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Applikationsbericht

A Rapid and Simple Clinical Research Method for Vitamin C in Serum by UPLC-QDa

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Abstract

We describe a clinical research method for the analysis of vitamin C in serum by ACQUITY UPLC H-Class PLUS and a single quadrupole mass detector, the ACQUITY QDa. A simple protein precipitation by Trichoroacetic acid (TCA) provides sufficient selectivity and analytical sensitivity for vitamin C to be analyzed by UPLC-QDa. From fat-soluble[1] to water-soluble vitamins, UPLC QDa is suitable as a vitamin analytical system for clinical research purposes.

Benefits

- Requires only 200 µl of serum
- Simple sample preparation
- An accurate and sensitive method for the analysis of vitamin C in serum with a total analysis time of 6 minutes

Introduction

Vitamin C or L-ascorbic acid is a water-soluble vitamin and essential nutrient for humans. Research analysis methods for vitamin C blood levels have been developed; HPLC with Electrochemical Detector (ECD) or Ultra-violet (UV) detection are commonly recommended as the method of choice for plasma vitamin C analysis.[2,3] Electrochemical detection is an attractive method for detection of electroactive species because of its inherent advantages of simplicity, ease of miniaturization, high analytical sensitivity, and relatively low cost.[1] However, both ECD and UV detection methods involve complicated sample preparation and mobile phase, dedicated column, and long analysis time. QDa detection not only provides analytically sensitive and selective analysis, but also with UPLC compatible speed and mass information for confirmation.

Experimental

200 µL of sample (calibrator, control, or unknown) was pipetted into a clean microtube. 600 µL of working internal standard (1.67 µg/ml of vitamin $^{13}C_6$ in 12 % TCA (v/v)) was added. The tubes were capped and placed on a multi-tube vortex at 1000 r.p.m. for 5 minutes before centrifugation at 11000 rpm for 5 minutes. 400 µL supernatant was transferred into the maximum recovery vial for the LC-MS analysis. Using an ACQUITY UPLC H-Class PLUS System with an Atlantis Premier BEH C_{18} AX Column (1.7 µm, 2.1 x 100 mm, P/N: 186009368 < https://www.waters.com/nextgen/us/en/shop/columns/186009368-atlantis-premier-beh-c18-ax-column-17--m-21-x-100-mm-1-pk.html>), a gradient elution of 95% mobile phase A (MP A) to 5% MP A was run to achieve separation. The injection to injection time was 6 minutes.

LC Conditions

LC system:	ACQUITY UPLC H-Class PLUS
Vials:	12 x 32 mm Amber Screw Neck Vial, Cap, preslit PTFE/Silicone septa, Maximum Recovery (P/N: 600000755CV)

Column:	Atlantis Premier BEH C_{18} AX Column, 2.1 x 100 mm, 1.7 µm (P/N: 186009368)
Column temp.:	40 °C
Sample temp.:	10 °C
Injection volume:	3 µL
Flow rate:	0.40 mL/min
Mobile phase A:	20 mM Ammonium formate in water
Mobile phase B:	20 mM Ammonium formate in MeOH

Gradient Table

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.40	95	5	Initial
2.00	0.40	25	75	4
2.50	0.40	5	95	6
3.50	0.40	5	95	6
4.00	0.40	95	5	6
6.00	0.40	95	5	6

MS Conditions (QDa Parameter)

MS system:	ACQUITY QDa
Ionization mode:	ESI-
Acquisition mode:	SIR
Capillary voltage:	0.8 kV
Cone voltage:	See channel details

	SIR, *m/z*	Cone (V)	Time
Vitamin C	175	20	0 to 2.5
	115	15	
Vitamin $^{13}C_6$ (IS)	181	20	
	119	15	

Data Management

LC-MS software:	MassLynx 4.2

Results and Discussion

Figure 1 shows the chromatograms of 0.5 µg/mL of vitamin C in the stripped serum. The analytical sensitivity

was investigated, demonstrating that quantitation of 0.5 µg/mL of vitamin C in serum is achievable (% RSD <20%, Bia <15%, S/N> 10). Figure 2 shows the calibration curve of the method. The method was shown to be linear in the range from 0.5 µg/ml to 20 µg/ml. Correlation coefficients (r^2) were >0.995 with all calibrator residuals <15%.

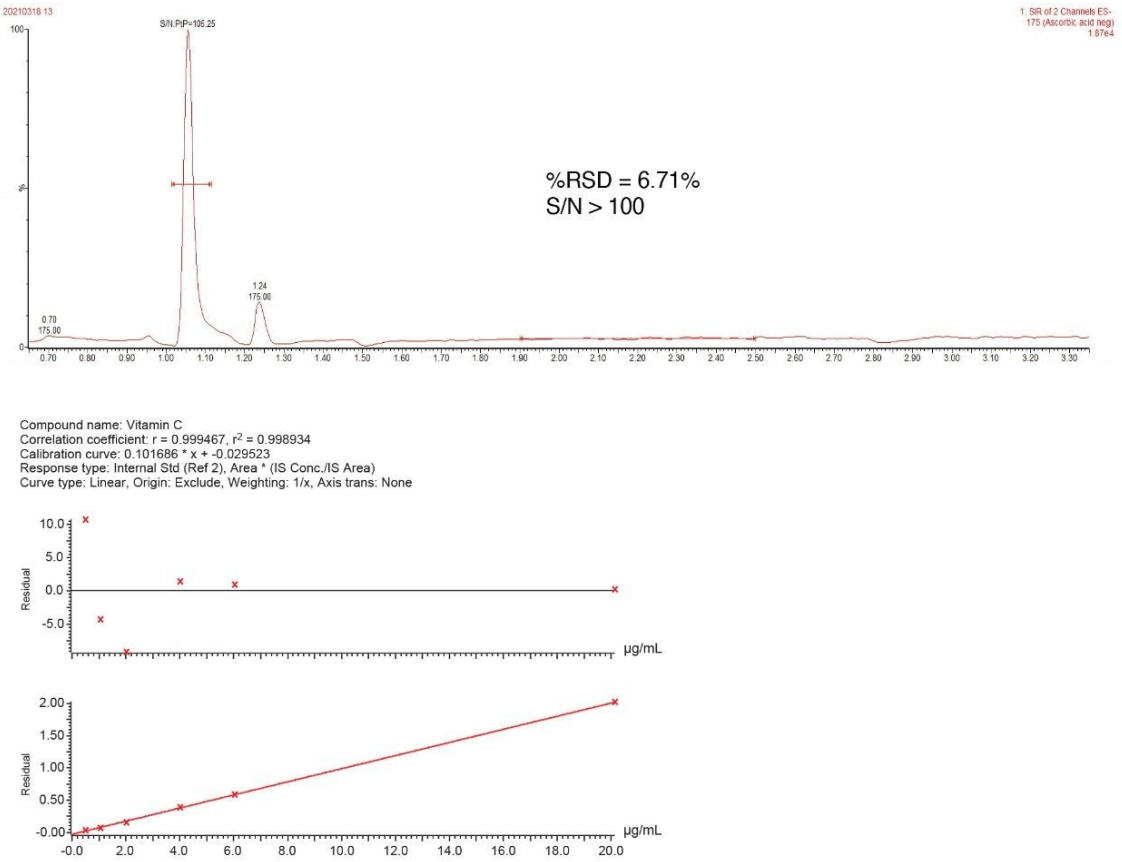

Figure 1. Chromatograms of 0.5 µg/mL of vitamin C in the serum and example of calibration curve of vitamin C.

Precision was assessed by extracting and analyzing 5 replicates from low to high concentration in the stripped serum over 5 days (n=25). Repeatability and total precision were ≤6% CV at all concentration levels tested and are summarized in Table 1. All calculated percentage recoveries were within ±15% for typical endogenous interferences tested when comparing test and control samples.

	Total QC precision (%RSD)			QC repeatability (%RSD)		
Vitamin C	QC1 (low)	QC2 (mid)	QC3 (high)	QC1 (low)	QC2 (mid)	QC3 (high)
	4.05	4.70	4.83	4.96	5.51	5.80

Table 1. The total precision and repeatability of QC. QC1: 0.80, QC2: 3.02, and QC3: 15.11 µg/mL.

Matrix factor results were within ±15% between test samples from six individuals and control samples compensated for by using an internal standard (Table 2). Significant matrix enhancement was observed for vitamin C, but it was also compensated for by the internal standard (adjusted matrix factor close to 1).

Compound	Spiked conc.	Matrix factor – absolute peak area (Range)	Matrix factor – adjusted conc. (Range)
Vitamin C	1 µg/mL	3.507 (3.393–4.609)	1.059 (0.959–1.065)
	15 µg/mL	1.992 (1.684–2.176)	0.975 (0.959–0.978)

Table 2. Result of matrix study.

Conclusion

We explore the application of a UPLC-single quadrupole MS system, the ACQUITY QDa Mass Detector, on a clinical research from fat-soluble vitamins to water-soluble vitamins. The simple preparation and short running time provide significant advantages over traditional HPLC methods. It well demonstrates that UPLC-QDa is suitable for use as a vitamin analytical system in clinical research analysis.

References

1. Analysis of Vitamin A and E in Serum by UPLC-QDa for Clinical Research. Waters Corporation, 2021 https://www.waters.com/webassets/cms/library/docs/720007164en.pdf < https://www.waters.com/webassets/cms/library/docs/720007164en.pdf> .

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3. Robitaille, L; Hoffer, L, J. A Simple Method for Plasma Total Vitamin C Analysis Suitable for Routine Clinical Laboratory Use. *Nutrition Journal*, 2016, 15:40.

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MassLynx MS Software <https://www.waters.com/513662>

720007322, Revised October 2021