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 path length 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 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 includes 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 × 19.6 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.

Experimental

Instrumentation, chromatographic conditions

1200 Infinity DAD2 G4212B with a 3.7-mm cell

Agilent 1200 Infinity DAD1 G4212B with a 60-mm cell or G4212B with a 10-mm cell

Agilent 1200 Infinity Column compartment G1316C

Agilent 1200 Infinity Standard Autosampler G1329B

Agilent 1200 Infinity ALS cooler G13390B

Agilent 1200 Infinity Binary Pump G1312B

Column: Agilent ZORBAX RRHT Eclipse plus C18, 4.6 × 150 mm, 1.8 μm (p/n 959904-902)

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 pantothentic acid and vitamin B6, 265/10 nm for vitamin C, niacinamide, and vitamin B12, 360/10 nm for vitamin B12 and B2.

Column temperature: 40 °C.

Sample preparation: Two vitamin effervescence 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 PreVac Syringe Filter, regenerated cellulose. 1.5 mL was transferred into an autosampler vial.

Results and Discussion

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

Analysis on a 1260 Infinity HDR DAD

Results and Discussion

Analysis on a 1260 Infinity HDR DAD with 360 nm

Final Results

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.

Software implementation

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

Experimental

The Agilent 1200 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 1200 Infinity HDR DAD. Vitamin B12 with a concentration of 2 μg/tablet was quantified as well as vitamin C with a concentration of 60 μg/tablet in one run.

Conclusions

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

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