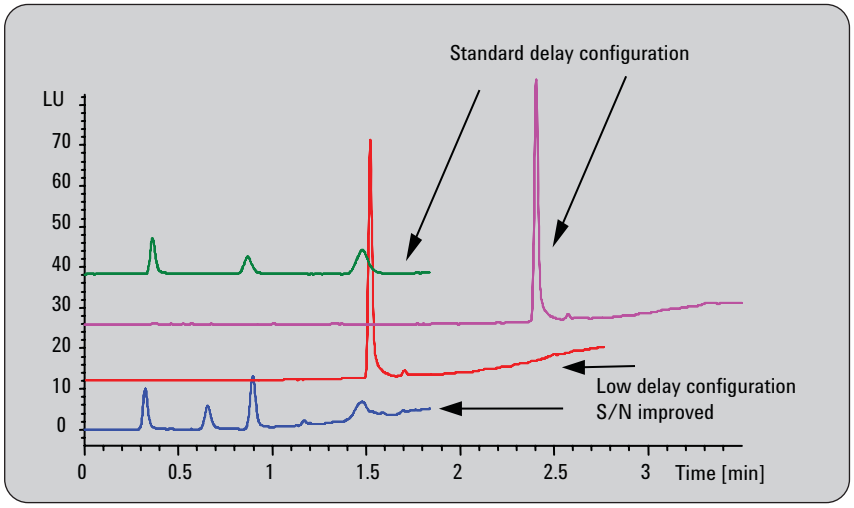

Figure 3
Analysis of vitamins B2 and B6 using standard and low delay volume configurations.

Chromatographic conditions	
Column:	ZORBAX SB C18, 1.8 µm, 50 x 2.1 mm
Concentration:	100 pg/µL Pyridoxamin, 90 pg/µL Pyridoxal, 180 pg/µL Pyridoxin, 210 pg/µL Riboflavin
Mobile phase:	H2O/ CH3CN = 100 /0
Gradient:	0 to 50 % ACN in 10min
Injection volume:	1 µL
Column temperature:	30 °C
FLD data rate:	9.75 Hz
Flow:	0.7 mL/min

Compound	Amount injected	S/N Standard delay volume config.	LOD calculated	S/N Low delay volume config.	LOD calculated
Pyrodoxamin	100 pg	21.1	9.5 pg	29.7	6.7
Pyridoxal	90 pg	10.2	18 pg	17.1	10.5
Pyridoxin	180 pg	14.4	25 pg	38.8	9.3
Riboflavin	210 pg	388.1	1.1 pg	450.7	0.9 pg

Table 1
LOD of vitamins using different Agilent 1200 Series RRLC system configurations.

tion from the column to the detector should be as short as possible, to avoid additional post column delay volume. The impact of the standard vs. the low delay configuration is shown in figure 3 and table 1.

One result is quite obvious, the run time is reduced from 3 min to 2 min in the low delay volume configuration. The signal-to-noise ratio has improved with the low delay volume, whereas the resolution has dropped, but the obtained resolution is still > 4, which is sufficient for reliable quantitation.

Conclusion

The analysis of vitamins B2 and B6 done in the year 2000 was compared to the same analysis in the year 2006 using the Agilent 1200 Series Rapid Resolution LC system and sub-2-µm particle columns. Resolution, limit of detection and speed could be improved significantly

References

1.
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