DEVELOPMENT AND VALIDATION OF UHPLC-MS/MS METHOD FOR SIMULTANEOUS QUANTIFICATION OF ASCORBIC AND DEHYDROASCORBIC ACIDS

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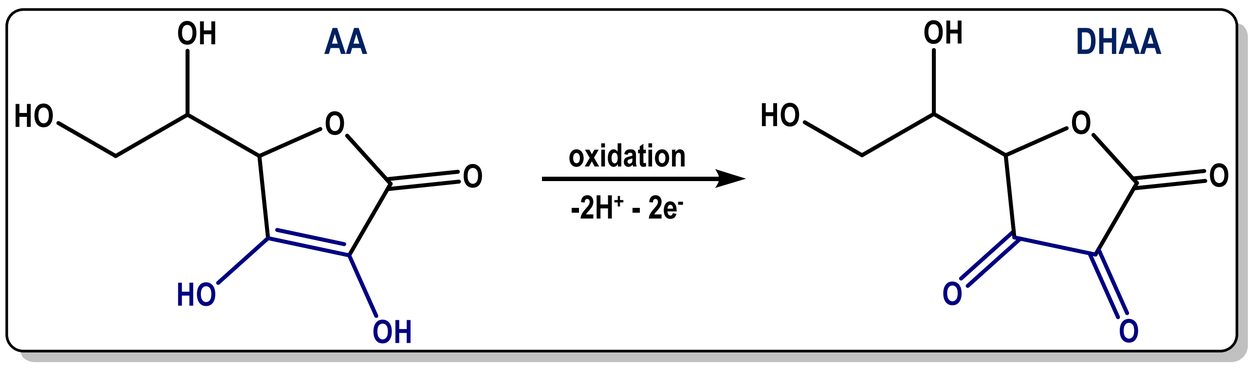
INTRODUCTION

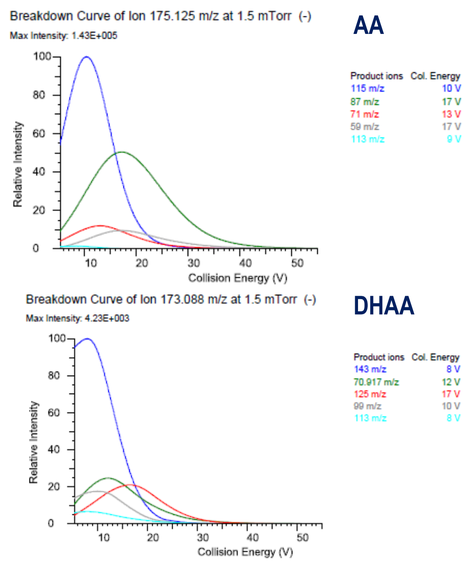
Ascorbic acid (AA) → the active form of vitamin C present in food and commonly used as a dietary supplement.
Under oxidative conditions, AA is readily converted to dehydroascorbic acid (DHAA) → also possesses biological activity.
When analysing vitamin C content, it is important to consider both AA and DHAA.



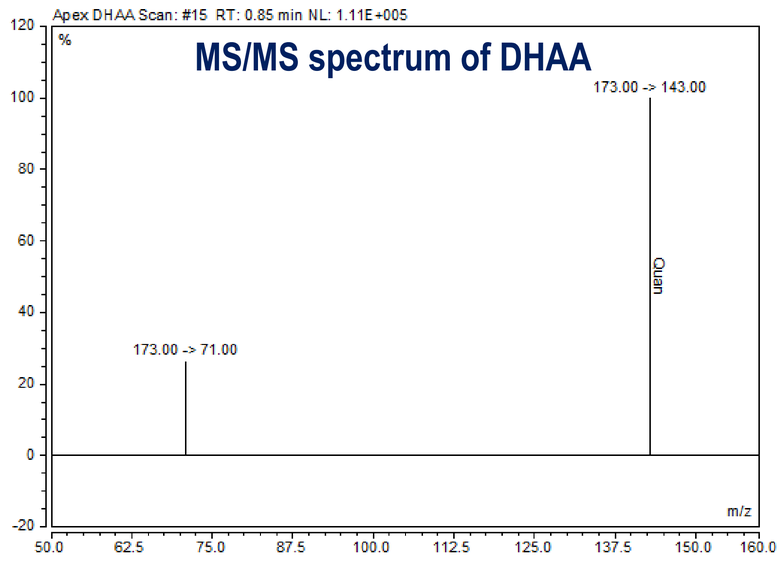
EXPERIMENTAL

Standard solutions → standards of AA and DHAA and water as a solvent
Instruments → UltiMate 3000RS and TSQ Quantis (Thermo Fisher Scientific)
The main problem in the AA analysis is related to its instability → solutions and samples were prepared and analysed within 1 hour, amber vials, autosampler temperature at 4 °C

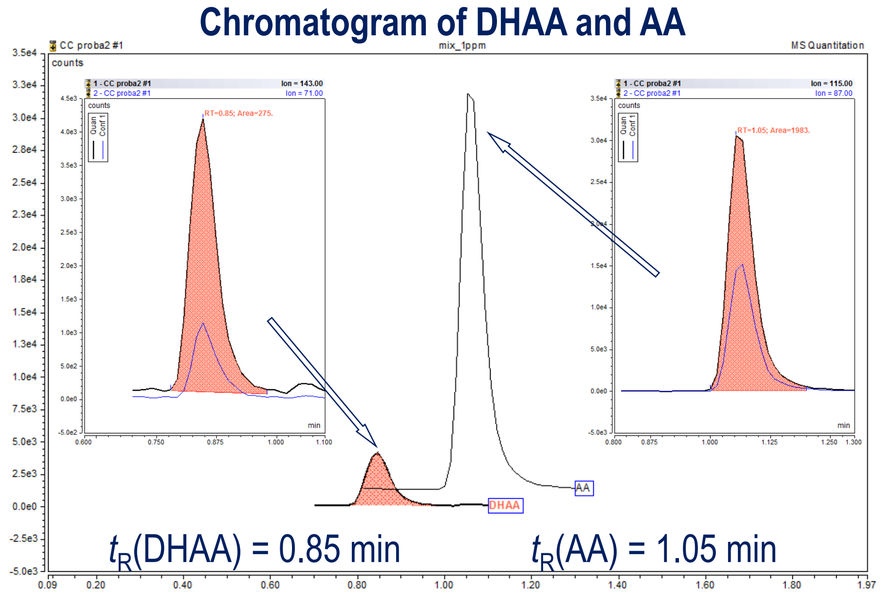
LC optimization	
Mobile phase	water
Organic modifier	0.1% acetic acid
Flow rate	0.7 mL/min
Pump	isocratic
Run Time	3 min
Stationary phase	Zorbax Eclipse XDB-C18 (Agilent)
Column dimensions	100 × 3.0 mm; 3.5 µm
Column temperature	20 °C
Sample injection volume	5 µL

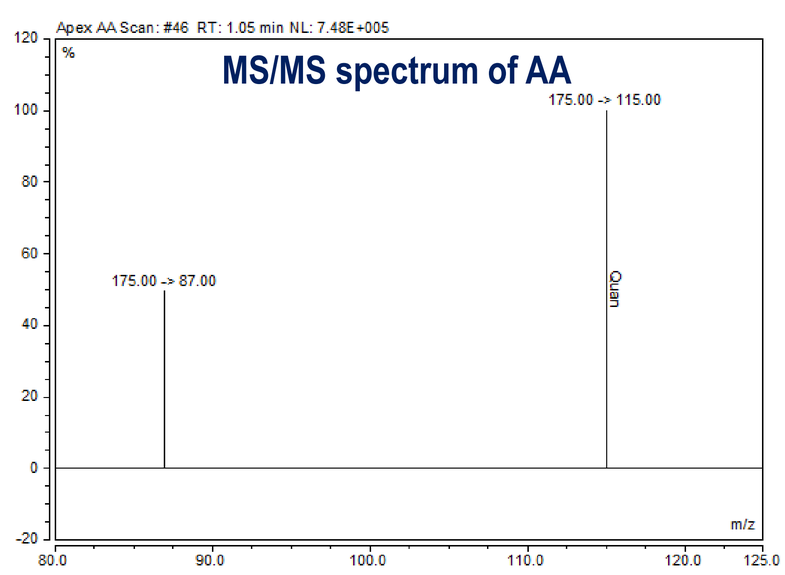


MS/MS optimization	AA	DHAA
Mode	ESI negative; MRM	
Quantifier (CE)	175 → 115 (10 V)	173 → 143 (8 V)
Qualifier (CE)	175 → 87 (17 V)	173 → 71 (12 V)
RF Lens Voltage	62 V	67 V
Spray Voltage	3 600 V	
Sheath gas	43.9 Arb	
Auxiliary gas	6.1 Arb	
Sweep gas	0 Arb	
CID gas	1.5 mTorr	
Ion Transfer Tube Temperature	325 °C	
Vaporizer Temperature	350 °C	



Chromatogram of DHAA and AA





Method validation	AA	DHAA	AA	DHAA
Calibration range	10–50 µg/mL (9 points)		0.50–5.0 µg/mL (5 points)	
Calibration curve equation	y=1555x+6882	y=94x+175	y=2352x-421	y=252x-97
Linearity (R^2)	0.9981	0.9992	0.9981	0.9988
Accuracy (RE)	0.09–9.61%	0.06–6.54%	1.20–5.84%	0.60–17.0%
Quality control solutions	10; 30; 45 µg/mL		2.5 µg/mL	
Intra-day precision (5 times)	15.91; 1.14; 2.69%	17.64; 3.40; 3.03%	0.86%	7.41%
Inter-day precision (5 days)	6.35; 2.88; 5.13%	16.34; 12.54; 8.92%	3.58%	3.01%
RSD (flow: ±0.1 mL/min)	< 2.39%	< 7.85%	3.38%	37.08%
RDS (column temp: ±4 °C)	< 5.52%	< 10.89%	2.95%	1.77%
RSD (stability: 1,2,3,5,24 h)	10.30; 5.33; 4.47% (5 h) **VS** > 38.02% (24 h)		5.87% (5 h) **VS** 41.03% (24 h)	
Limit of Detection	2.1 ng/mL	18.7 ng/mL	*Specificity* → technology inherent justification *System suitability* → no carry-over *Repeatability* → 0.10% (AA) and 3.47% (DHAA)	
Limit of Quantification	7.2 ng/mL	62.3 ng/mL		

Real samples analysis	Declared amount of vitamin C	Measured amount AA	Measured amount DHAA
Cedevita	213 mg/100 g	203	7
$\sigma_{Cedevita}$ (n =3)		3.4	0.2
Naturel Vitamin C 500 mg direkt	500 mg/ 3 g (1 bag)	603	48
$\sigma_{Naturel}$ (n =3)		6.8	3.9

CONCLUSION

UHPLC-MS/MS is a suitable technique for the simultaneous quantification of ascorbic and dehydroascorbic acids due to its high sensitivity and selectivity.
The developed method is simple (no need for oxidation/reduction or derivatization) and fast (3 minutes).
The method validation parameters were proven satisfactory and the procedure presented was successfully applied to analyse supplements with a declared amount of vitamin C.

References

[1] L. Nováková, P. Solich, D. Solichová, *Trac-Trends Anal. Chem.* 27 (2008) 942-958. [2] ICH Harmonised Guideline: Validation of analytical procedures Q2(R2), 2022.

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