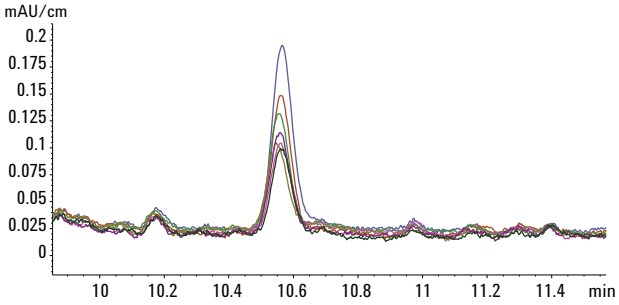
Analysis of High- and Low-Dosed Vitamins in a Single Run Using the Agilent 1200 Infinity Series High Dynamic Range Diode Array Detector Solution

Application Note

Food Testing & Agriculture



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Abstract

The Agilent 1200 Infinity Series High Dynamic Range Diode Array Detector (HDR DAD) solution expands the linear dynamic range by a factor of 30. By combining the signals from two diode array detectors with different path length Agilent Max-Light flow cells, the 1200 Infinity Series HDR DAD solution facilitates detection and quantification of main and trace components in a single run without exceeding the linear range of the HDR DAD solution. This Application Note analyzed water soluble vitamins from vitamin tablets. All vitamins were separated from each other and from matrix peaks. High-dosed vitamin C and low-dosed vitamin B12 were quantified in a single run.

Agilent Technologies

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 (S/N) 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[1,2]. 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.

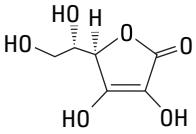
Experimental

The following instruments, conditions, and compounds were used.

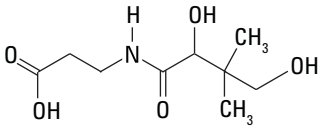
Instrumentation

Instrument	Part no.
Agilent 1260 Infinity Diode Array Detector 2	G4212B with a 3.7-mm cell
Agilent 1260 Infinity Diode Array Detector 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

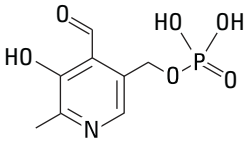
Compounds analyzed



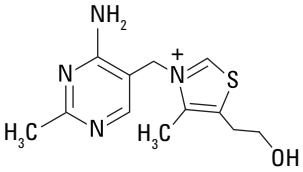
Vitamin C



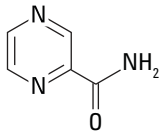
Pantothenic acid



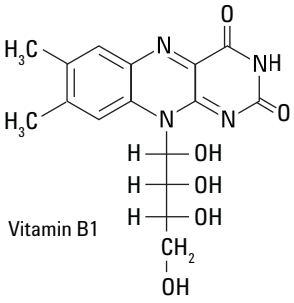
Vitamin B6



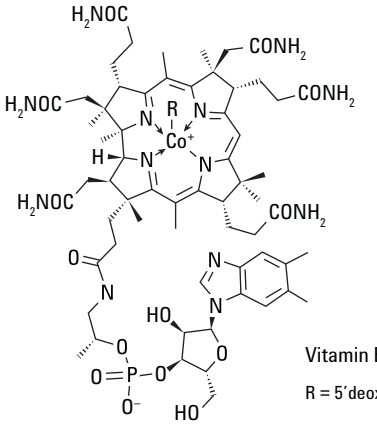
Vitamin B2



Niacinamid



Vitamin B1



Vitamin B12

R = 5′deoxyadenosyl, ME, OH, CN

Sample preparation

- Two vitamin effervescent tablets were placed in 20 mL of water and 20 mL of ethanol. Ethanol is the optimum solvent for niacinamid and vitamin B12.
- The solution was stirred for 10 minutes in the dark.
- A 20-mL aliquot was filtered using two Agilent Captiva Premium Syringe Filters, regenerated cellulose, p/n 5190-5111.
- 1.5 mL was transferred into an autosampler vial and the sequence was started immediately.

Software

Agilent OpenLAB CDS ChemStation Edition, revision C.01.05

Chromatographic conditions

Parameter	Value
Column	Agilent ZORBAX RRHT Eclipse plus C18, 4.6 × 150 mm, 1.8 µm (p/n 959994-902)
Mobile phases	A) water + 4 g/L K_2HPO_4 + 0.5 g/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 %
Flow	1.2 mL/min
Stop time	14 minutes
Post time	5 minutes
Injection volume	2 µL, sample temperature was kept at 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

Concentration and injected amount

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

Principle and functionality of the 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 pathlength 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 Infinity or 1290 Infinity DADs together, see Figure 2. 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. 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.

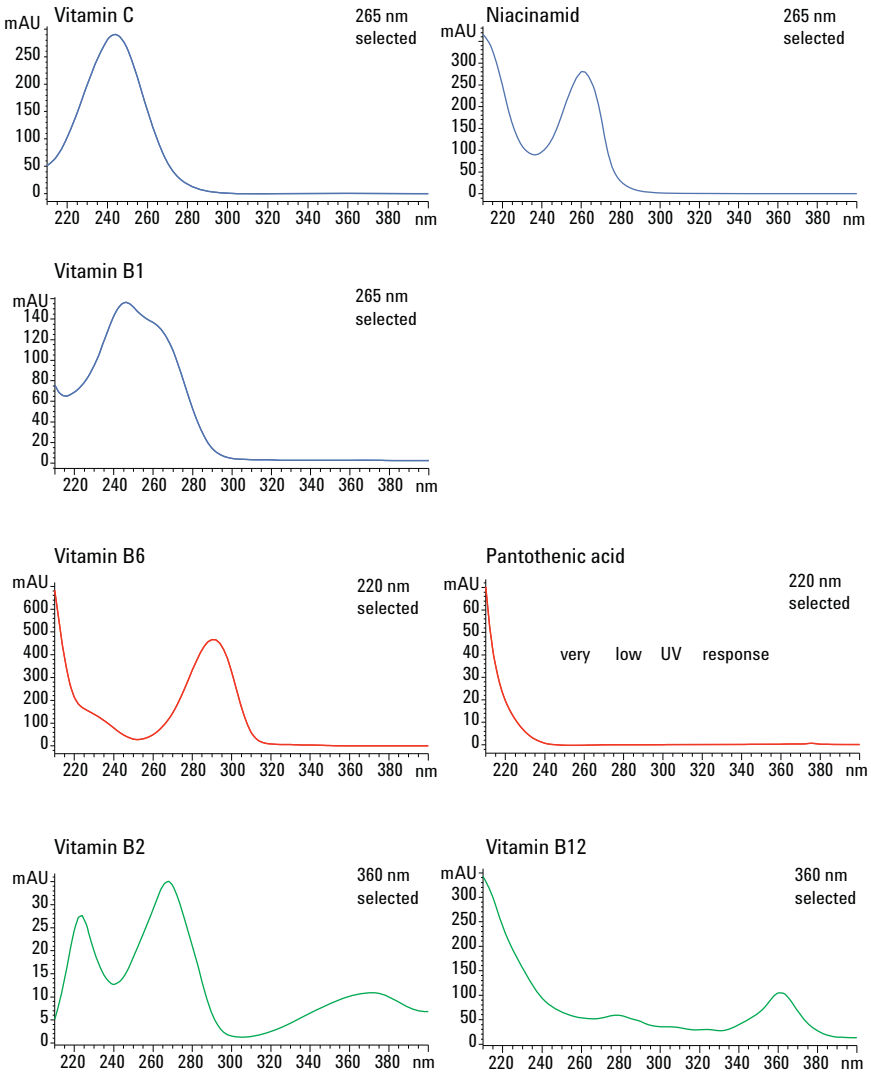


Figure 1. Compound spectra.

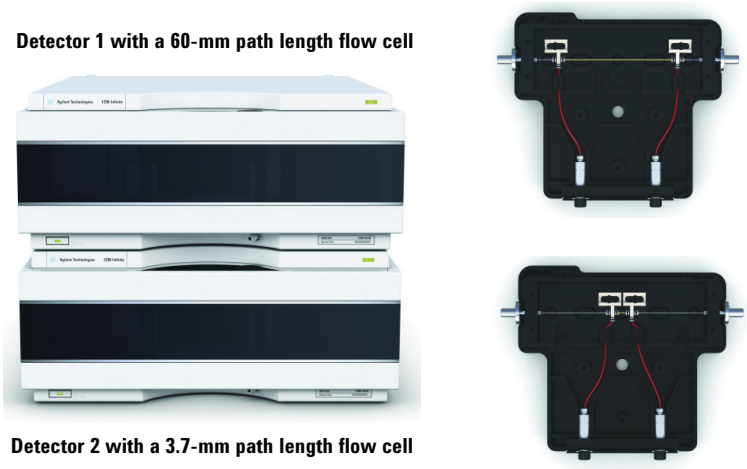


Figure 2. Agilent 1200 Infinity Series HDR DAD solution with two clustered detectors.

The high dynamic range (HDR) tool is configured during instrument configuration. Both detectors are clustered and the delay volume of the capillary connecting both detectors is filled in. In the user interface, both detectors appear as one detector, see Figure 3.

Results and Discussion

Optimizing the separation

In vitamin tablets, the concentration of vitamin B12 is very low, approximately 2 to 5 µg/tablet. Therefore, selective detection is 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 exposed to light 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.

Pantothenic acid shows very low UV absorbance, see Figure 1. Even though it was present at 18 mg/tablet, the peak height only showed approximately 15 mAU at 220 nm. Good separation from other vitamins and matrix peaks was also mandatory.

Several experiments with different columns, mobile phases, gradients, and different pHs were evaluated. The best separation from each other and from matrix peaks was achieved by using the column, mobile phase, and gradient as described under Chromatographic conditions.

The ion pairing reagent was needed to obtain good peak shape and more retention on the reversed phase material. Methanol gave better separation than acetonitrile. To elute vitamin B12 earlier, the organic percentage was increased to 50 % after 11 minutes.

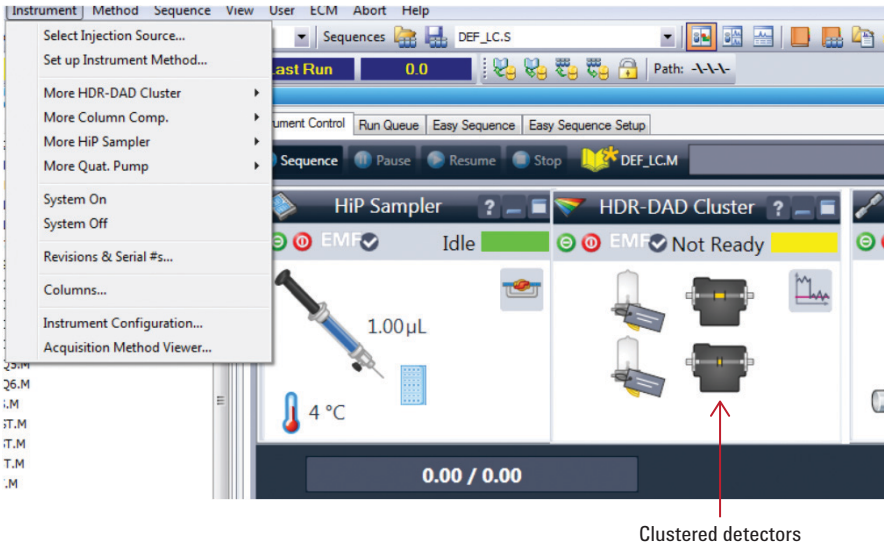


Figure 3. Configuring the Agilent 1200 Infinity Series HDR DAD tool in the Agilent ChemStation.

Analysis of vitamins with different detector configurations

To prove that using the Infinity HDR-DAD, vitamin B12, and, for example, vitamin C can be quantified in one run, three different detector configurations were used.

Analysis of standard and sample with:

- Agilent 1260 Infinity DAD with 10-mm cell
- Agilent 1260 Infinity DAD with 60-mm cell
- Agilent 1260 Infinity HDR DAD with 3.7 and 60-mm cell

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

Quantitation of pantothenic acid and B6 at 220 nm, quantitation of vitamin C, niacinamid, and B1 at 265 nm, and quantitation of B2 at 360 nm was possible with excellent precision for retention times and areas. The precision of retention times was between 0.0112 % RSD for vitamin C, and 0.033 % RSD for vitamin B1. The area precision was between 0.29 % RSD and 0.46 % RSD.

No quantitation of B12 at 360 nm was feasible. With an S/N ratio of 4.4, which is very close to the detection limit with S/N = 3, reliable quantitation is very difficult. The S/N ratio should be at least 10 for quantitation. For better overview, the results are summarized in Table 1.

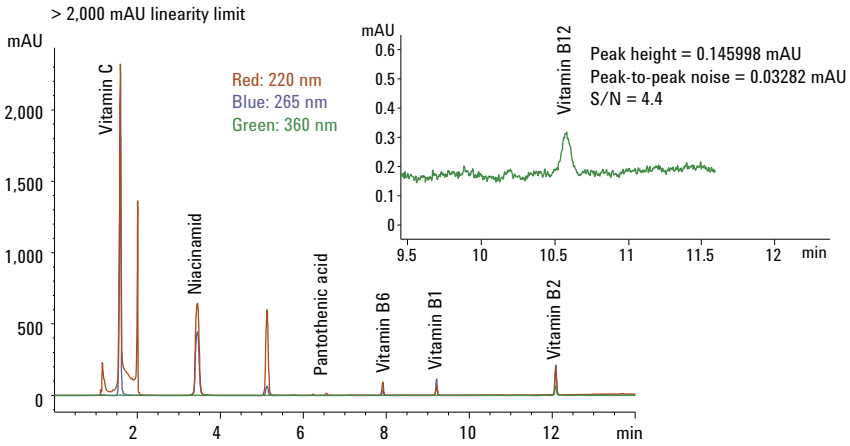


Figure 4. Analysis of water soluble vitamins with an Agilent 1260 Infinity DAD with a 10-mm path length cell.

Table 1. Summary of results for Figure 4 (✓ Quantification possible; ✕ Quantification not possible).

Compound	Quantification with an Agilent 1260 Infinity DAD with a 10-mm cell
Vitamin C	✓
Niacinamid	✓
Pantothenic acid	✓
Vitamin B6	✓
Vitamin B1	✓
Vitamin B12	✕
Vitamin B2	✓

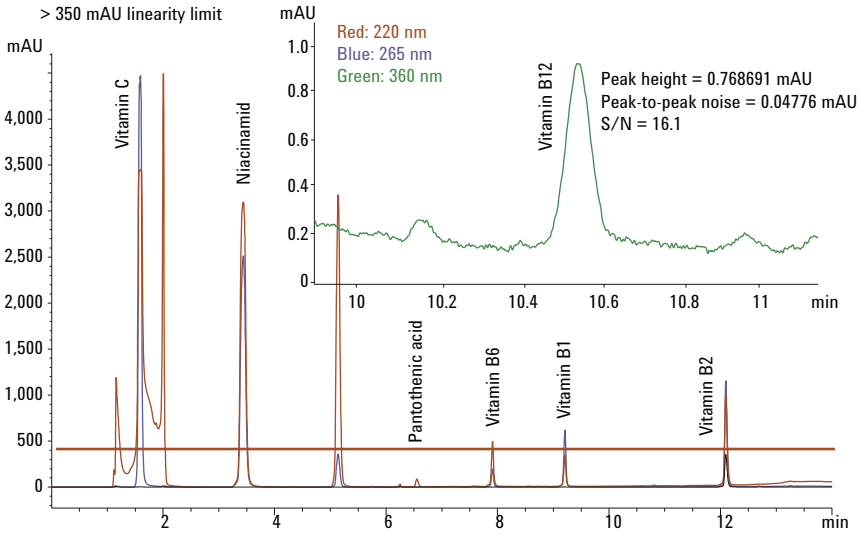


Figure 5. Analysis of vitamins with an Agilent 1260 Infinity DAD with a 60-mm path length cell.

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

Quantitation of vitamin B12 at 360 nm with a S/N = 16.1 was feasible as well as the quantitation of pantothenic acid, B6, B1, and B2, see Table 2.

No quantitation of vitamin C and niacinamid was possible. Both were out of the linear range of the DAD with the 60-mm cell.

Analysis with a 1260 Infinity HDR DAD

Quantitation of all vitamins was possible due to the enhanced linear range of the 1260 Infinity HDR DAD. One injection was sufficient to analyze and evaluate low-dosed and high-dosed vitamins, see Table 3.

One remaining challenge was the decomposition of vitamin B12 after dissolution, see Figure 7. Each run took approximately 20 minutes, and after three injections approximately 50 % was decomposed even though the sample temperature was kept at 4 °C, and the vials were completely filled to exclude oxygen as much as possible.

We assume that decomposition of vitamin B12 started immediately after dissolution during sample preparation. Further decomposition was observed in the first and second injections. After three injections, the area counts became more stable. Preparing the sample in the dark, and applying a protective gas might result in less decomposition and better area precision. The precision of retention times was 0.055 % RSD over seven runs. After stabilization of the area counts, the area precision was approximately 11.5 % RSD evaluating runs 4 to 7. The study on degradation of vitamin B12 in dietary supplements[3] notes that if vitamin C is present in the preparation, it influences the decomposition of vitamin B12.

Table 2. Summary of results for Figures 4 and 5 (✓ Quantification possible; ✕ Quantification not possible).

Compound	Quantification with an Agilent 1260 Infinity DAD with a 10-mm cell	Quantitation with an Agilent 1260 Infinity DAD with a 60-mm cell
Vitamin C	✓	✕
Niacinamid	✓	✕
Pantothenic acid	✓	✓
Vitamin B6	✓	✓
Vitamin B1	✓	✓
Vitamin B12	✕	✓
Vitamin B2	✓	✓

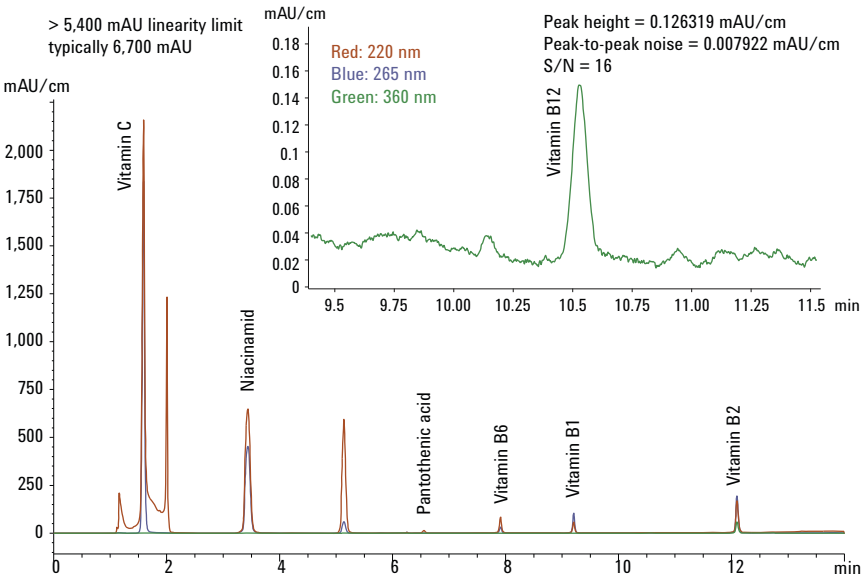


Figure 6. Analysis of vitamins with an Agilent 1260 Infinity HDR DAD.

Table 3. Summary of results for Figures 4, 5, and 6 (✓ Quantification possible; ✕ Quantification not possible).

Compound	Quantification with an Agilent 1260 Infinity DAD with a 10-mm cell	Quantification with an Agilent 1260 Infinity DAD with a 60-mm cell	Quantification with an Agilent 1260 Infinity DAD HDR Solution
Vitamin C	✓	✕	✓
Niacinamid	✓	✕	✓
Pantothenic acid	✓	✓	✓
Vitamin B6	✓	✓	✓
Vitamin B1	✓	✓	✓
Vitamin B12	✕	✓	✓
Vitamin B2	✓	✓	✓

Conclusion

The Agilent 1260 Infinity 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 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.

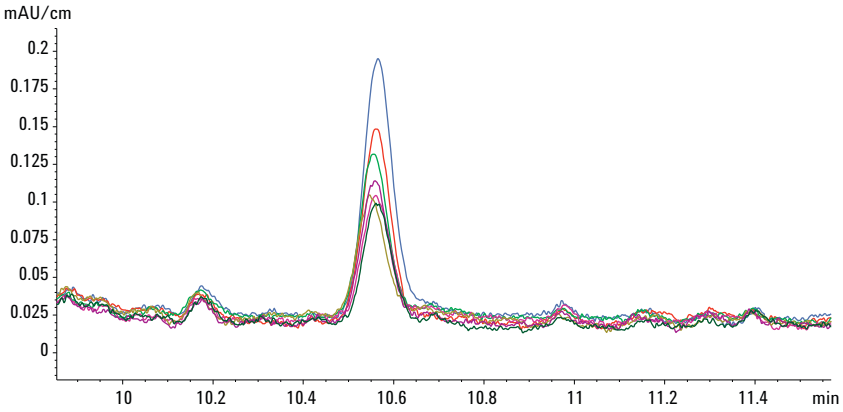


Figure 7. Decomposition of vitamin B12 over time.

References

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2. Rada Amidzic *et al*, "RP-HPLC Determination of vitamins B1,B3,B6, and B12 in multivitamin tablets", *J.Serb.Chem.Soc*. 70 (10) 1229-1235 (**2005**).

3. Yamada K, Shimodaira M, Chida S, Yamada N, Matsushima N, Fukuda M, Yamada S., Department of Liberal Arts and Sciences, School of Health Sciences, Sapporo Medical University, Sapporo, Japan. oyama@sapmed.ac.jp, "Degradation of vitamin B12 in dietary supplements." *Int J Vitam Nutr Res*. Jul-Sep; 78(4-5):195-203. doi: 10.1024/0300-9831.78.45.195. (**2008**).

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