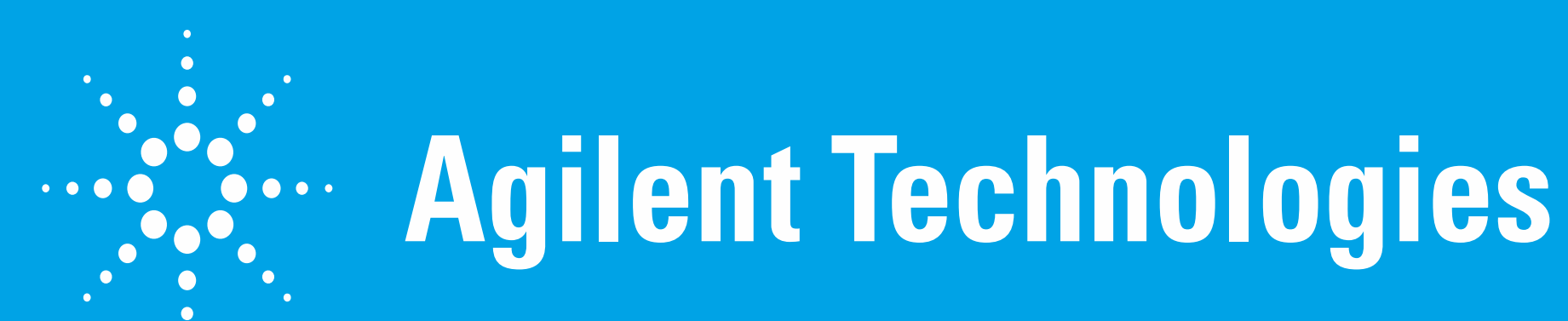


Analysis of high- and low-dosed vitamins simultaneously using the Agilent 1200 Infinity Series HDR Detector Solution

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Introduction

When using conventional diode array detectors in HPLC, the analysis of main and trace components often requires two separate analyses to be able to quantify all components.

This challenge can be solved by using the 1200 Infinity HDR DAD solution. The detector design facilitates the analysis of main and trace components simultaneously in a single run. This is possible due to the enhanced dynamic range for main compounds and the improved signal-to-noise ratios for measured trace components using the HDR-DAD solution.

In vitamin tablets, some vitamins are present at high concentrations, whereas, for example, vitamin B12 is present in very low concentrations.

In the literature for vitamin B12, different sample preparation procedures and different chromatographic methods are used to be able to detect and quantify vitamin B12 in vitamin preparations.

Until now, it was not possible by applying one sample preparation and one chromatographic method to detect and quantify all water soluble vitamins present in a vitamin tablet. The combination of an Agilent 1200 Infinity LC with the 1200 Infinity Series HDR DAD solution offers a perfect solution.

Principle and functionality

1200 Infinity Series HDR DAD solution

The 1200 Infinity Series HDR DAD solution expands the linear dynamic range by a factor of more than 30. By combining the signals from two diode array detectors with different path length Max-Light flow cells, the HDR DAD solution enables detection and quantification of components with significantly different concentrations in a single run. The HDR DAD solution clusters two 1260 or 1290 Infinity DADs together. Detector 1 is equipped with a 60-mm path length cell for analyzing low concentration components and Detector 2 is equipped with a 3.7-mm path length cell for analyzing high concentration compounds. The 60-mm cell must be installed in the first detector and the 3.7-mm cell in the second detector. The resulting HDR DAD signal is one combined signal, normalized to 10-mm path length. The HDR DAD linear range is typically as wide as 0.6×10^{-6} to 6.7 AU/cm. A conventional 1200 Infinity Series Diode Array Detector has a maximum linear range of 7×10^{-6} to 2 AU/cm

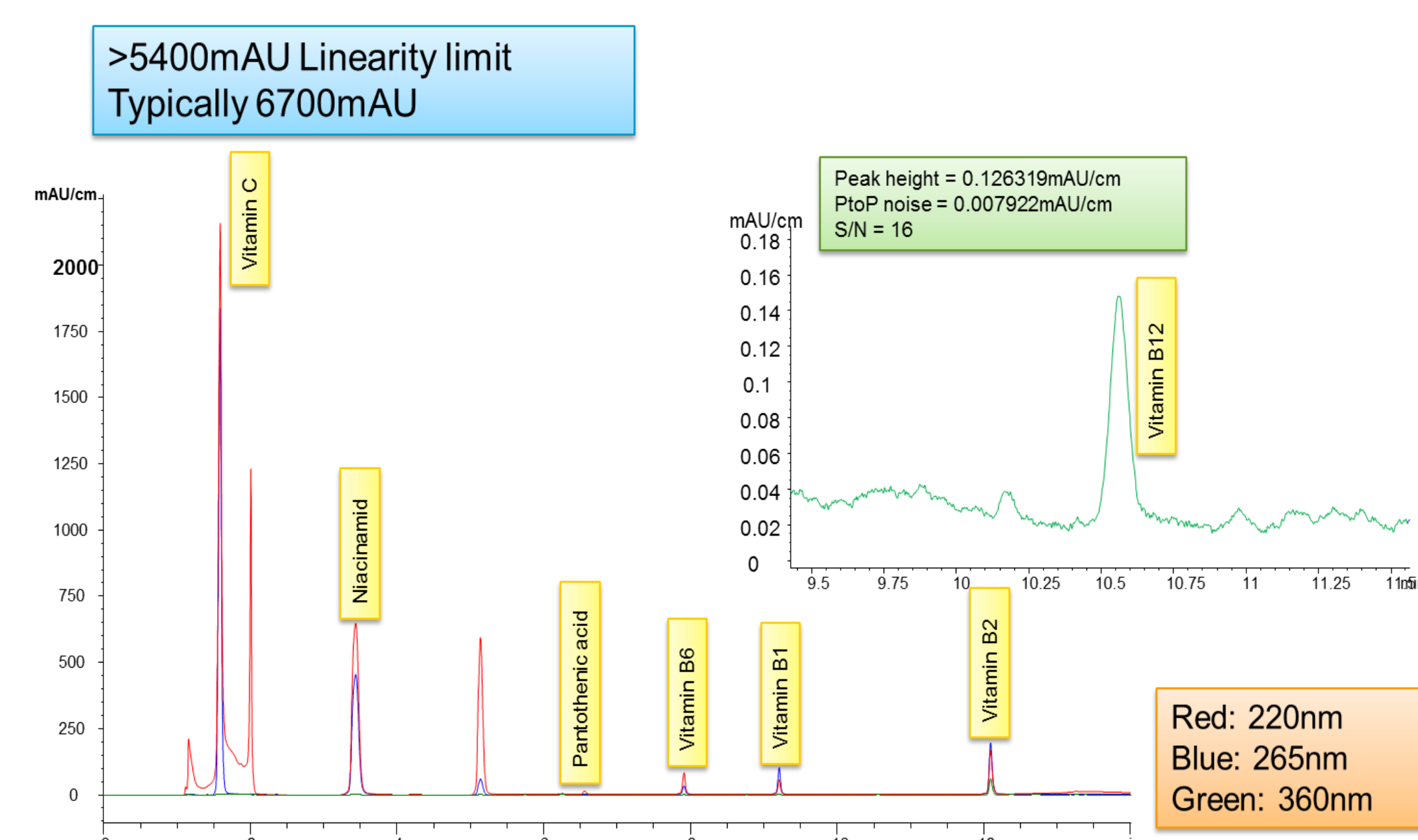
Results and Discussion

Optimizing the separation

In vitamin tablets, the concentration of vitamin B12 is very low, approximately 2 to 5 µg/tablet. Selective detection is, therefore, mandatory. Vitamin B12 is detected with high selectivity at 360 nm. Further coelution of vitamin B12 with matrix compounds must be avoided. In addition, vitamin B12 tends to decompose if it is exposed to light and/or oxygen, which causes additional problems during data evaluation. Other vitamins, for example vitamin C, are present in 30,000 times higher concentrations than vitamin B12. Vitamin C is a very polar compound and tends to elute with low retention on reversed phase material. This creates a need to start the gradient at a very low organic percentage.

Results and Discussion

Analysis on a 1260 Infinity HDR-DAD



Experimental

Instrumentation, chromatographic conditions

Agilent 1260 Infinity DAD2 G4212B with a 3.7-mm cell
Agilent 1260 Infinity DAD 1 G4212B with a 60-mm cell or G4212B with a 10-mm cell
Agilent 1260 Infinity Column compartment G1316C
Agilent 1260 Infinity Standard Autosampler G1329B
Agilent 1260 Infinity ALS cooler G1330B
Agilent 1260 Infinity Binary Pump G1312B
Column: Agilent ZORBAX RRHT Eclipse plus C18, 4.6 × 150 mm, 1.8 µm (p/n 959994-902)
Mobile phases: A=water+4g/L K2HPO4+0.5g/L hexanesulfonate, pH 3 with phosphoric acid, B = methanol
Gradient: at 0 minutes 3% B, at 1 minute 3% B, at 10 minutes 34% B, at 11 minutes 50% B. Flow:1.2 mL/min. Stop time:14 min. Post time:5 min. Injection volume: 2 µL, sample temperature 4 °C. UV: 220/10 nm for pantothenic acid and vitamin B6, 265/10 nm for vitamin C, niacinamid, and vitamin B1, 360/10 nm for vitamin B12 and B2, Ref: off, 20 Hz. Column temperature: 40°C.

Sample preparation: Two vitamin effervescent tablets were placed in 20 mL water and 20 mL ethanol. The solution was stirred for 10 minutes in the dark. 20 mL were filtered using two Agilent Captiva Premium Syringe Filter, regenerated cellulose. 1.5 mL was transferred into an autosampler vial.

1290 Infinity HDR DAD

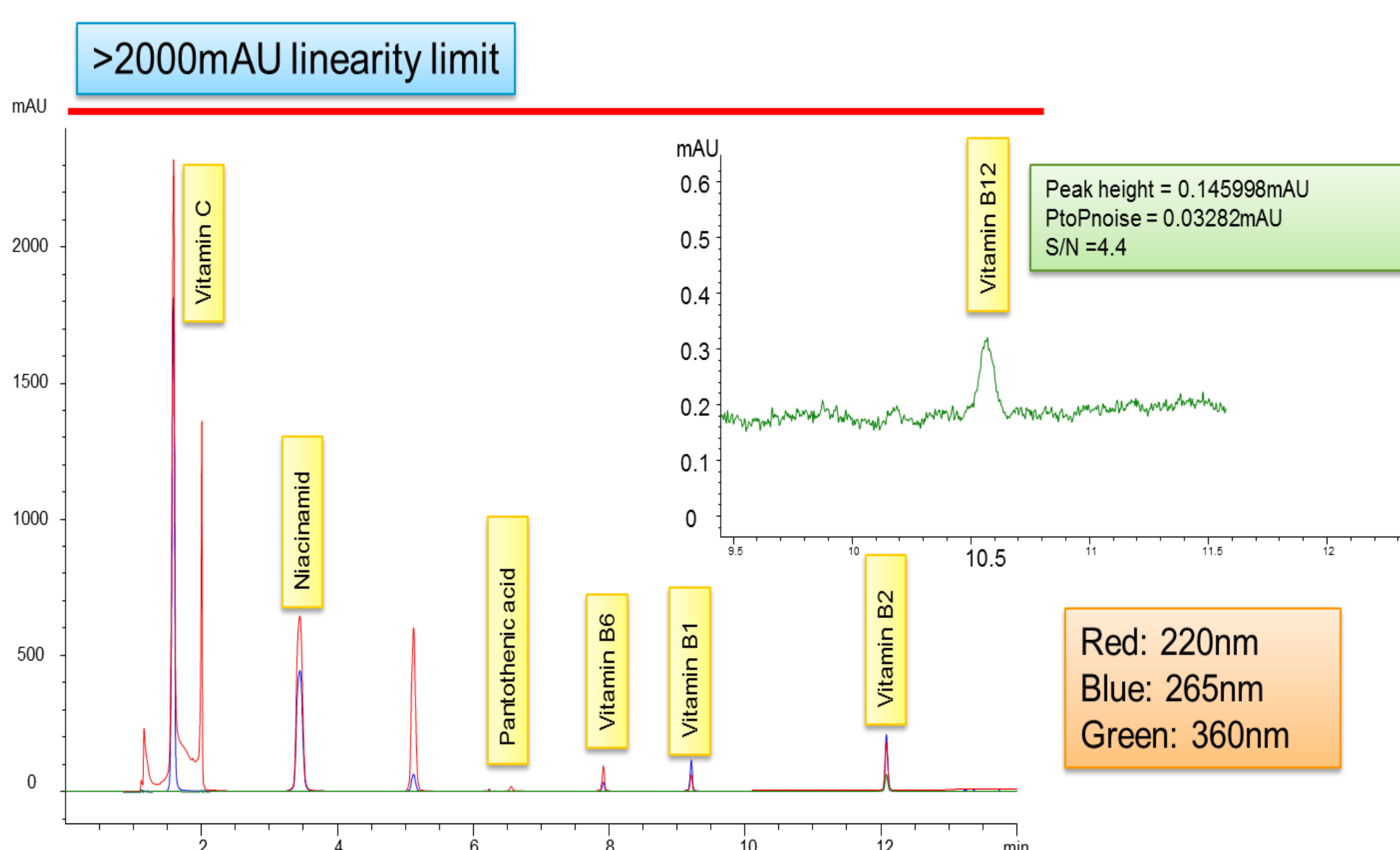
Instrument design

For detection of trace components, the HDR DAD signal is based on the signal acquired by the 60-mm cell. The 3.7-mm cell is used to provide the HDR DAD signal for the main component, which is typically out of the linear range of the 60-mm cell. For peaks between trace and main component absorbance range, a combination of both signals is used combined by a weighting function.



Results and Discussion

Analysis on a 1260 Infinity DAD with a 10-mm path length cell



Final Results

Compound	Quantitation with 1260 Infinity DAD with 10mm cell	Quantitation with 1260 Infinity DAD with 60mm cell	Quantitation with 1260 Infinity DAD-HDR
Vitamin C			
Niacinamid			
Pantothenic acid			
Vitamin B6			
Vitamin B1			
Vitamin B12			
Vitamin B2			

Experimental

Concentration and injected amounts

In vitamin tablets, the concentration of vitamin B12 is very low, approximately 2 to 5 µg/tablet.

Selective detection is, therefore, mandatory. Vitamin B12 is detected with high selectivity at 360 nm.

Further co-elution of vitamin B12 with matrix compounds must be avoided. In addition, vitamin B12 tends to decompose if it is exposed to light and/or oxygen, which causes additional problems during data evaluation.

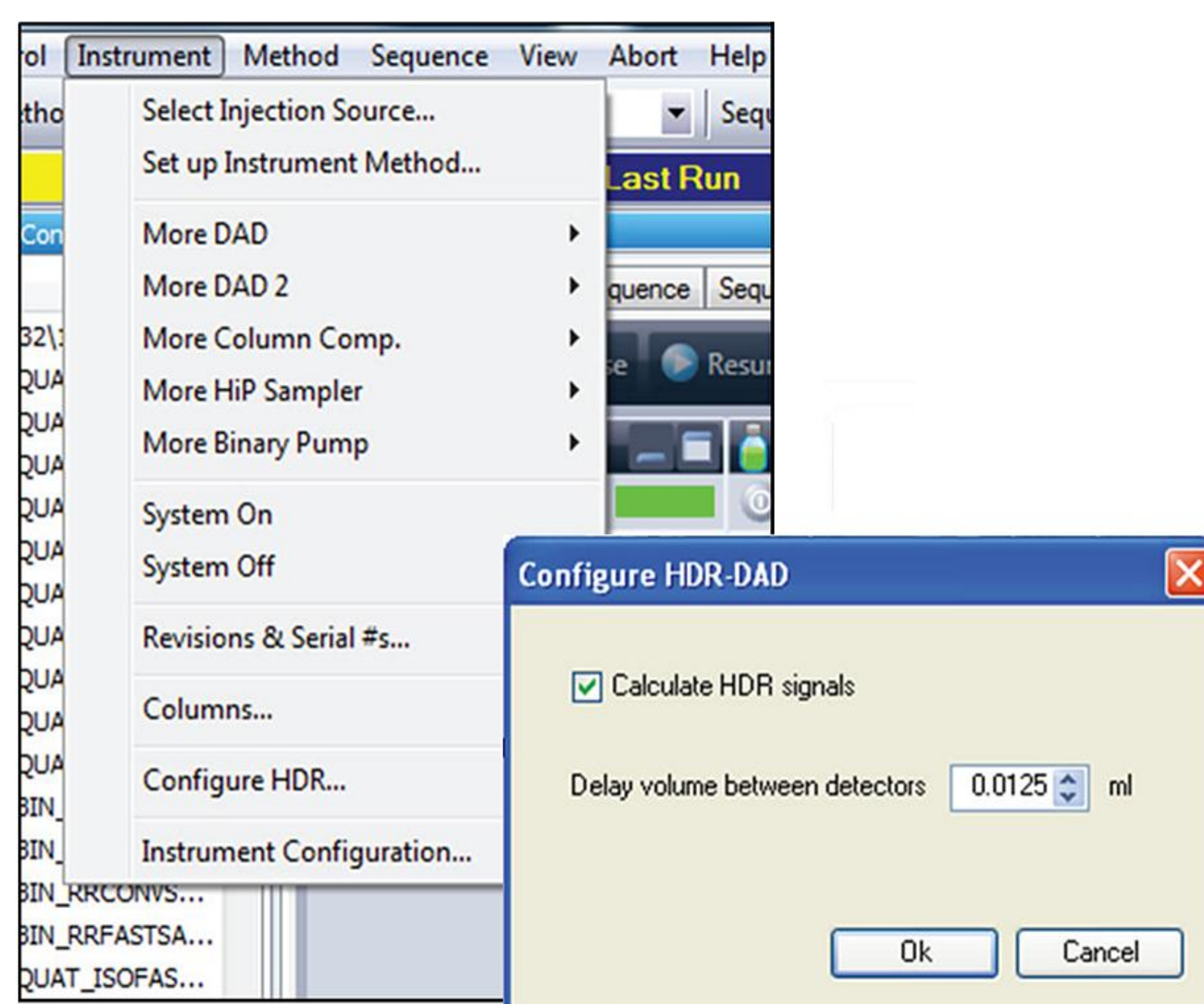
Other vitamins, for example vitamin C, are present in 30,000 times higher concentrations than vitamin B12.

Vitamin C is a very polar compound and tends to elute with low retention on reversed phase material. This creates a need to start the gradient at a very low organic percentage.

Compound	Concentration	Injected amount
Vitamin C	60 mg/tablet	6,000 ng
Niacinamid	18 mg/tablet	1,800 ng
Pantothenic acid	6 mg/tablet	600 ng
Vitamin B6	2 mg/tablet	200 ng
Vitamin B2	1.6 mg/tablet	160 ng
Vitamin B1	1.4 mg/tablet	140 ng
Vitamin B12	2 µg/tablet	0.2 ng

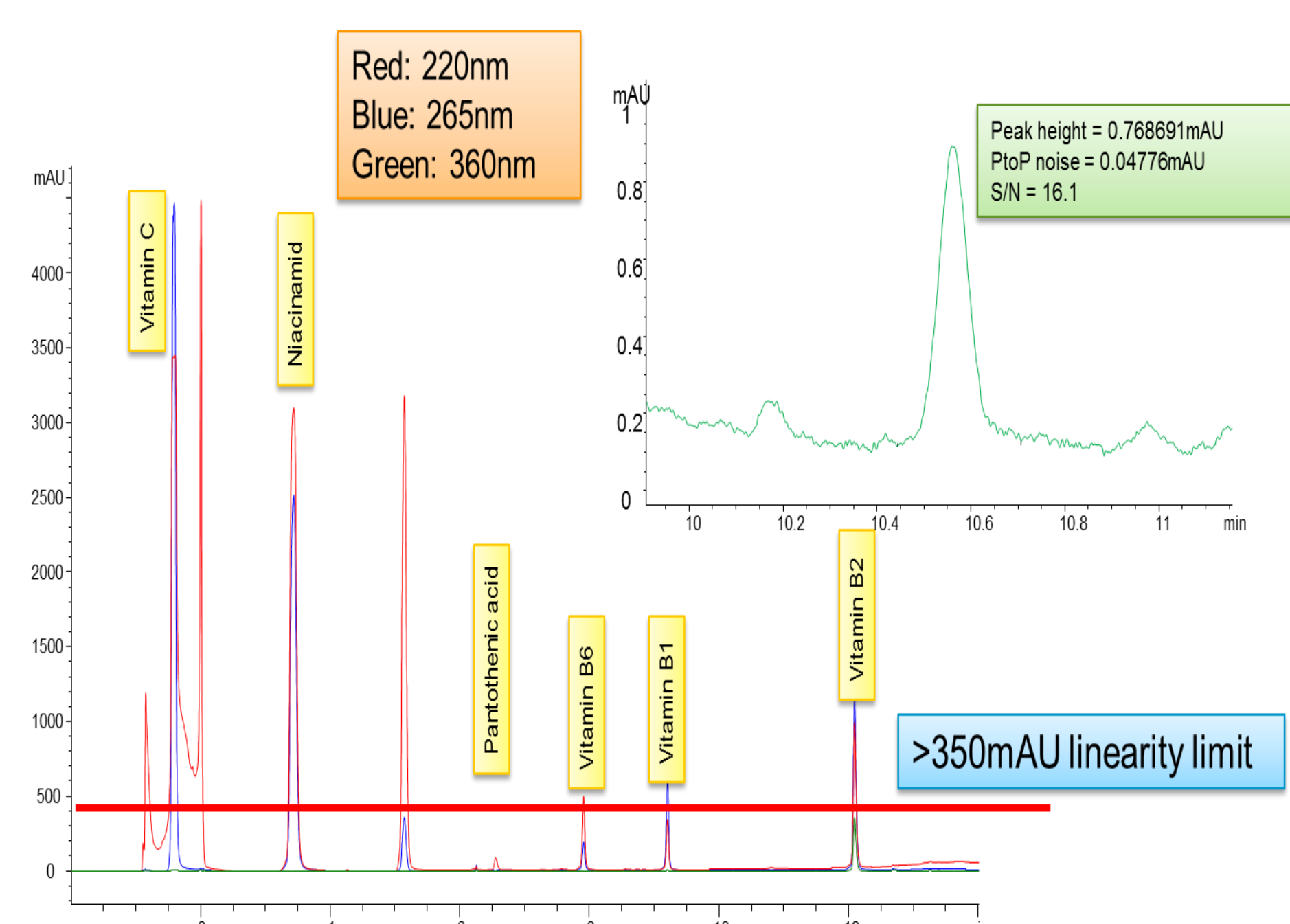
Software implementation

Configuring the Agilent 1200 Infinity Series HDR DAD tool in the ChemStation



Results and Discussion

Analysis on a 1260 Infinity DAD with a 60-mm path length cell



Conclusions

The Agilent 1260 Infinity High Dynamic Range DAD (HDR-DAD) allowed the analysis of main and trace compounds in one single run due to an increased linear dynamic range by a factor of >30.

The analysis of all water soluble vitamins in vitamin tablets was possible by using the Agilent 1260 Infinity HDR-DAD. Vitamin B12 with a concentration of 2 µg/tablet was quantified as well as vitamin C with a concentration of 60 mg/tablet in one run.