

Electronic Supplementary Material

Quantitative analysis of azaspiracids in *Azadinium spinosum* cultures

Authors

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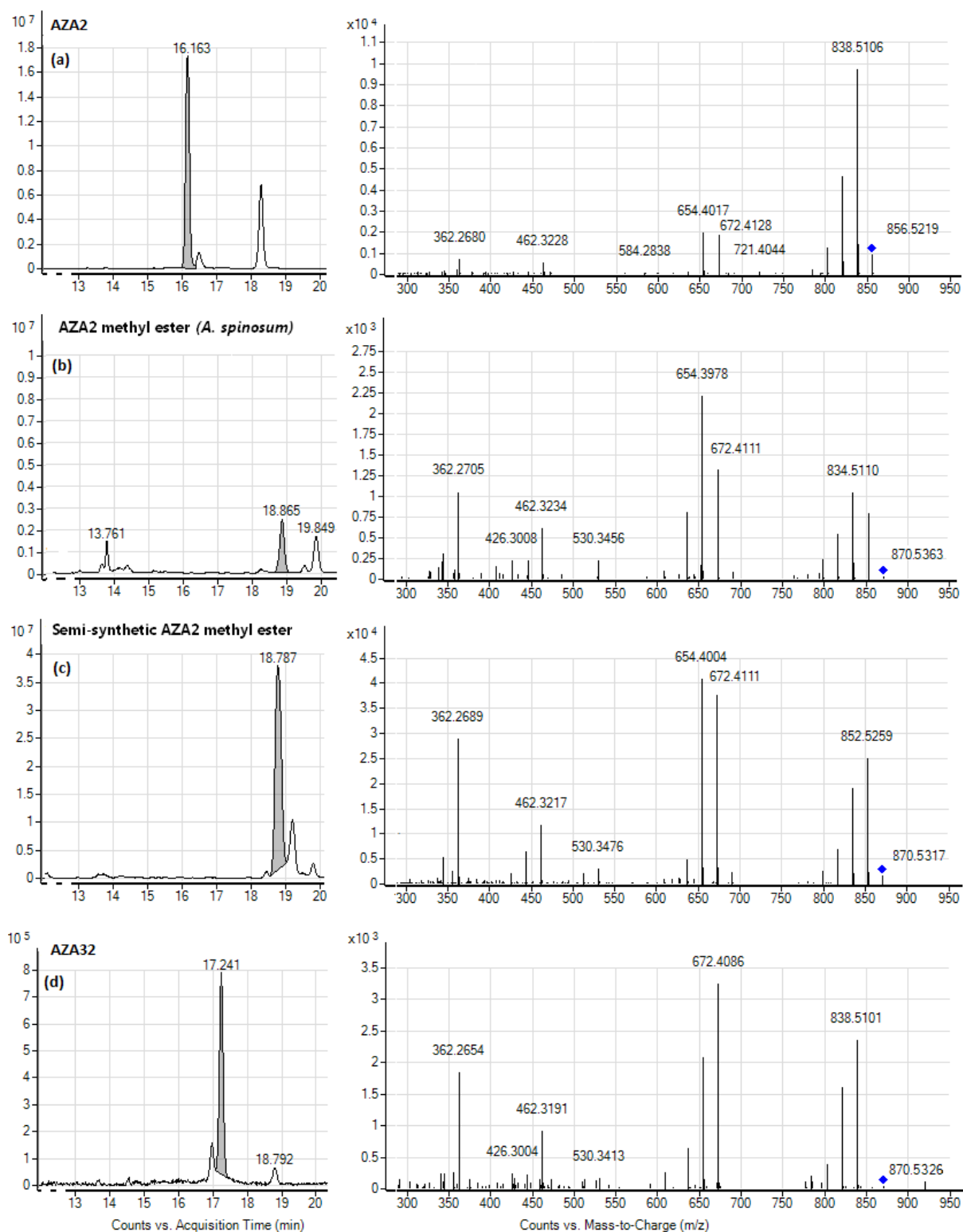


Fig. S1 LC-MS chromatograms and targeted high resolution MS/MS spectra, obtained with an Agilent 6540 QTOF, for AZA2 and derivatives: (a) AZA2; (b) AZA2 methyl ester in an *A. spinosum* extract; (c) semi-synthetic AZA2 methyl ester produced from AZA2 via derivatization with diazomethane, and; (d) AZA32 obtained from long-term storage of AZA2 in MeOH

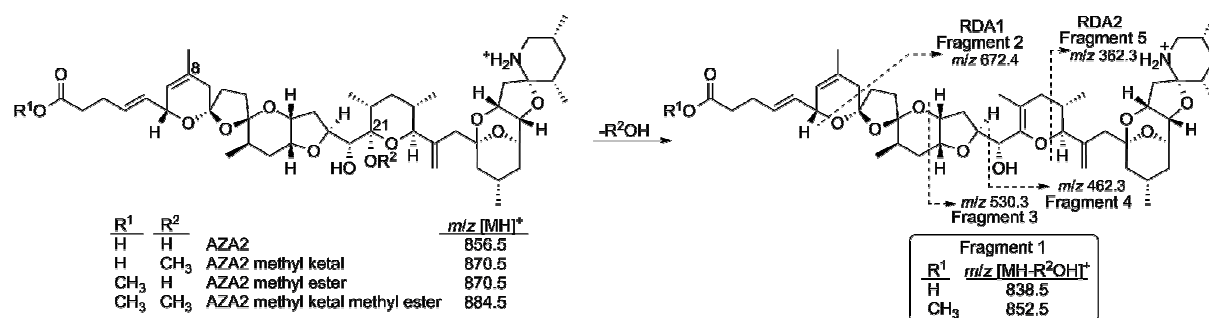


Fig. S2 Structure and m/z for $[M + H]^+$ ions of AZA2, AZA2 methyl ketal, AZA2 methyl ester, AZA2 bismethyl ketal ester and MS/MS fragmentation with indicated groups

Table S3 High resolution LC-MS and LC-MS/MS data (measured m/z and Δ (ppm)) for AZA2, its methyl ester (extraction artefact from *A. spinosum*, and semi-synthetic), and AZA32 (AZA2 methyl ketal). Fragment ions correspond to Fig. S2

Ion		AZA2	AZA2 methyl ester (artefact)	AZA2 methyl ester (semi-synthetic)	AZA32
[MH] ⁺	Formula m/z (Δ)	C ₄₈ H ₇₄ NO ₁₂ ⁺ 856.5219 (1.57)	C ₄₉ H ₇₆ NO ₁₂ ⁺ 870.5363 (0.11)	C ₄₉ H ₇₆ NO ₁₂ ⁺ 870.5317 (5.17)	C ₄₉ H ₇₆ NO ₁₂ ⁺ 870.5326 (4.14)
[MH-ROH] ⁺ (Fragment 1)	Formula m/z (Δ)	C ₄₈ H ₇₁ NO ₁₁ ⁺ 838.5106 (0.72)	C ₄₉ H ₇₃ NO ₁₂ ⁺ 852.5241 (1.81)	C ₄₉ H ₇₃ NO ₁₂ ⁺ 852.5241 (0.3)	C ₄₈ H ₇₂ NO ₁₁ ⁺ 838.5101 (0.12)
Fragment 2 (RDA 1)	Formula m/z (Δ)	C ₃₈ H ₅₉ NO ₉ ⁺ 672.4128 (3.27)	C ₃₈ H ₅₉ NO ₉ ⁺ 672.4111 (0.74)	C ₃₈ H ₅₉ NO ₉ ⁺ 672.4111 (0.74)	C ₃₈ H ₅₉ NO ₉ ⁺ 672.4086 (2.97)
Fragment 3*	Formula m/z (Δ)	C ₃₁ H ₄₉ NO ₆ ⁺ 530.3419 (10.8)	C ₃₁ H ₄₉ NO ₆ ⁺ 530.3456 (3.77)	C ₃₁ H ₄₉ NO ₆ ⁺ 530.3476 (0.00)	C ₃₁ H ₄₉ NO ₆ ⁺ 530.3413 (11.9)
Fragment 4	Formula m/z (Δ)	C ₂₇ H ₄₅ NO ₅ ⁺ 462.3228 (3.03)	C ₂₇ H ₄₅ NO ₅ ⁺ 462.3234 (4.33)	C ₂₇ H ₄₅ NO ₅ ⁺ 462.3217 (0.65)	C ₂₇ H ₄₅ NO ₅ ⁺ 462.3191 (4.97)
Fragment 5 (RDA 2)	Formula m/z (Δ)	C ₂₂ H ₃₇ NO ₃ ⁺ 362.2680 (2.76)	C ₂₂ H ₃₇ NO ₃ ⁺ 362.2705 (4.14)	C ₂₂ H ₃₇ NO ₃ ⁺ 362.2689 (0.28)	C ₂₂ H ₃₇ NO ₃ ⁺ 362.2654 (9.94)

*The abundance of the 530 ion was very low, explaining the somewhat higher mass errors for this group

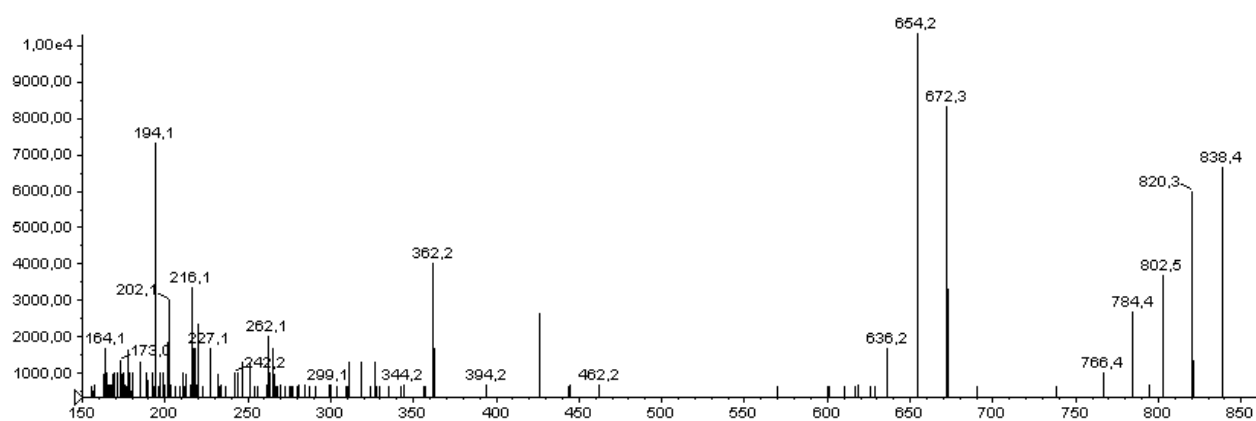


Fig. S4 AZA1 methyl ester spectrum previously reported by Krock *et al.* obtained through extraction of *A. spinosum* and analysed with an API4000 Qtrap (CUR: 10 psi, CAD: Medium, IS: 5500 V, TEM: 0, GS1: 10, GS2: 0, DP: 100, CES: 0, CE: 70 V)

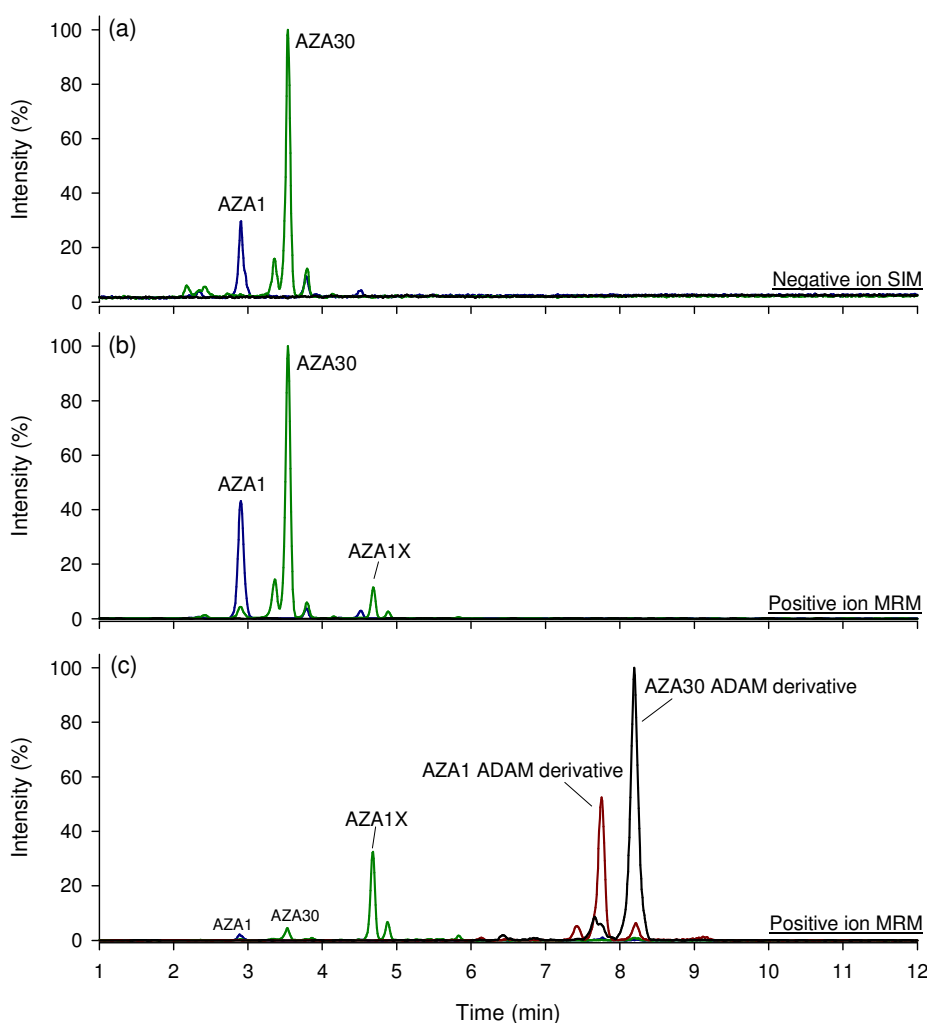


Fig. S5 Gradient elution LC-MS(/MS) analysis of a sample containing AZA1, AZA30 and AZA1 methyl ester: (a) negative ion SIM mode ($[M-H]^-$ of 840.5, 854.5, 868.5) showing AZA1 and AZ30, while AZA1 methyl ester is not detected; (b) positive ion MRM mode ($[M+H]^+$ of 842→672, 856→672, 1032→672, 1046→672) showing AZA1, AZA30 and AZA1 methyl ester (AZA1X), and; (c) the same sample following ADAM derivatization, showing successful derivatization of AZA1 and AZA30 as their (9-anthryl)methyl esters, whereas AZA1 methyl ester is unaffected (because it is already esterified, and so is unable to react)

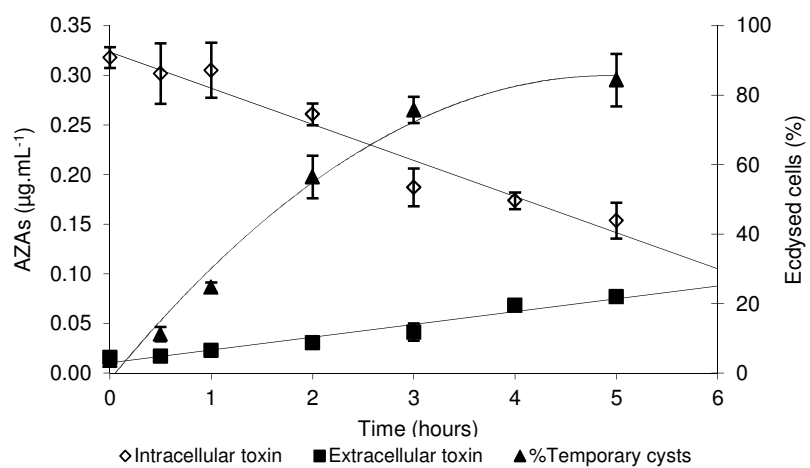


Fig. S5 Effect of residence time in a sampling tube on *A. spinosum*, prior to extraction, on the measured intracellular and extracellular azaspiracid content

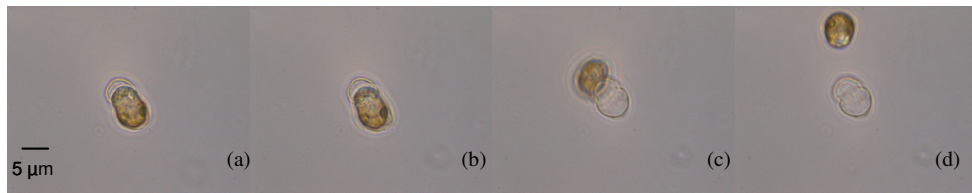


Fig. S6 a-d Different phases of *A. spinosum* dehiscence, b-c, protoplast extrusion from the theca