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アプリケーションノート

LC-MS Analysis of Vitamin B_{12}

Hongjian Jiang, Zhao Gui Pin, Kate Yu

Waters Corporation

Abstract

This application note describes a simple LC-MS method for the analysis of a Vitamin B_{12} standard.

Benefits

With the use of the Waters Atlantis C_{18} Column, Vitamin B_{12} was well retained, with a good peak shape

Introduction

Vitamin B_{12} (cyanocobalamine) is a water-soluble vitamin that has important physiological function in the human body. Vitamin B_{12} has the ability to make folic acid available to bone marrow, which appears to be necessary for red blood cell formation. A Vitamin B_{12} deficiency leads to a degeneration of both the sensory and motor columns in the spinal cord with loss of sensation and paralysis. As a result, people need approximately 1–2 µg of Vitamin B_{12} per day.

The determination of Vitamin B_{12} is important, but can be challenging largely because of its chemical instability and the complexity of the matrices in which it is usually found. Ideally, methods for Vitamin B_{12} analysis should be simple, selective and sensitive to overcome the above issues. The most commonly reported method for Vitamin B_{12} analysis is reversed-phase HPLC separation with various detection schemes such as

UV, fluorescence, chemiluminescence, etc. However, most of these methods are tedious and time consuming. Phosphate buffers are usually called for in order to obtain sufficient LC separation.

In this application note, we describe a simple LC-MS method for the analysis of a Vitamin B_{12} standard. A Waters Atlantis C_{18} Column, with superior retention for polar compounds and the ability to operate in 100% aqueous conditions, was used for the separation. A Waters Micromass ZQ 4000 single quadrupole mass spectrometer was used for detection. With the selectivity and sensitivity offered by the MS detector, the method simply used a binary acetonitrile/water gradient without the need for a buffer or ion pairing reagents.

Experimental

LC Conditions

LC system:	Alliance HT Separations Module
Column:	Atlantis C_{18} 2.1 x 150 mm, 3.5 µm
Flow rate:	0.2 mL/min
Mobile phase:	Acetonitrile (A) Water (B)
Injection volume:	10 µL

Gradient

Time(min)	%A	%B	Curve
0	0	100	1
1	0	100	1
8	40	60	6

Time(min)	%A	%B	Curve
10	60	40	6
11	0	100	11

MS Conditions

Mass spectrometer:	Micromass ZQ 4000 Mass Spectrometer
Ion mode:	ESI+
Capillary voltage:	3.5 kV
Cone voltage:	80 V
Source temp.:	105 °C
Desolvation temp.:	180 °C
Desolvation gas flow:	365 L/Hour
Cone gas flow:	50 L/Hour
Inter channel delay:	0.02 s
Inter scan delay:	0.02 s
Dwell time:	0.08 s

Results and Discussion

Full scan spectra of Vitamin B_{12} were obtained during the optimization of the MS parameters, as shown in Figure

1. Acquisition parameters were optimized in order to select the proper ion for selected ion monitoring (SIR) experiments and to determine an optimum cone voltage. The protonated molecule $[M+H]^+$ at *m/z* 1356 was obtained at a cone voltage of 50 V with good intensity (4.41e5). However, the Vitamin B_{12} showed fragmentation into many other ions, which reduced the intensity of the $[M+H]^+$ ion. The spectrum at 80 V was cleaner with higher ion intensity (6.14e5) at *m/z* 913. As a result, the ion at *m/z* 913 was chosen to be the ion to monitor for the SIR experiments and cone voltage of 80 V was chosen as the optimum for the ion at *m/z* 913.

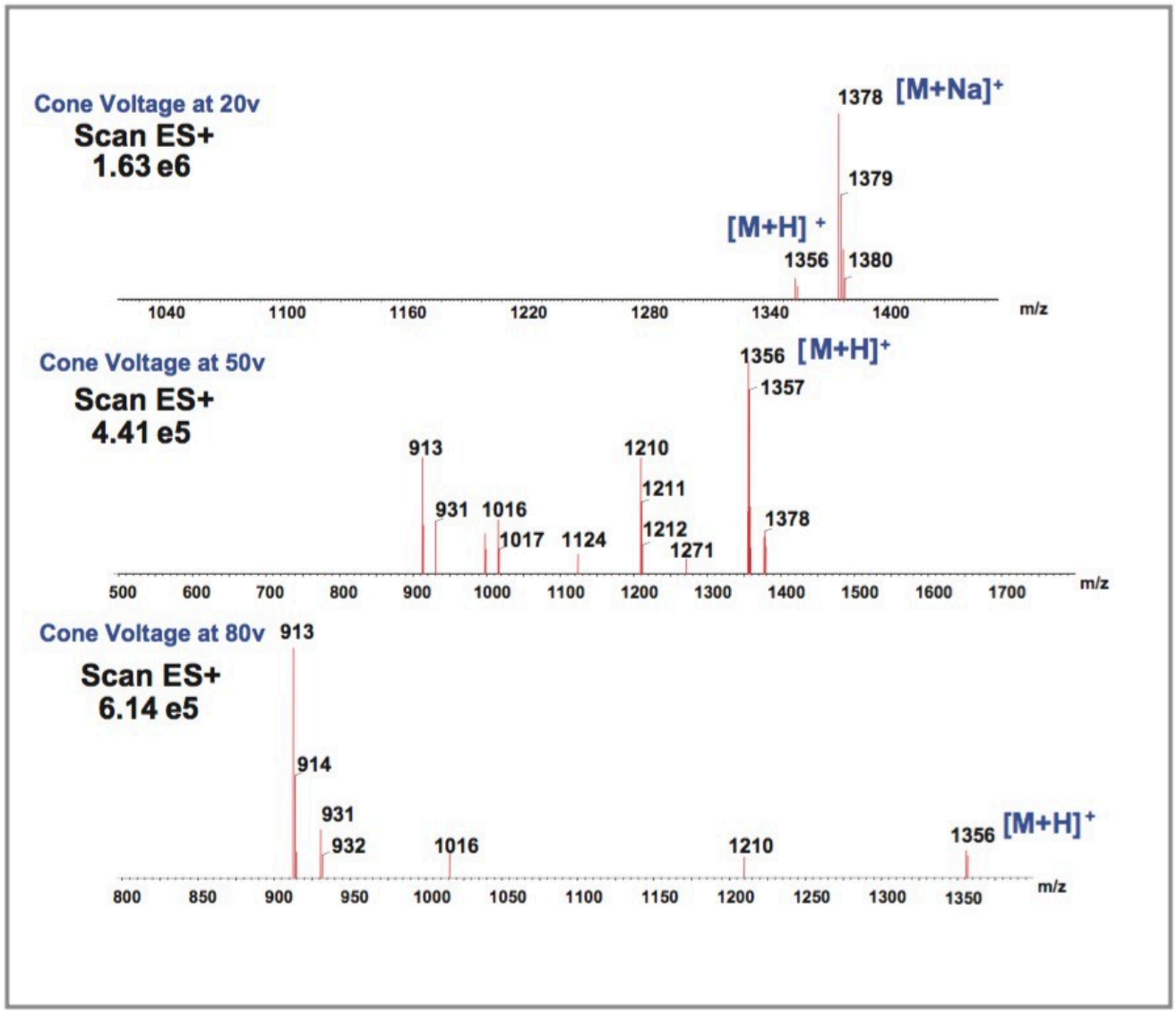

Figure 1. Full scan MS spectra for Vitamin B_{12} at different cone voltages.

Figure 2 shows the calibration curve of Vitamin B_{12}. The linear range was from 5 to 4000 ppb. With the Waters Atlantis C_{18} Column, this water-soluble vitamin was well retained with an excellent peak shape. Figure 3 shows the SIR chromatograms of Vitamin B_{12} at *m/z* 913 at different concentrations. The signal-to-noise ratio at 2 ppb was 5, and at 5 ppb was 50.

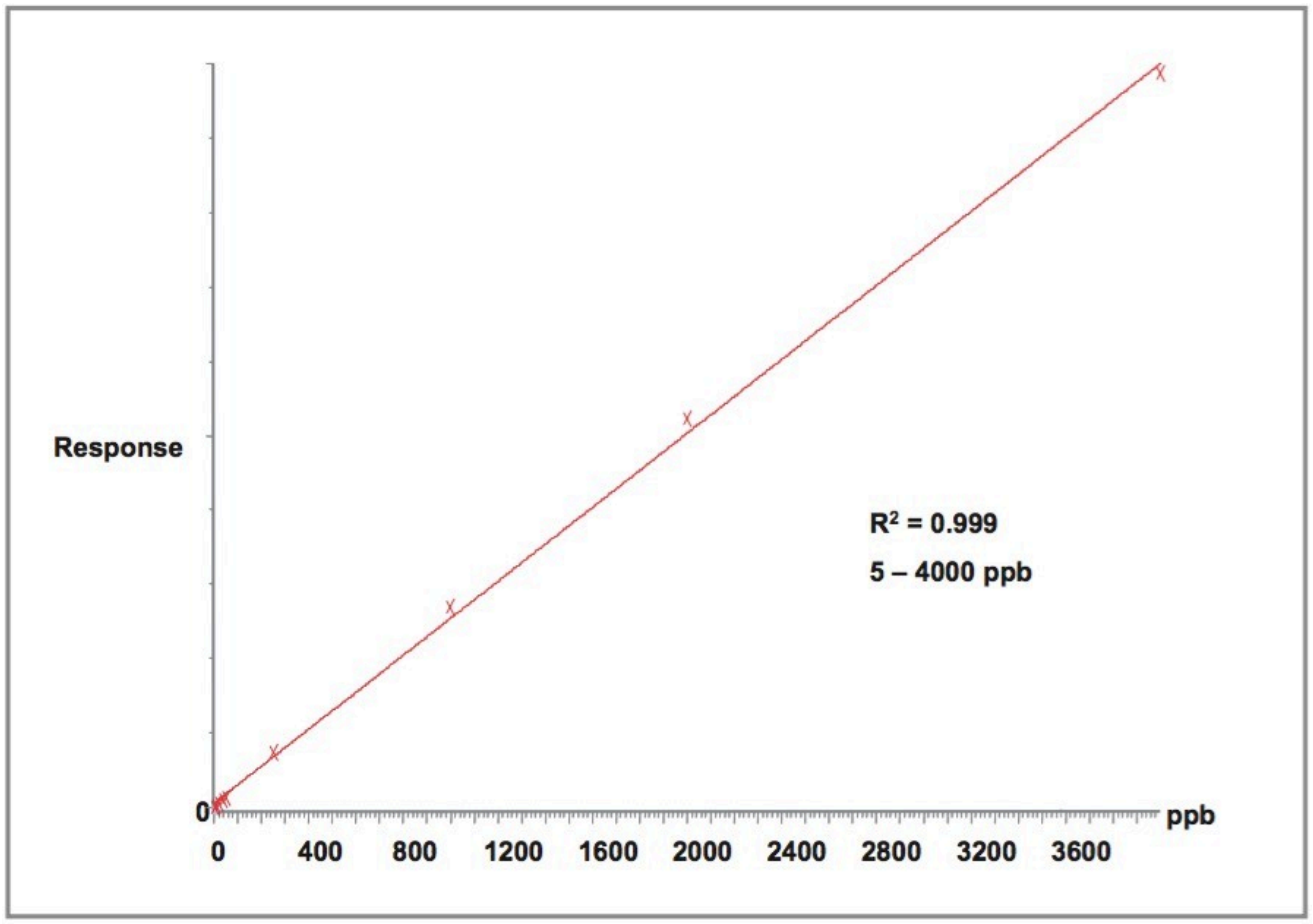

Figure 2. Calibration curve of Vitamin B_{12}.

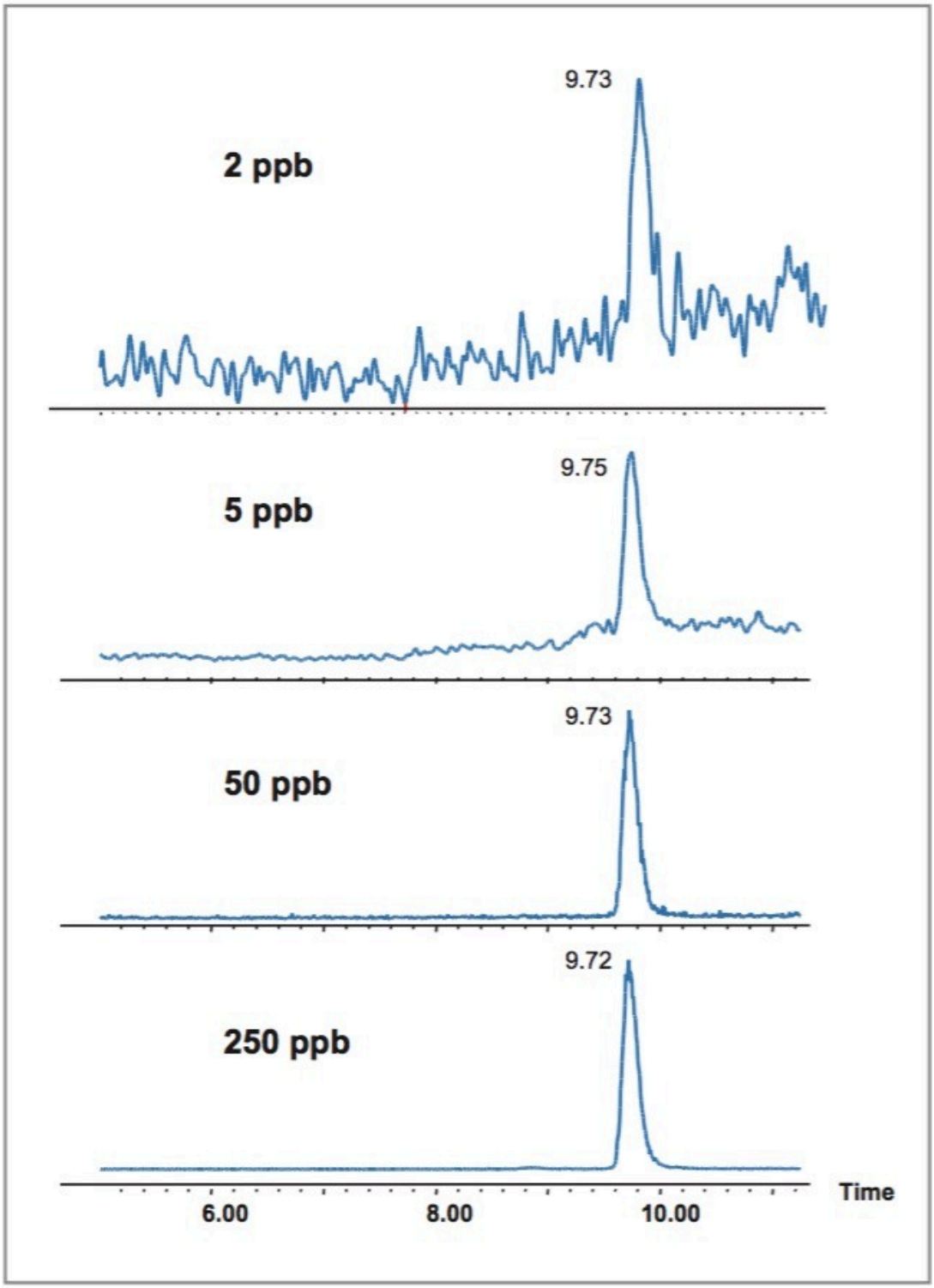

Figure 3. SIR chromatograms of Vitamin B_{12} at m/z 913.

Conclusion

A simple LC-MS quantification method for Vitamin B_{12} was described. With the use of the Waters Atlantis C_{18} Column, Vitamin B_{12} was well retained, with a good peak shape. A simple binary gradient was used without adding buffer. The quantification linear range was from 5n to 4000 ppb. The limit of detection with a 10 µL injection was 2 ppb.

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