

Study of Mono and Di-O-caffeoylquinic Acid Isomers in *Acmella oleracea* Extracts by HPLC-MS/MS and Application of Linear Equation of Deconvolution Analysis Algorithm for Their Characterization

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Supplementary Materials

Table S1. Chromatographic parameters for each analyte obtained with the proposed HPLC-MS analysis. Rt, retention times \pm error (2 standard deviation or 2 SD); Base Width, base peaks width; N, efficiency; R, resolution between contiguous analytes. The void time measured for the chromatographic system used was 1.02 min.

	Rt (min)	2 SD (min)	Base Width (min)	2 SD (min)	N	R
3-CQA	5.93	0.04	0.38	0.05	3895	-
5-CQA	8.58	0.04	0.43	0.06	6675	6.6
4-CQA	9.29	0.04	0.31	0.05	14358	2.2
3,4-diCQA	17.53	0.04	0.30	0.05	54601	-
3,5-diCQA	18.01	0.04	0.32	0.05	50680	1.6
4,5-diCQA	19.39	0.04	0.37	0.05	43955	4.0

Table S2. Results of the calibration curve obtained for Ri signal of chlorogenic acid (or 5-CQA), defined as linear regressions parameters (slope and y-intercept), the determination coefficient (R^2) and the estimated LOD and LOQ values.

Compound	MS/MS signal	Slope (counts/mg L ⁻¹)	y-Intercept counts)	R ²	LOD (mg L ⁻¹)	LOQ (mg L ⁻¹)
5-CQA	Ri	6.2 10 ⁶	8.5 10 ⁴	0.999	0.1	0.4

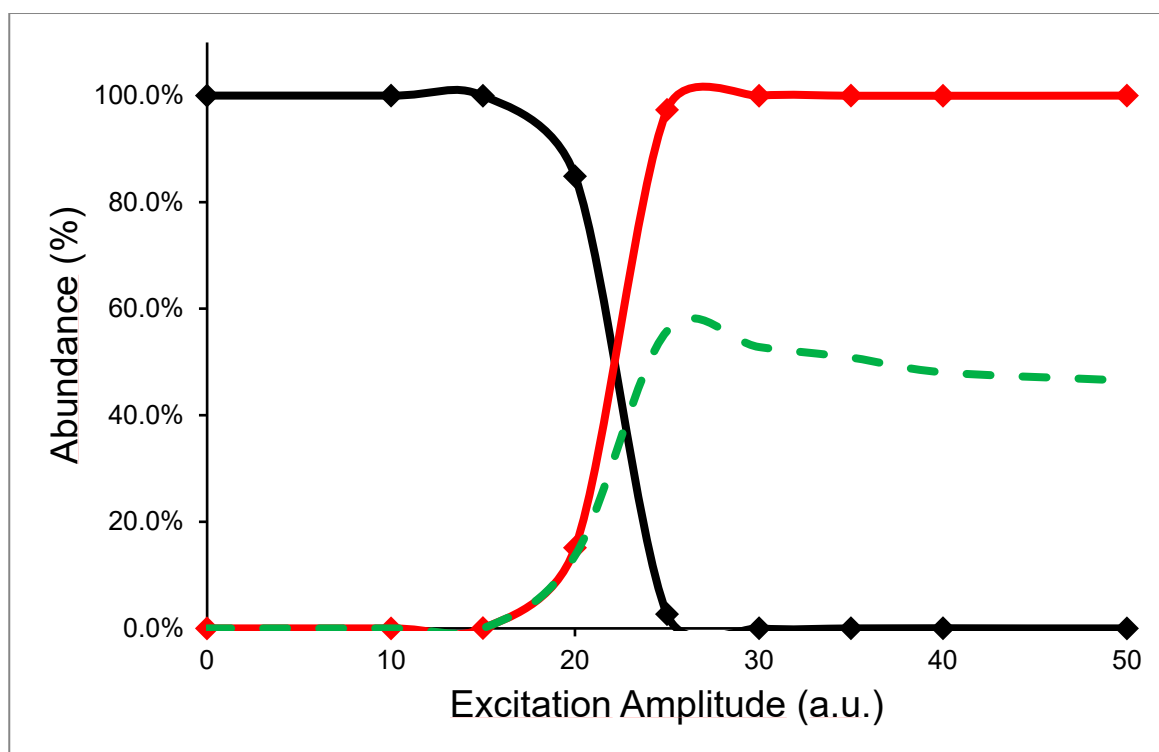


Figure S1. Precursor SY curve (black line), PiF (red line) and PiY (green dashed line) of the 3-CQA isomer at ExT 50 ms.

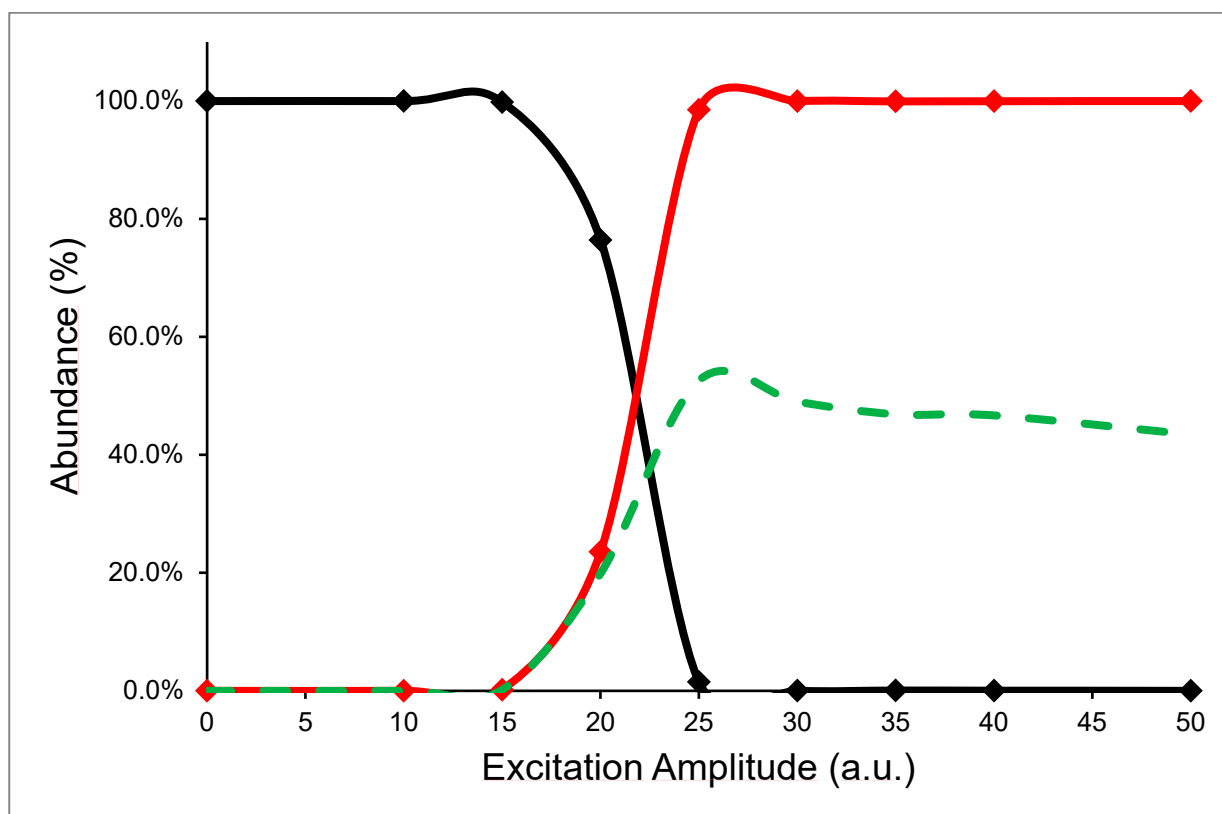


Figure S2. Precursor SY curve (black line), PiF (red line) and PiY (green dashed line) of the 4-CQA isomer at ExT 50 ms.

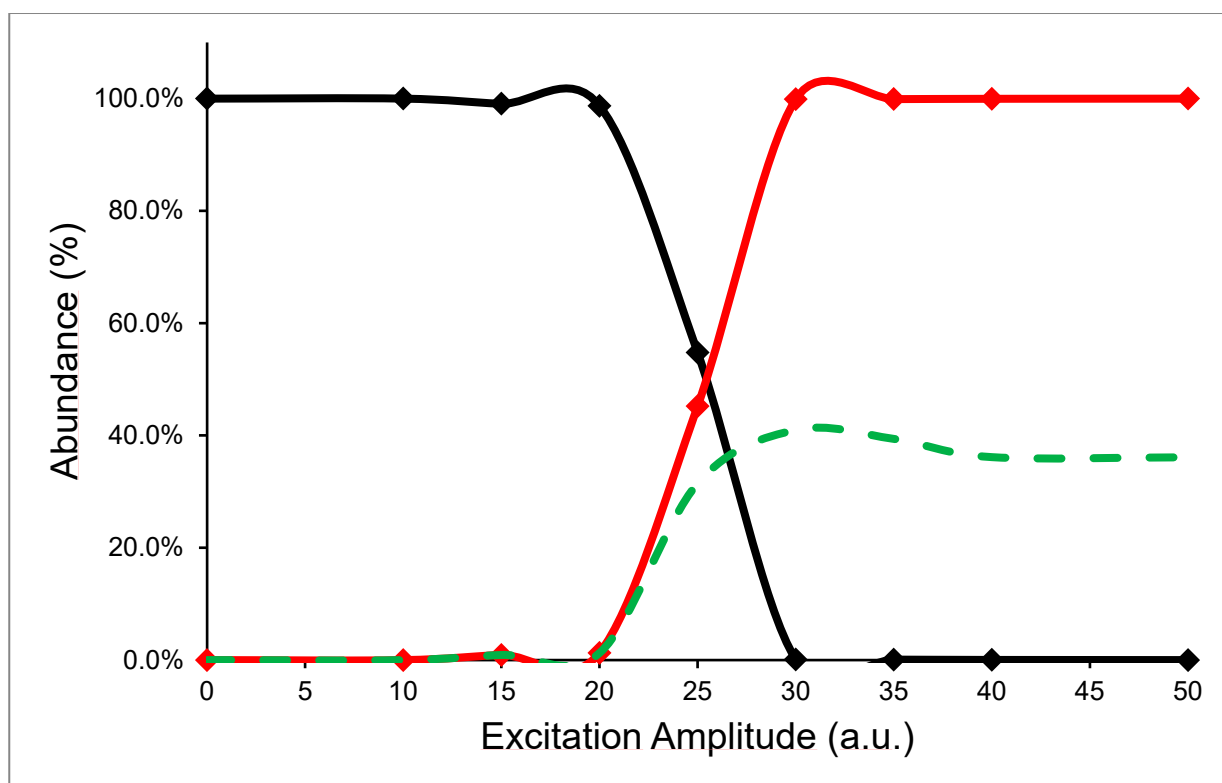


Figure S3. Precursor SY curve (black line), PiF (red line) and PiY (green dashed line) of the 3,4-diCQA isomer at ExT 50 ms.

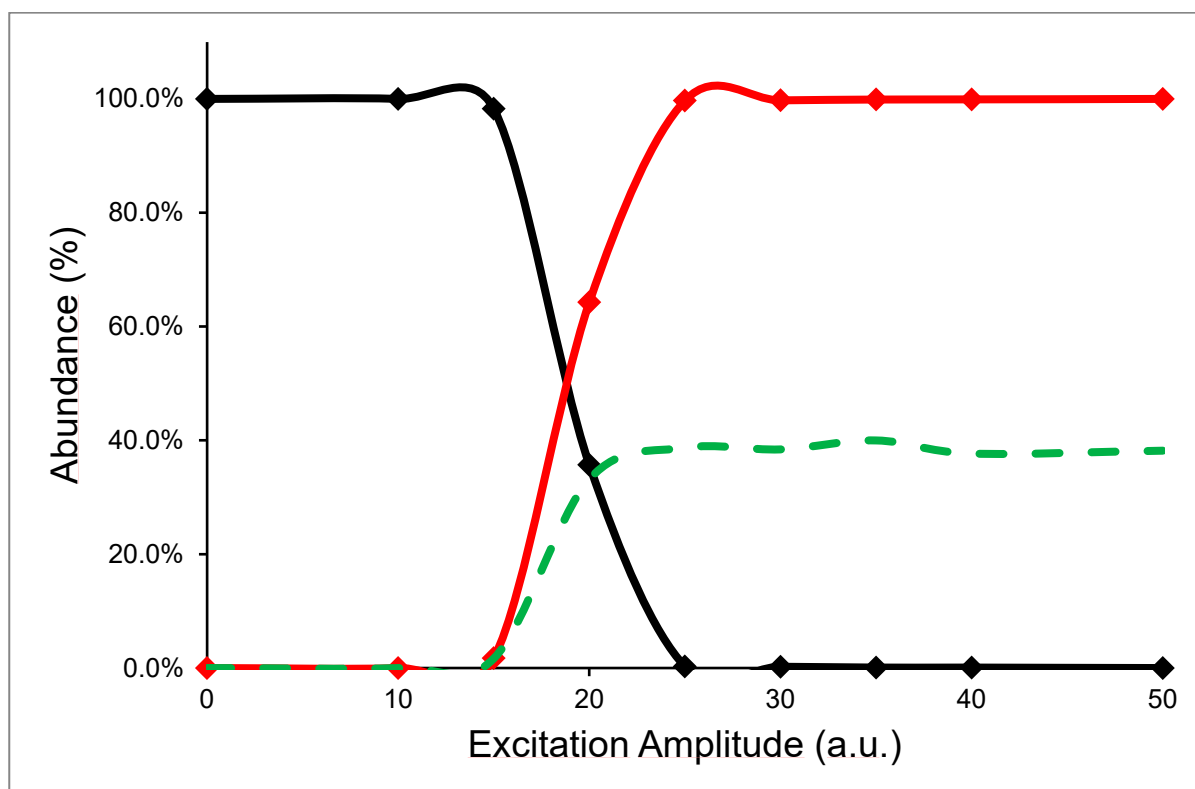


Figure S4. Precursor SY curve (black line), PiF (red line) and PiY (green dashed line) of the 3,5-diCQA isomer at ExT 50 ms.

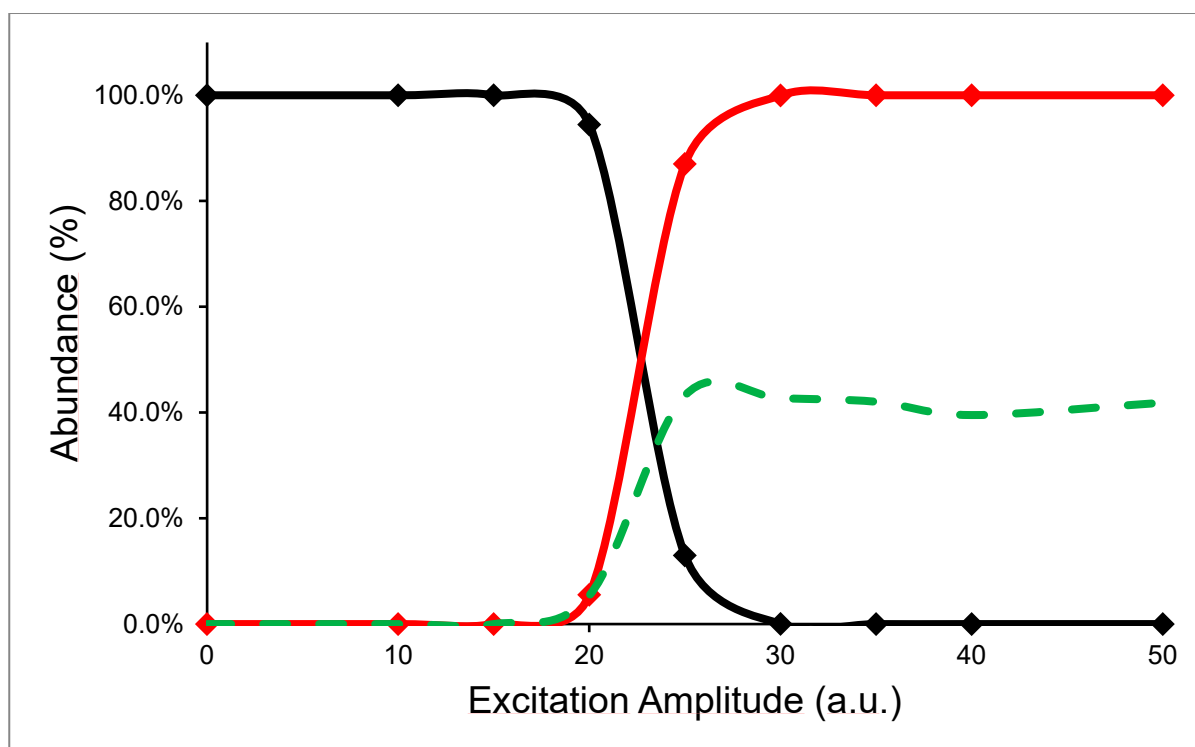


Figure S5. Precursor SY curve (black line), PiF (red line) and PiY (green dashed line) of the 4,5-diCQA isomer at ExT 50 ms.

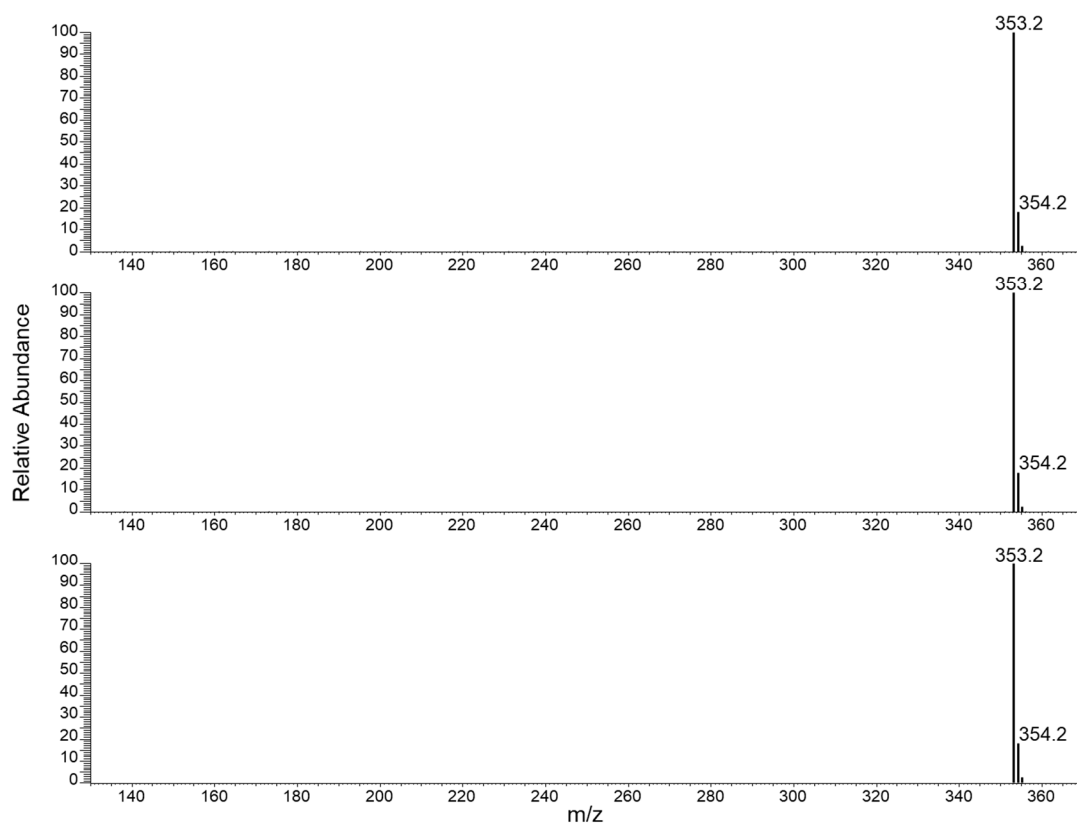


Figure S6. ESI negative ions spectra of 3-CQA (upper), 5-CQA (middle) and 4-CQA (bottom).

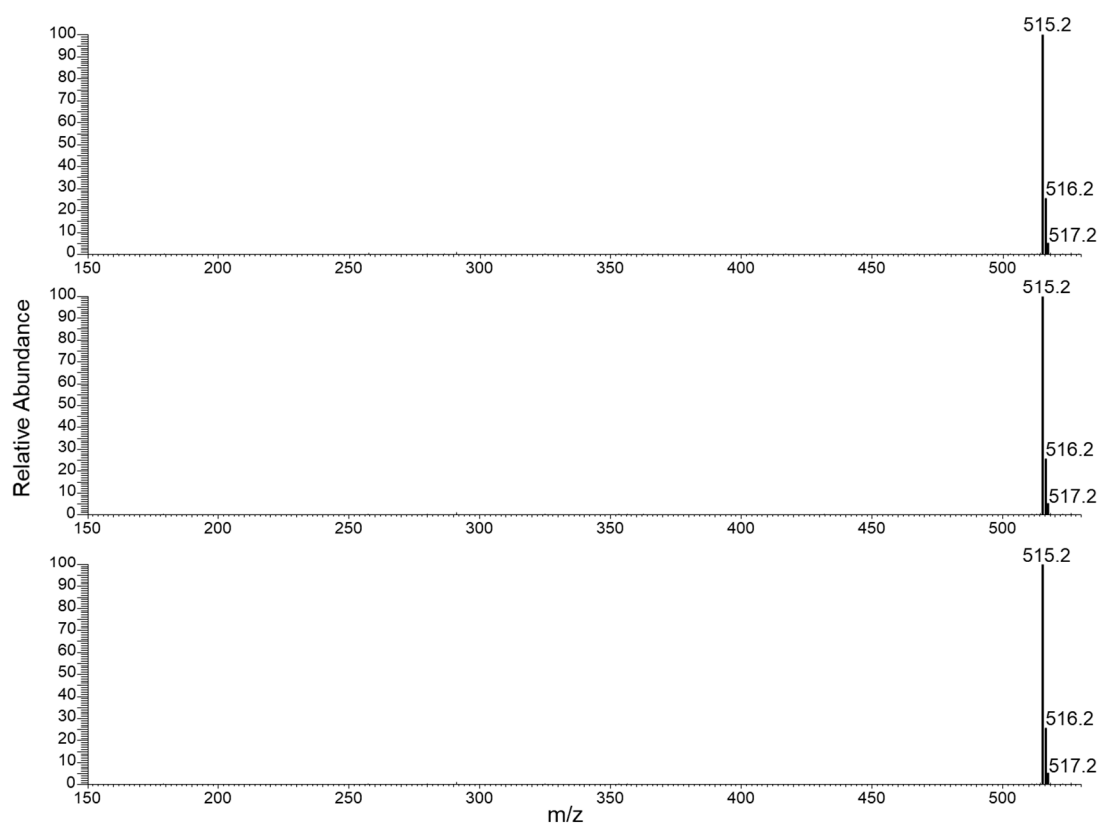


Figure S7. ESI negative ions spectra of 3,4-diCQA (upper), 3,5-diCQA (middle) and 4,5-diCQA (bottom).

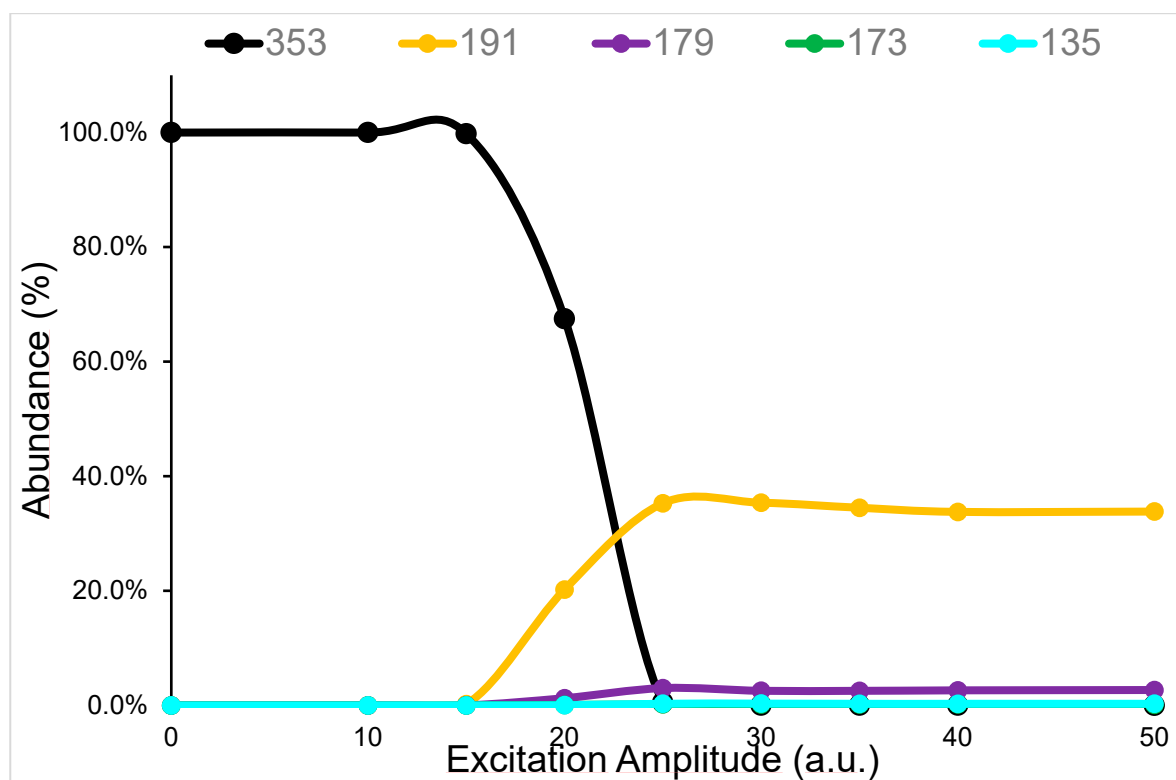


Figure S8. The breakdown curves of 5-CQA isomer obtained by elaboration of ERMS data.

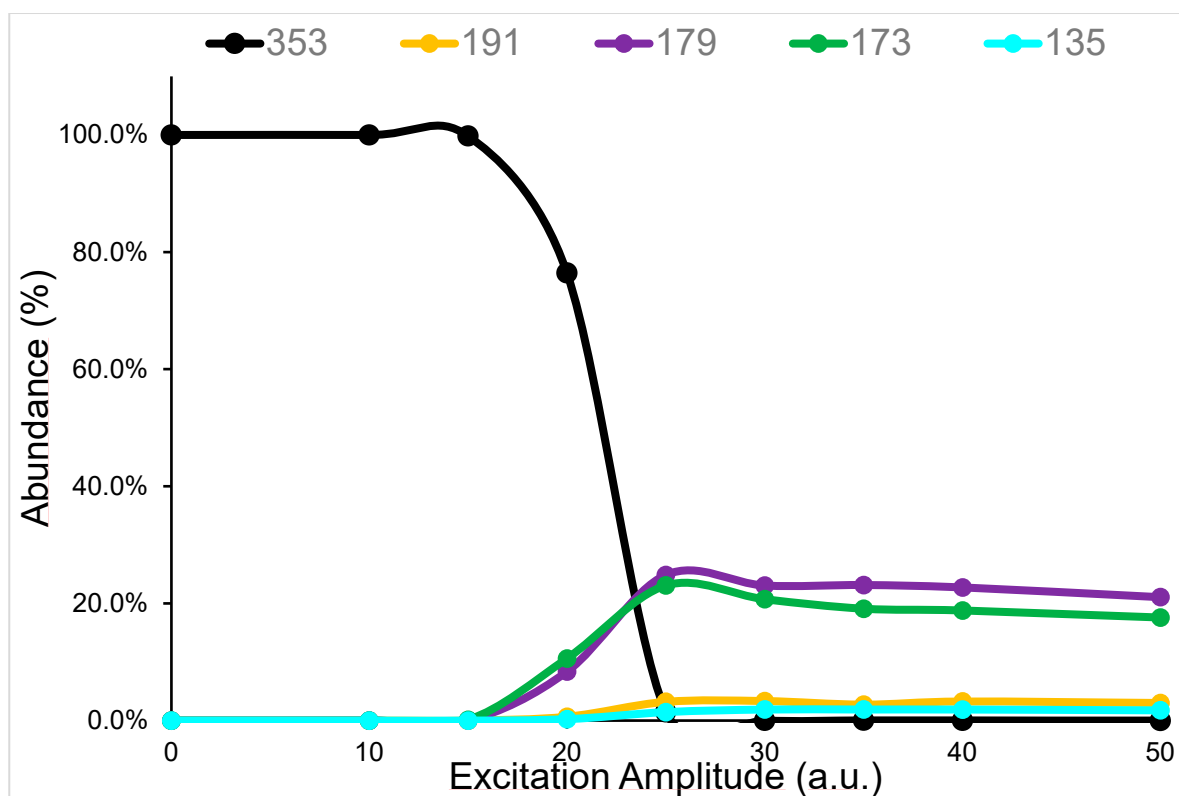


Figure S9. The breakdown curves of 4-CQA isomer obtained by elaboration of ERMS data.

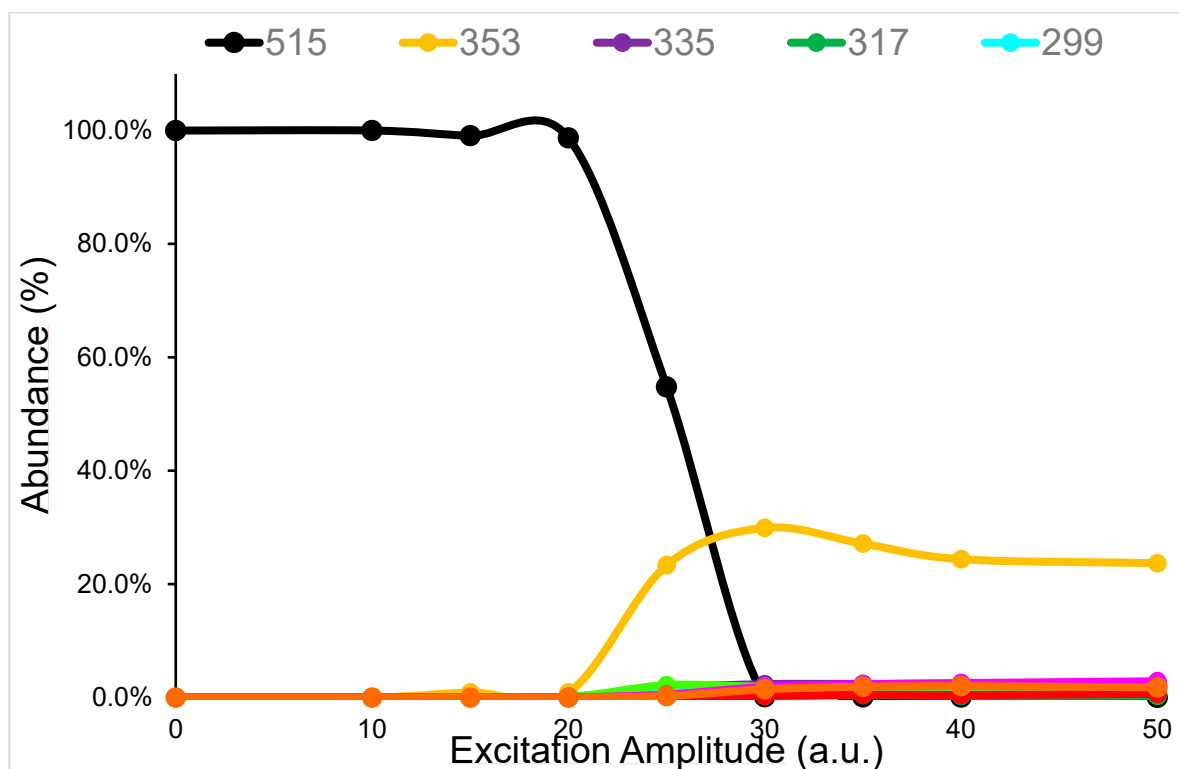


Figure S10. The breakdown curves of 3,4-diCQA isomer obtained by elaboration of ERMS data.

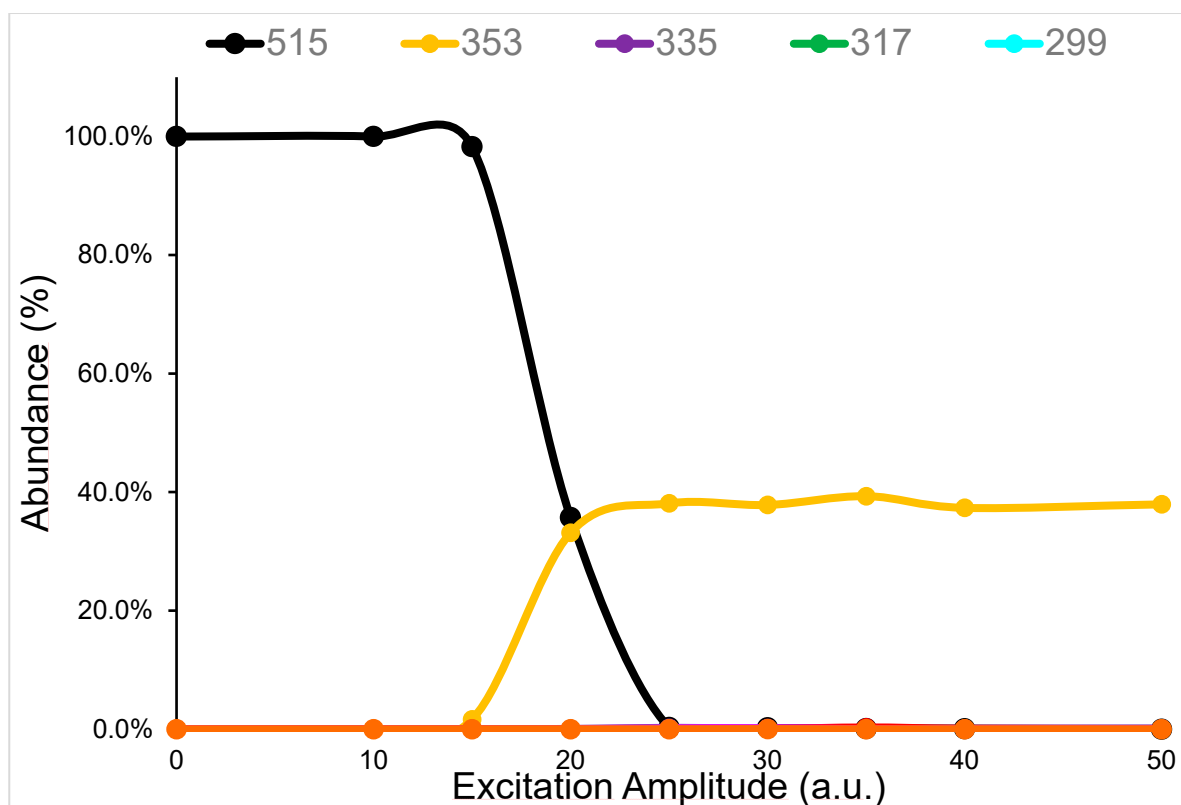


Figure S11. The breakdown curves of 3,5-diCQA isomer obtained by elaboration of ERMS data.

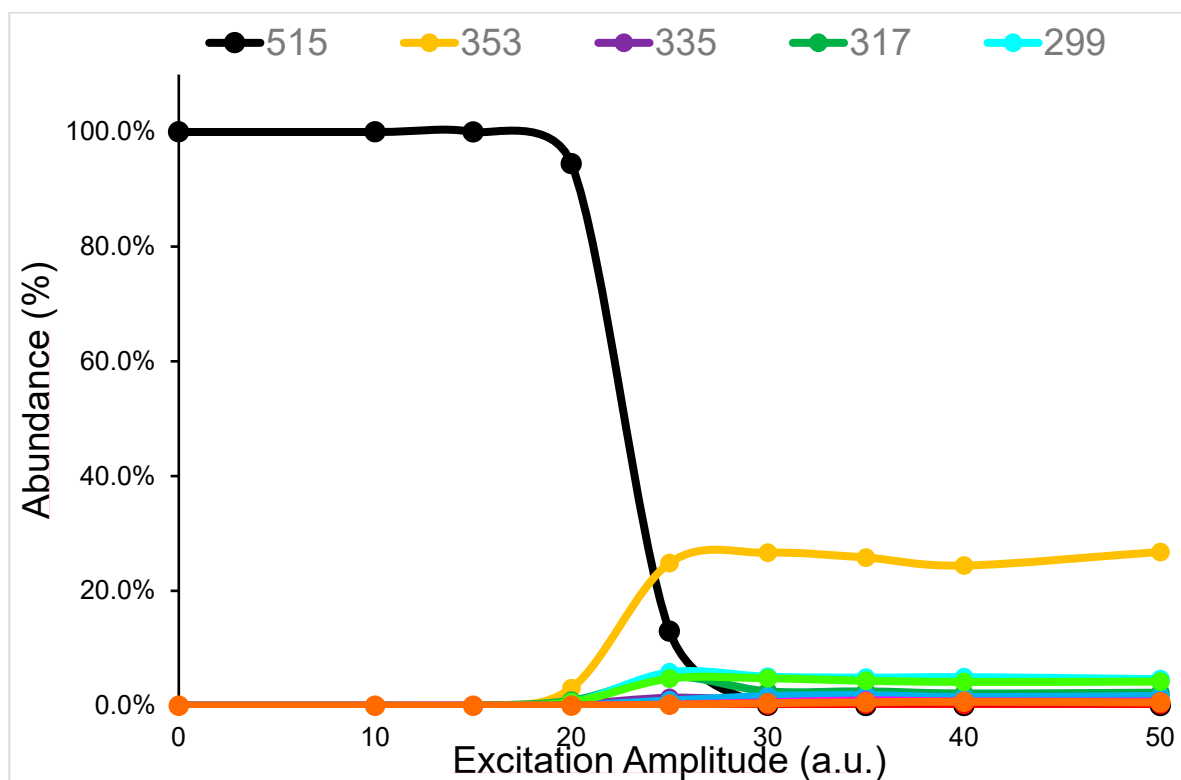


Figure S12. The breakdown curves of 4,5-diCQA isomer obtained by elaboration of ERMS data.

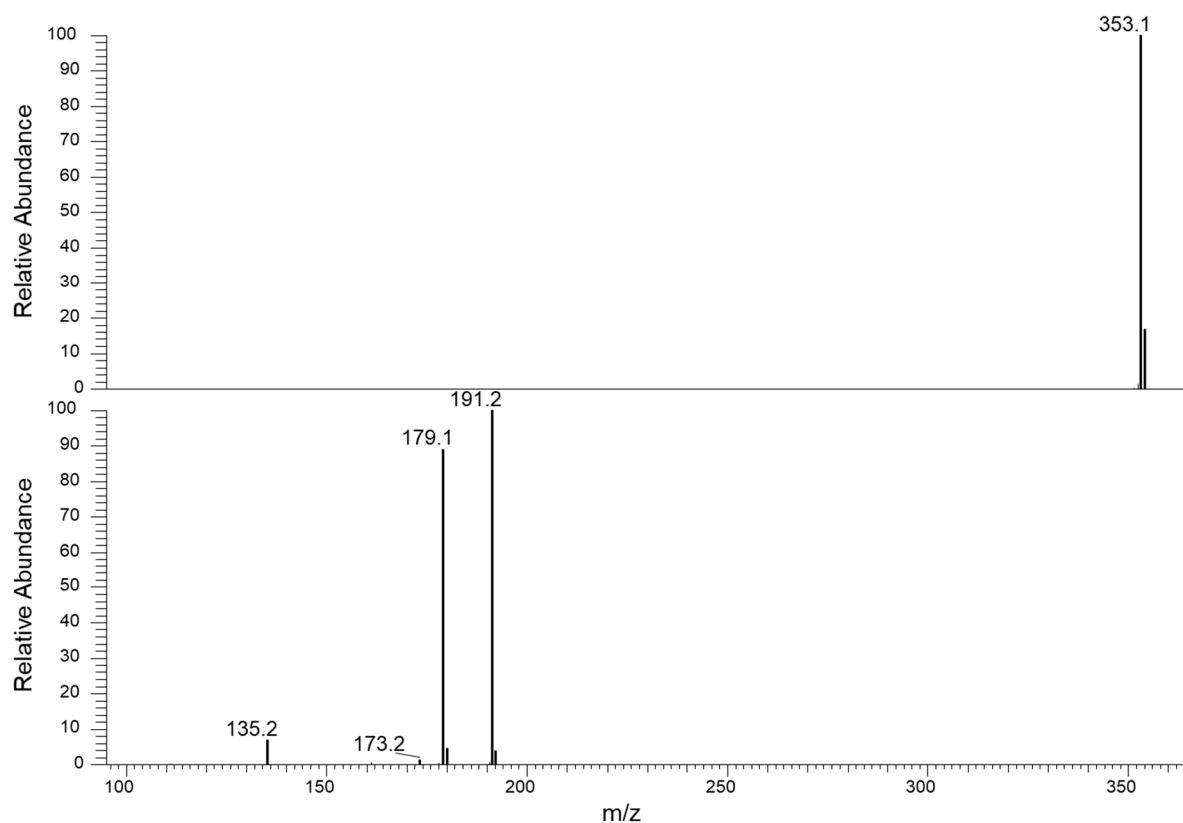


Figure S13. Comparison of the MS/MS spectra obtained at low ExA (MS/MS event 1, above), used to monitor the Ri signal, and at high ExA (MS/MS event 2, below), to acquire the Pis signals of 3-CQA.

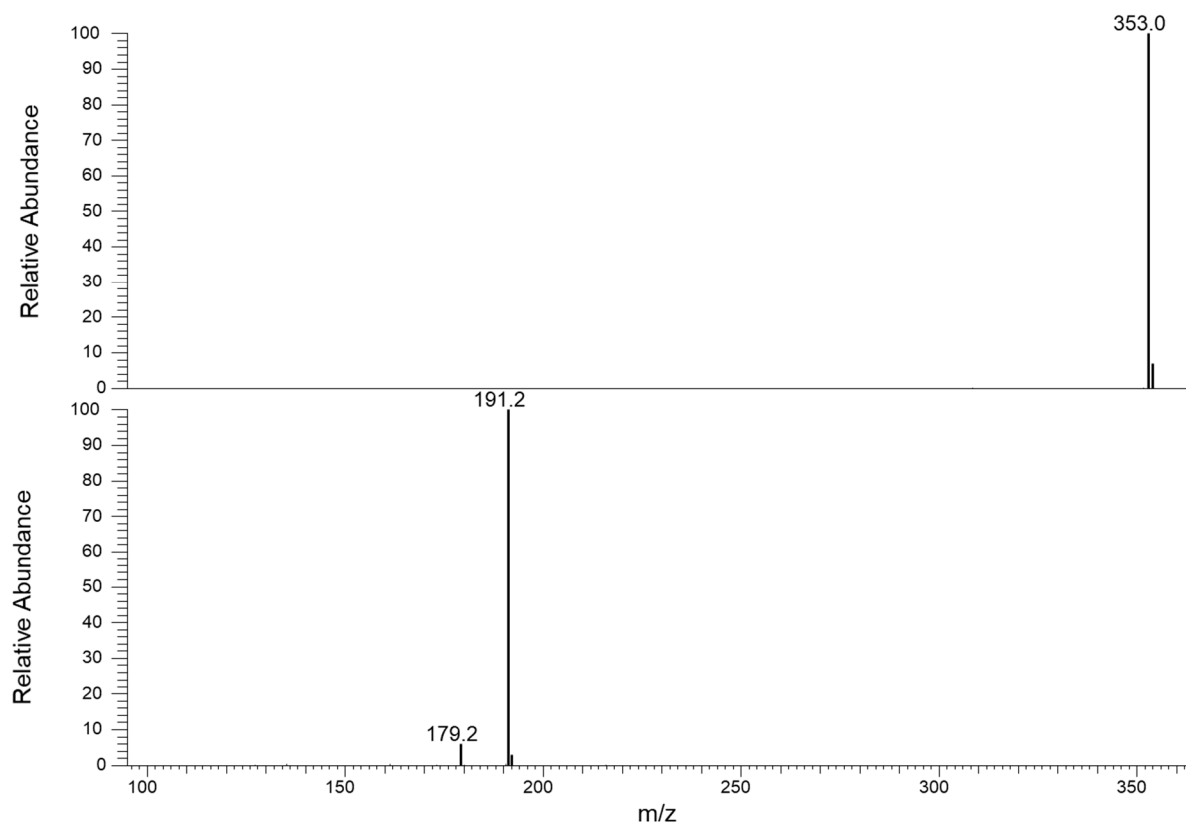


Figure S14. Comparison of the MS/MS spectra obtained at low ExA (MS/MS event 1, above), used to monitor the Ri signal, and at high ExA (MS/MS event 2, below), to acquire the Pis signals of 5-CQA.

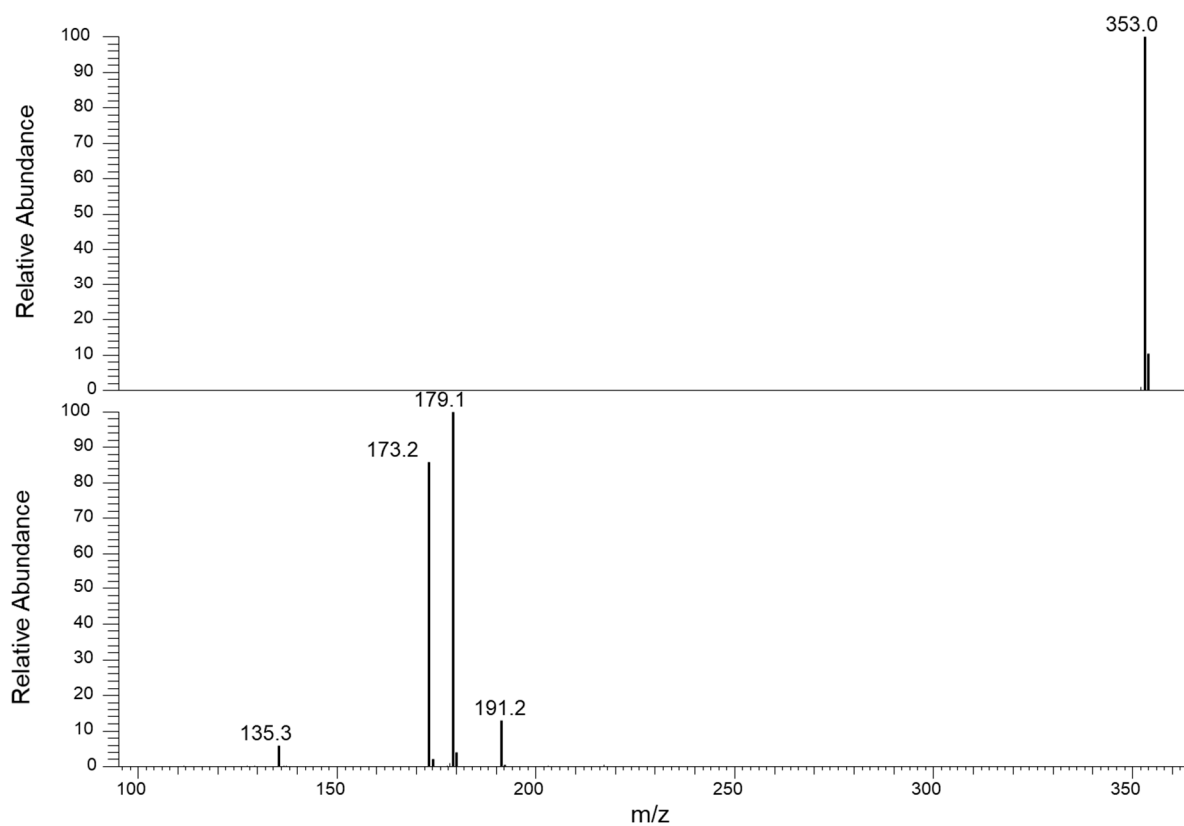


Figure S15. Comparison of the MS/MS spectra obtained at low ExA (MS/MS event 1, above), used to monitor the Ri signal, and at high ExA (MS/MS event 2, below), to acquire the Pis signals of 4-CQA.

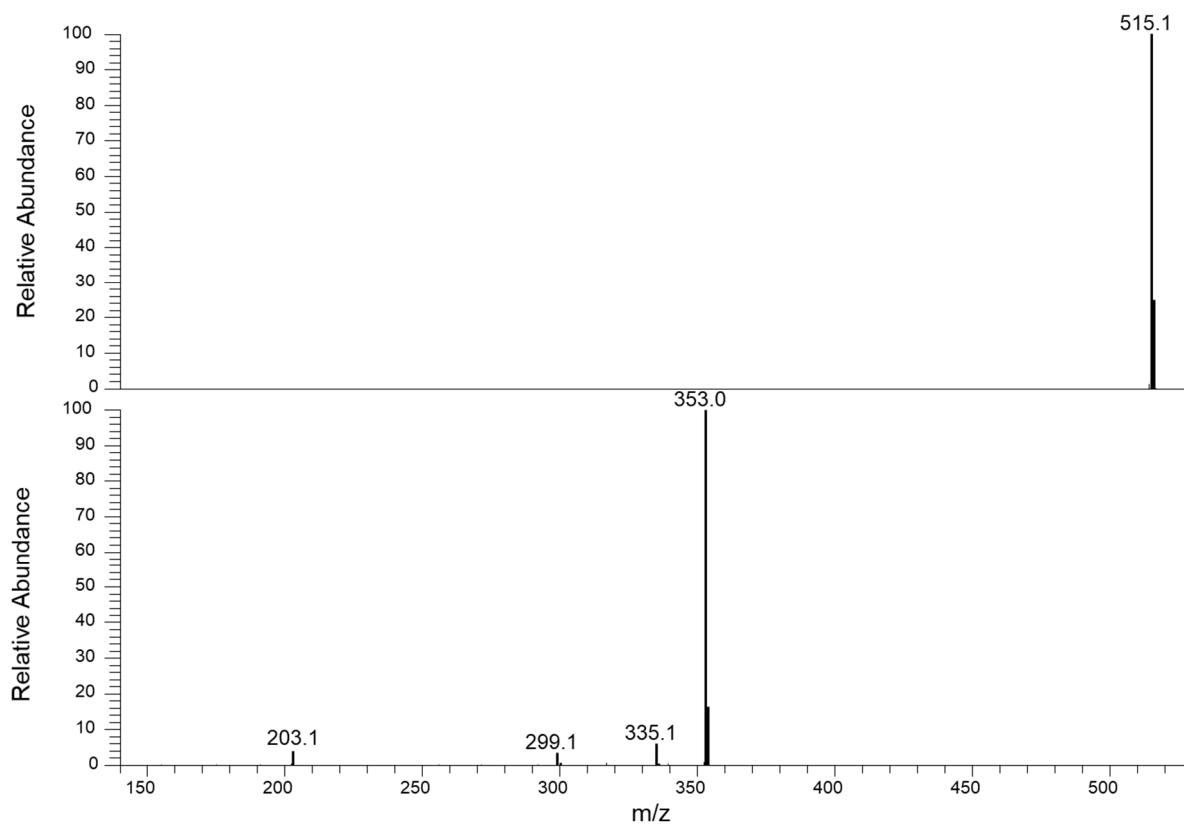


Figure S16. Comparison of the MS/MS spectra obtained at low ExA (MS/MS event 1, above), used to monitor the Ri signal, and at high ExA (MS/MS event 2, below), to acquire the Pis signals of 3,4-diCQA.

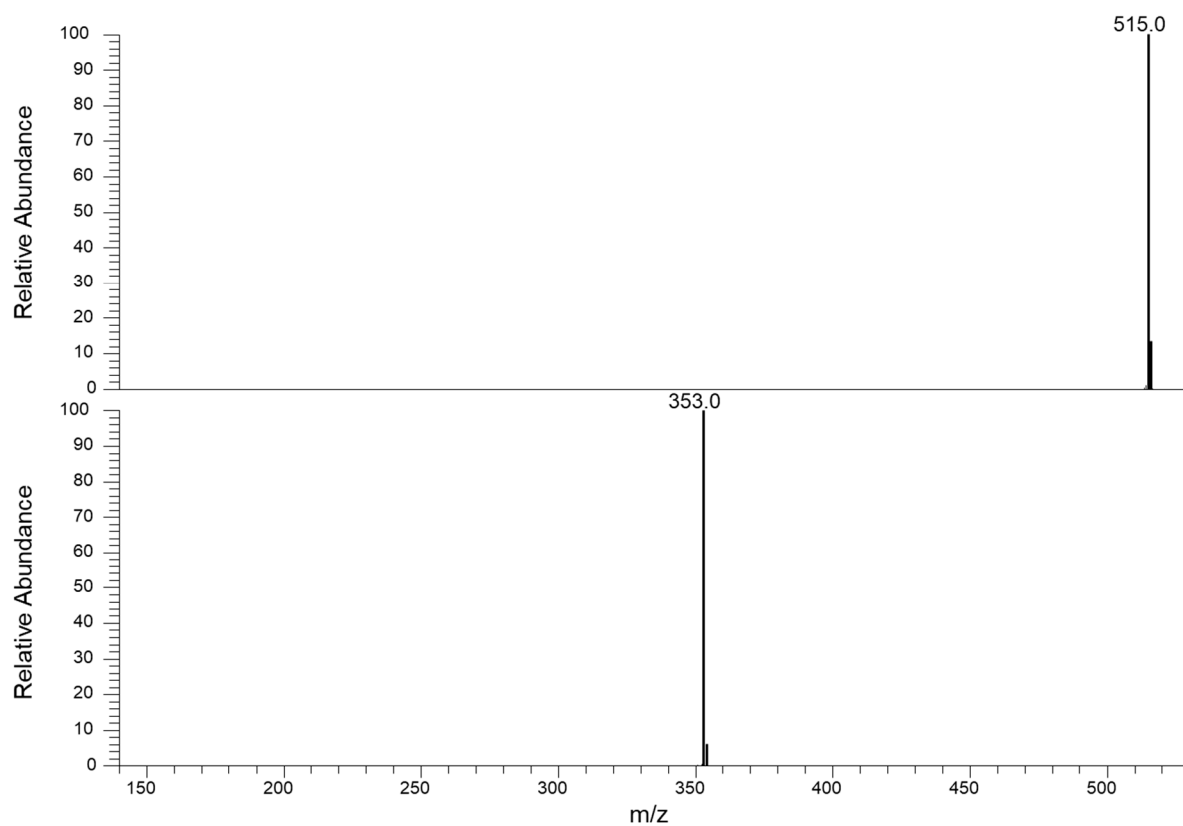


Figure S17. Comparison of the MS/MS spectra obtained at low ExA (MS/MS event 1, above), used to monitor the Ri signal, and at high ExA (MS/MS event 2, below), to acquire the Pis signals of 3,5-diCQA.

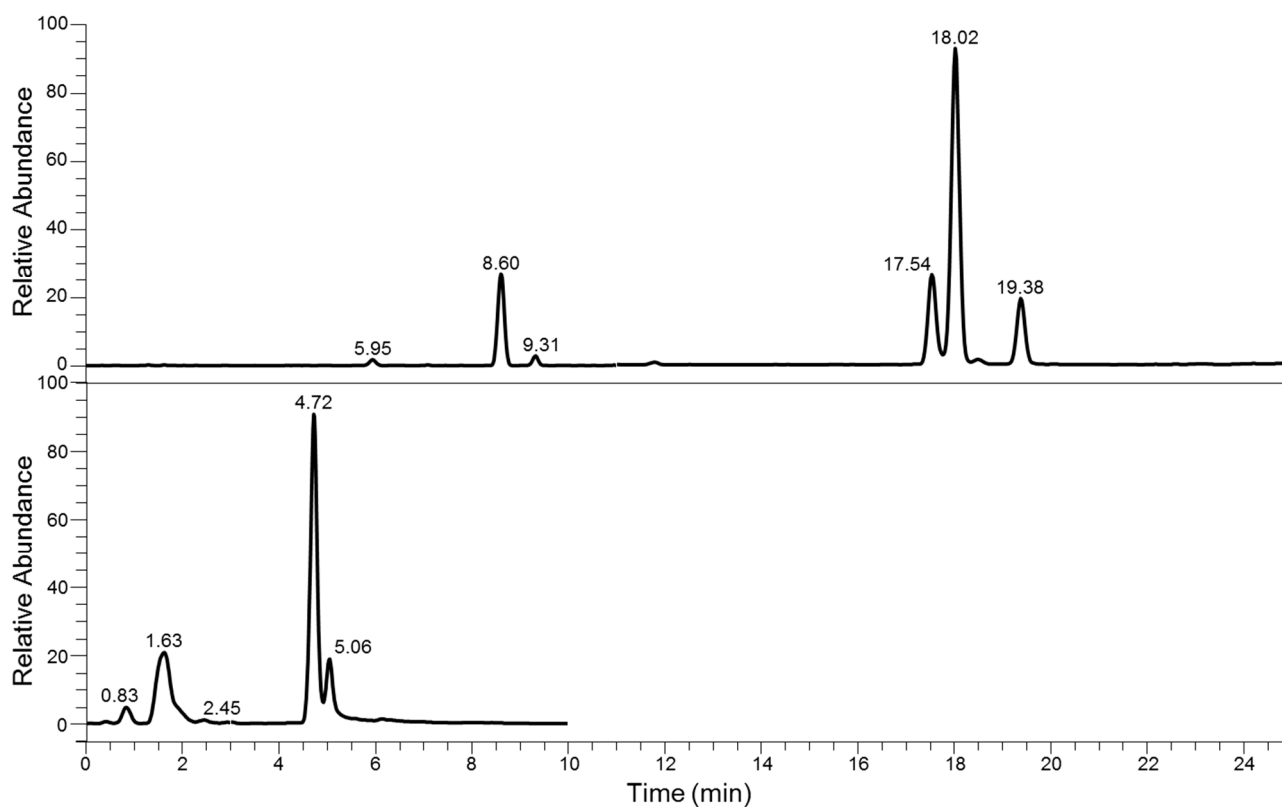


Figure S18. Comparison between the HPLC-MS/MS analysis of Acmella WS with ChromSys 1 (above) and ChromSys 2 (bottom).

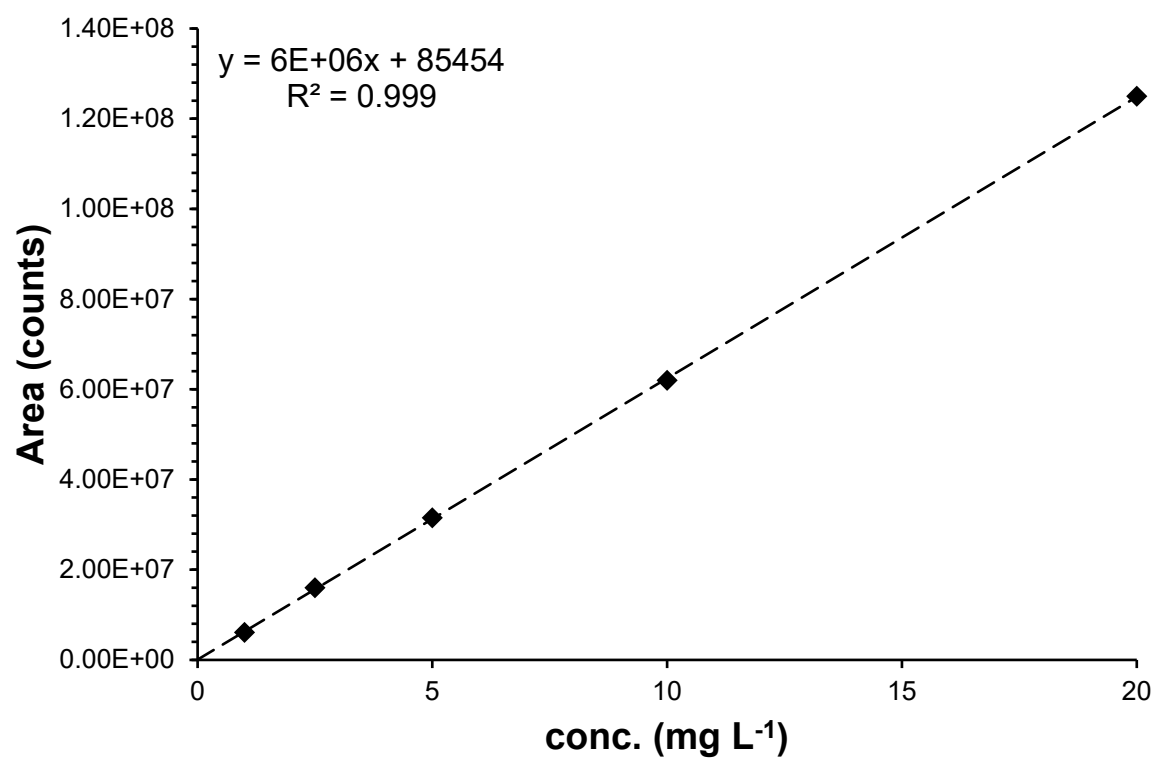


Figure S19. The calibration curve of 5-CQA.

LEDA algorithm

Equation S1. LEDA_{CQA} matrix for CQAs isomers speciation:

$$\begin{bmatrix} Area_{191}/Area_{Ri} = \left(\frac{Pi_{191}}{Ri}\right)_{isomer1} [\%]_{isomer1} + \left(\frac{Pi_{1191}}{Ri}\right)_{isomer2} [\%]_{isomer2} + \left(\frac{Pi_{191}}{Ri}\right)_{isomer3} [\%]_{isomer3} \\ Area_{179}/Area_{Ri} = \left(\frac{Pi_{179}}{Ri}\right)_{isomer1} [\%]_{isomer1} + \left(\frac{Pi_{179}}{Ri}\right)_{isomer2} [\%]_{isomer2} + \left(\frac{Pi_{179}}{Ri}\right)_{isomer3} [\%]_{isomer3} \\ Area_{173}/Area_{Ri} = \left(\frac{Pi_{173}}{Ri}\right)_{isomer1} [\%]_{isomer1} + \left(\frac{Pi_{173}}{Ri}\right)_{isomer2} [\%]_{isomer2} + \left(\frac{Pi_{173}}{Ri}\right)_{isomer3} [\%]_{isomer3} \\ Area_{135}/Area_{Ri} = \left(\frac{Pi_{135}}{Ri}\right)_{isomer1} [\%]_{isomer1} + \left(\frac{Pi_{135}}{Ri}\right)_{isomer2} [\%]_{isomer2} + \left(\frac{Pi_{135}}{Ri}\right)_{isomer3} [\%]_{isomer3} \end{bmatrix}$$

$$\begin{bmatrix} Area_{191}/Area_{Ri} \\ Area_{179}/Area_{Ri} \\ Area_{173}/Area_{Ri} \\ Area_{135}/Area_{Ri} \end{bmatrix} = A$$

$$\begin{bmatrix} \left(\frac{Pi_{191}}{Ri}\right)_{isomer1} & \left(\frac{Pi_{191}}{Ri}\right)_{isomer2} & \left(\frac{Pi_{191}}{Ri}\right)_{isomer3} \\ \left(\frac{Pi_{179}}{Ri}\right)_{isomer1} & \left(\frac{Pi_{179}}{Ri}\right)_{isomer2} & \left(\frac{Pi_{179}}{Ri}\right)_{isomer3} \\ \left(\frac{Pi_{173}}{Ri}\right)_{isomer1} & \left(\frac{Pi_{173}}{Ri}\right)_{isomer2} & \left(\frac{Pi_{173}}{Ri}\right)_{isomer3} \\ \left(\frac{Pi_{135}}{Ri}\right)_{isomer1} & \left(\frac{Pi_{135}}{Ri}\right)_{isomer2} & \left(\frac{Pi_{135}}{Ri}\right)_{isomer3} \end{bmatrix} = K$$

$$[[\%]_{isomer1} \quad [\%]_{isomer2} \quad [\%]_{isomer3}] = X$$

Equation S2. LEDA_{diCQA} matrix for diCQAs isomers speciation:

$$\begin{bmatrix} Area_{353}/Area_{Ri} = \left(\frac{Pi_{353}}{Ri}\right)_{isomer1} [\%]_{isomer1} + \left(\frac{Pi_{2033}}{Ri}\right)_{isomer2} [\%]_{isomer2} + \left(\frac{Pi_{353}}{Ri}\right)_{isomer3} [\%]_{isomer3} \\ Area_{335}/Area_{Ri} = \left(\frac{Pi_{335}}{Ri}\right)_{isomer1} [\%]_{isomer1} + \left(\frac{Pi_{335}}{Ri}\right)_{isomer2} [\%]_{isomer2} + \left(\frac{Pi_{335}}{Ri}\right)_{isomer3} [\%]_{isomer3} \\ Area_{317}/Area_{Ri} = \left(\frac{Pi_{317}}{Ri}\right)_{isomer1} [\%]_{isomer1} + \left(\frac{Pi_{317}}{Ri}\right)_{isomer2} [\%]_{isomer2} + \left(\frac{Pi_{317}}{Ri}\right)_{isomer3} [\%]_{isomer3} \\ Area_{203}/Area_{Ri} = \left(\frac{Pi_{203}}{Ri}\right)_{isomer1} [\%]_{isomer1} + \left(\frac{Pi_{203}}{Ri}\right)_{isomer2} [\%]_{isomer2} + \left(\frac{Pi_{203}}{Ri}\right)_{isomer3} [\%]_{isomer3} \\ Area_{203}/Area_{Ri} = \left(\frac{Pi_{203}}{Ri}\right)_{isomer1} [\%]_{isomer1} + \left(\frac{Pi_{203}}{Ri}\right)_{isomer2} [\%]_{isomer2} + \left(\frac{Pi_{203}}{Ri}\right)_{isomer3} [\%]_{isomer3} \end{bmatrix}$$

$$\begin{bmatrix} Area_{353}/Area_{Ri} \\ Area_{335}/Area_{Ri} \\ Area_{317}/Area_{Ri} \\ Area_{299}/Area_{Ri} \\ Area_{203}/Area_{Ri} \end{bmatrix} = A$$

$$\begin{bmatrix} \left(\frac{Pi_{353}}{Ri} \right)_{isomer1} & \left(\frac{Pi_{353}}{Ri} \right)_{isomer2} & \left(\frac{Pi_{353}}{Ri} \right)_{isomer3} \\ \left(\frac{Pi_{335}}{Ri} \right)_{isomer1} & \left(\frac{Pi_{335}}{Ri} \right)_{isomer2} & \left(\frac{Pi_{335}}{Ri} \right)_{isomer3} \\ \left(\frac{Pi_{317}}{Ri} \right)_{isomer1} & \left(\frac{Pi_{317}}{Ri} \right)_{isomer2} & \left(\frac{Pi_{317}}{Ri} \right)_{isomer3} \\ \left(\frac{Pi_{299}}{Ri} \right)_{isomer1} & \left(\frac{Pi_{299}}{Ri} \right)_{isomer2} & \left(\frac{Pi_{299}}{Ri} \right)_{isomer3} \\ \left(\frac{Pi_{203}}{Ri} \right)_{isomer1} & \left(\frac{Pi_{203}}{Ri} \right)_{isomer2} & \left(\frac{Pi_{203}}{Ri} \right)_{isomer3} \end{bmatrix} = K$$

$$[[\%]_{isomer1} \quad [\%]_{isomer2} \quad [\%]_{isomer3}] = X$$

A general equation to solve each overdetermined LEDA matrix:

Traspose K matrix = K'

Inverse $(K \times K') = (K \times K')^{-1}$

$$X = (A \times K') \times (K \times K')^{-1} \quad \text{(Equation S3)}$$