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A Quantitative Detection Method for 9 Water-Soluble Vitamins in Human Serum/Plasma was Established

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Introduction

Vitamins are a class of trace substances necessary for the maintenance of normal physiological functions. Vitamins can be roughly divided into water-soluble and fat-soluble vitamins in terms of chemical properties. Water-soluble vitamins are mainly B vitamins. Vitamin deficiency can occur when vitamins required for health are missing. For example, vitamin B1 deficiency can cause beriberi, and vitamin B12 deficiency can lead to megaloblastic anemia. Therefore, vitamin testing is of great significance for assessing the nutritional status of the human body and the diagnosis of related diseases. In this study, the Agilent Infinity LC Clinical Edition/K6460 TQ/LC MS system was used to establish a detection method for nine water-soluble vitamins in human serum to quickly and comprehensively evaluate the status of water-soluble vitamins contained in the human body.

Experimental

Sample Preparation

Step 1 Standards Working Solution Preparation

Dilute the standard with water to the concentration level shown in the following figure. Table 1.

Vitamin B (ng/ml)	S1	S2	S3	S4	S5	S6	S7
VB1 (thiamine hydrochloride)	1	3	5	10	25	50	100
VB2 (riboflavin)	0.5	1.5	2.5	5	12.5	25	50
VB3-NH3 (niacin)	0.5	1.5	2.5	5	12.5	25	50
VB3-COOH (niacinamide)	0.5	1.5	2.5	5	12.5	25	50
VB5 (pantothenic acid)	5	15	25	50	125	250	500
VB6-COOH (4-pyridoxic acid)	1	3	5	10	25	50	100
VB7 (biotin)	0.25	0.75	1.25	2.5	6.25	12.5	25
VB9-5-MTHF (5-methyltetrahydrofolate)	1	3	5	10	25	50	100
VB12 (methylmalonic)	0.1	0.3	0.5	1	2.5	5	10

Table 1.The Concentration of the 9 Water-Soluble Vitamins

Accurately transfer 10 μ L of the above standard series solution into a 90 μ L water-soluble vitamin blank serum sample, vortex mix, and use it as the standards working solution, store at -20 ° C, away from light, for future use.

Experimental

Step 2 Isotope Labelled Internal Standard Preparation

Preparation of isotope labelled internal standard solution (300ng/mL): An internal standard stock solution of the 9 water-soluble vitamins compounds at a concentration of 300 µg/mL was prepared with MeOH, and then an internal standard working solution with a concentration of 300 ng/mL was prepared with MeOH, and stored in the dark at -20 °C for backup.

Step 3 Sample Preparation

Pipette the standards working solution, human serum sample, 100 µL in a 1.5 mL centrifuge tube, add 300 µL of the isotope labelled internal standard working solution, close the lid, vortex for 5 min, place the sample in a benchtop high-speed refrigerated centrifuge after the completion of shaking, centrifuge at 13000 rpm at 4°C for 5 min, after the end of refrigeration centrifugation, aspirate all the supernatant in a clean 1.5 mL centrifuge tube, and dry with nitrogen. After blow-drying, pipette 100 µL of ultrapure water in a centrifuge tube, vortex for 1 min, centrifuge at 13,000 rpm for 5 min at 4°C, and pipette 85 µL of the upper clear solution for analysis .

LC Method

Agilent 1260 Infinity UHPLC binary pumps, well plate sample with thermostat, temperature-controlled column compartment.

Parameter	Value	
Column:	Agilent Pursuit 3 PFP (2.1 x 100 mm)	
Column temperature:	40°C	
Injection volume:	2 µL	
Autosampler temperature:	4°C	
Mobile phase:	A: 10mmol Ammonium acetate in water B: MeOH	
Flow:	0.3 mL/min	
Gradient:	Time	B%
	0	1
	3	1
	5	10
	9	50
	9.5	98
	12	98
	12.1	1
	16	1

Table 2. LC Parameters

Experimental

MS Method

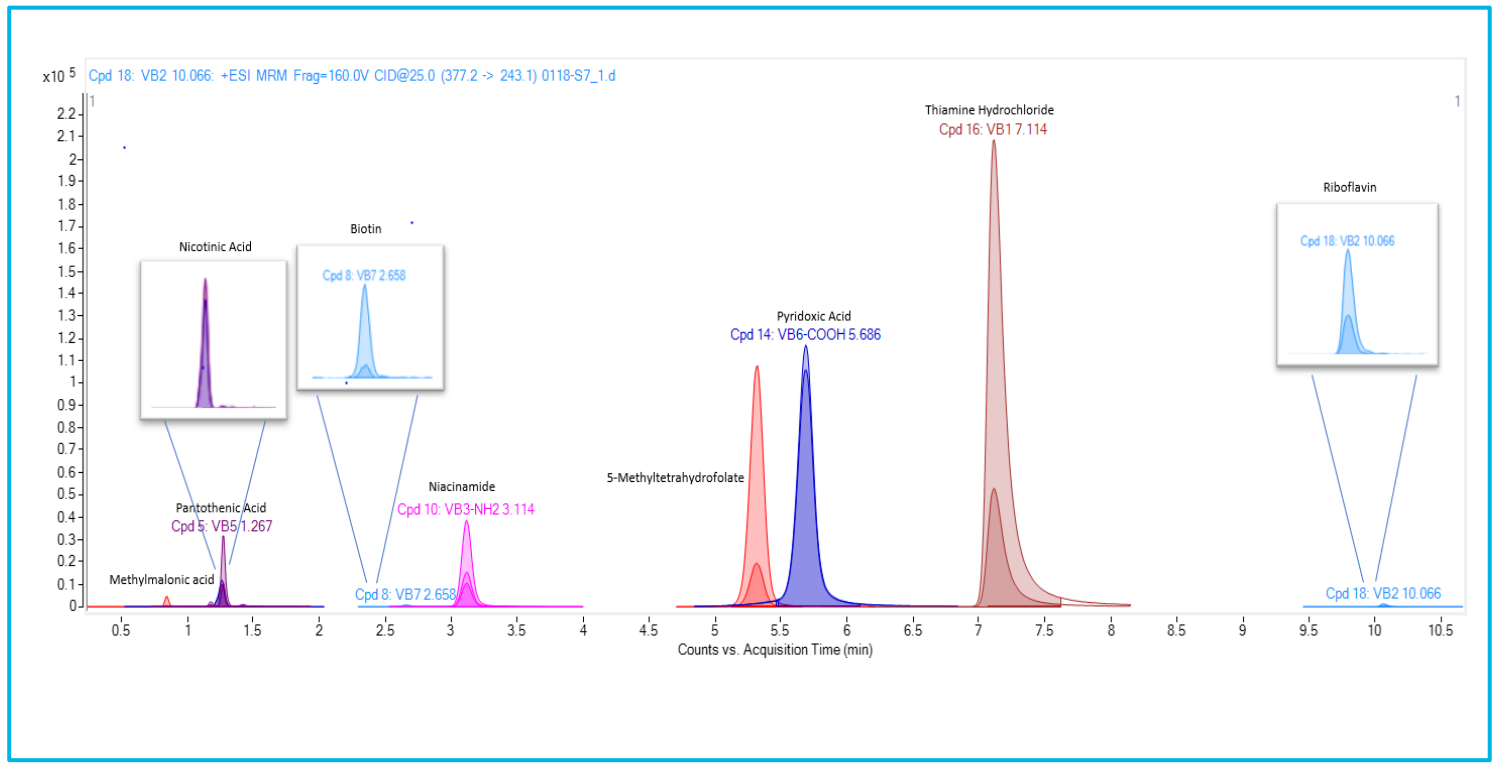
Agilent K6460 QQQ LC/MS with Agilent JetStream Technology	
Ion mode:	Positive/Negative
Drying gas temperature	250 °C
Drying gas flow:	7 L/min
Press:	25 psi
Sheath gas temperature:	350 °C
Sheath gas flow:	12 L/min
Vcap:	3000 V (+)/2500 V (-)
Detection mode:	dMRM
MRM parameter:	Table 4

Table 3. MS Parameters

Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	Fragmentor (V)	CE (V)	Polarity
VB1	265.2	122.1	90	15	Positive
VB2	377.2	243.1	160	25	Positive
VB3-COOH	124.1	80.1	120	25	Positive
VB3-NH3	123.1	80.1	120	25	Positive
VB5	220.1	90.1	95	15	Positive
VB6-COOH	184.2	166.1	100	10	Positive
VB7	245.2	227.1	100	10	Positive
VB9-5MeTHF	460.2	313.2	130	18	Positive
VB12	117.1	73.1	60	10	Negative

Table 4. MRM Parameters

Results and Discussion



Results and Discussion

Limit of Quantification

Limit of quantification test results show that the limits of quantification for the 9 water-soluble vitamins are as follows: VB1 1ng/ml , VB2 0.5 ng/ml ,VB3 -COOH 0.5ng/ml ,VB3 -NH4 0.5ng/ml , VB5 5ng/ml ,VB6-COOH 1ng/ml ,VB7 0.25ng/ml ,VB9-5MeTHF 1ng/ml ,VB12 2ng/ml. The signal response is good and can be accurately quantified to meet the needs of clinical research.

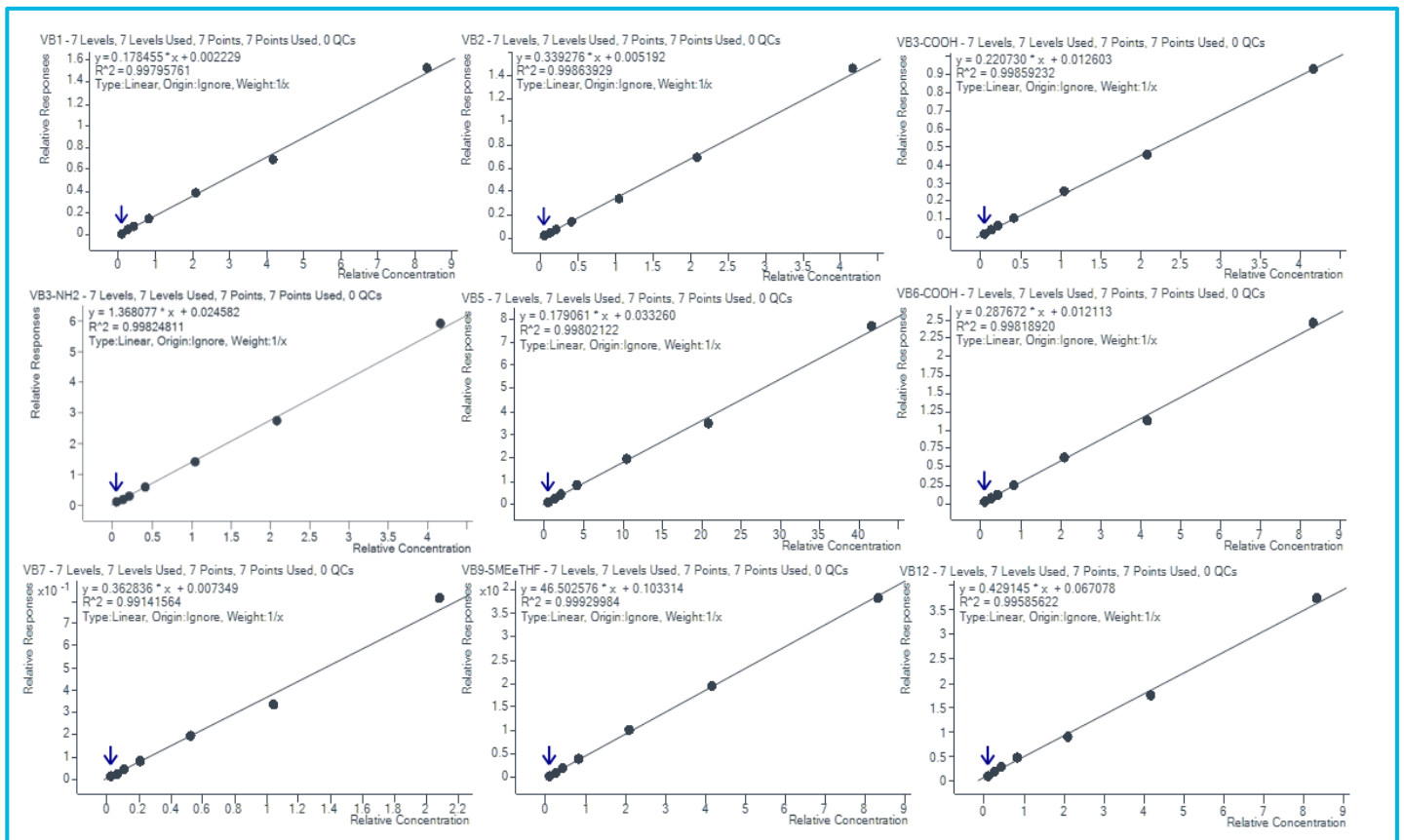


Figure 2. Standards Curves for 9 Water-Soluble Vitamins

Calibration Curve

Preparation of 9 water-soluble vitamins calibrants was done in 1% bovine serum albumin and were prepared as zero samples.

VB1: 1 - 100 ng/mL, linear correlation coefficient R greater than 0.99; VB2: 0.5-50 ng/mL, linear correlation coefficient R greater than 0.99; VB3-COOH: 0.5-50 ng/mL, linear correlation coefficient R greater than 0.99; VB3-NH2: 0.5-50 ng/mL, linear correlation coefficient R greater than 0.99; VB5: 5-500 ng/mL, linear correlation coefficient R greater than 0.99; VB6-COOH: 1-100 ng/mL, linear correlation coefficient R greater than 0.99, VB7: 0.25-25 ng/mL, linear correlation coefficient R greater than 0.99, VB9-5M-THF: 1-100 ng/mL, linear correlation coefficient R greater than 0.99, VB12: 2-200 ng/mL, linear correlation coefficient R greater than 0.99.

Accuracy

Bio-Rad Lyphocheck's reference materials are used to determine the accuracy of 9 water-soluble vitamins, this method determined 9 kinds of water-soluble vitamins, and the accuracy was satisfactory 85%-115%.

Conclusions

This work uses the Agilent Infinity LC Clinical Edition/K6460 TQ/LC MS to establish a quantitative detection method for 9 water-soluble vitamins in human serum. The experimental results show that the method has good sensitivity, stability and accuracy, and is suitable for the quantitative analysis of VB1, VB2, VB3-COOH, VB3-NH3, VB5, VB6-COOH, VB7, VB9-5M-THF), and VB12. Method validation should be carried out according to the relevant guidelines.

References

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2. Henderson, J.W.Jr., Longy, W.J., *Exceptional Selectivity of Agilent ZORBAX Eclipse Plus Phenyl-Hexyl Columns to Separate Estrogens.* 2009 Mar 26;5990-9130 EN

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