

Combining an OSMAC approach and untargeted metabolomics to profile compounds exhibiting anti-HIV-1 activities in an endophytic fungus, *Penicillium rubens* P03MB2

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Affiliation:

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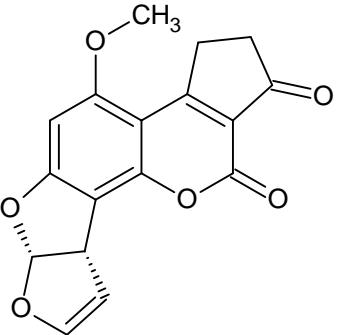
³NMISA, National Metrology Institute of South Africa CSIR Campus, Building 5, Meiring Naude Road, Brummeria Pretoria, South Africa.

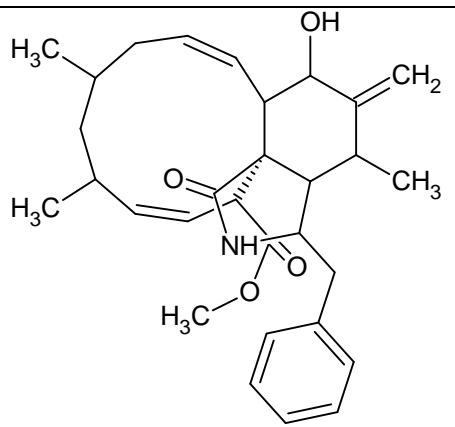
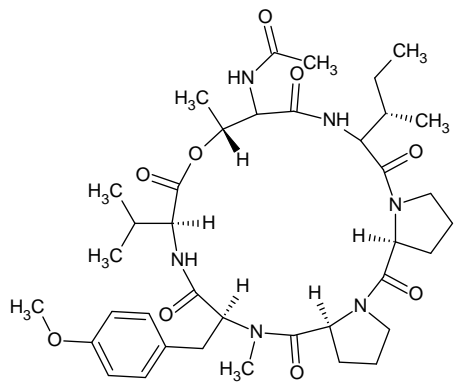

⁴Rand Water, Scientific Services Division, PO Box 1170, Johannesburg, 2000, South Africa

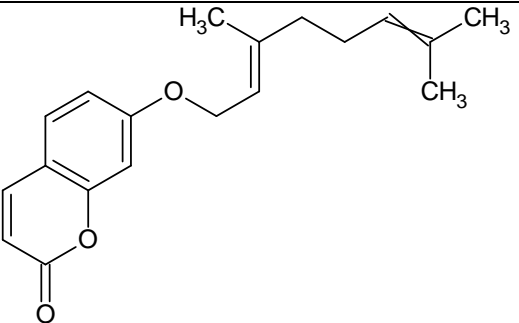
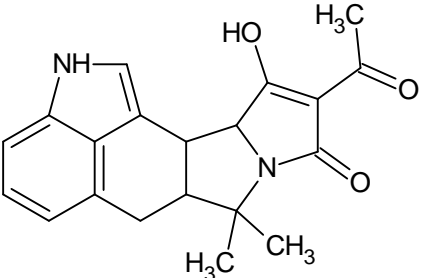
⁵HIV Pathogenesis Programme, Doris Duke Medical research institute, School of Laboratory Medicine and Medical Sciences, University of KwaZulu-Natal, Durban. South Africa.

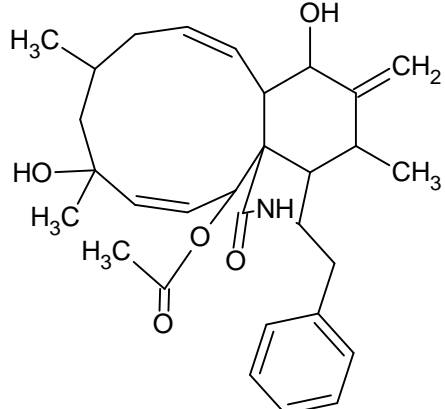
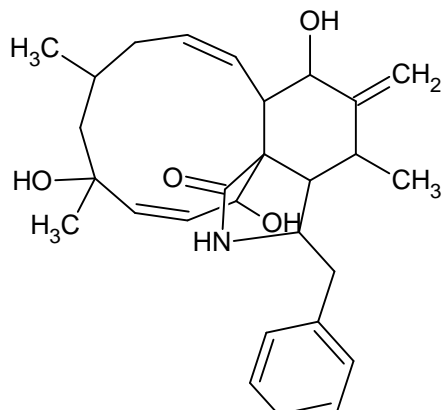
⁶Unit for Environmental Sciences and Management, North-West University, Potchefstroom, 2520, South Africa

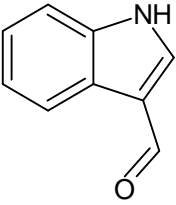
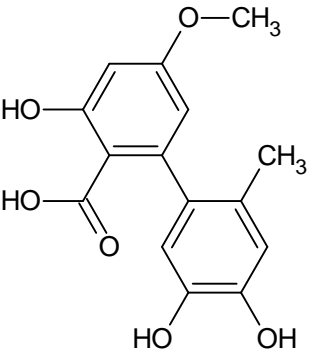
Table S1: Compounds identified from a feature-based molecular network of *P. rubens* P03MB2 extracts

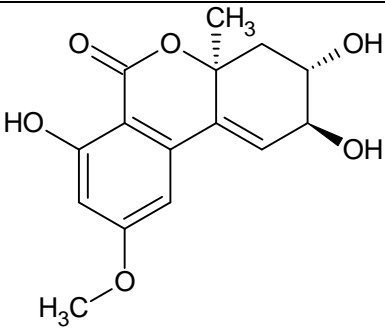
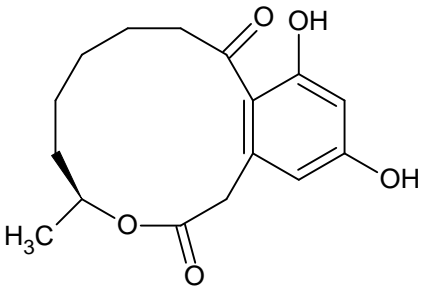
PubChem ID	Compound name (structure)	Compound class	Parent mass	Rt (min)	Molecular formula	MZ Error (ppm)	Fractions
186907	Aflatoxin B1 	Polyketides	313.068	8.914	C ₁₇ H ₁₂ O ₆	10.235	Oats MCX 45% *Oats MCX 95%
146157142	Antibiotic L-696,474	Pseudoalkaloids	478.285	16.251	C ₃₀ H ₃₉ NO ₄	20.737	Oats MCX 5% Oats MCX 95% *Rice MCX 95%

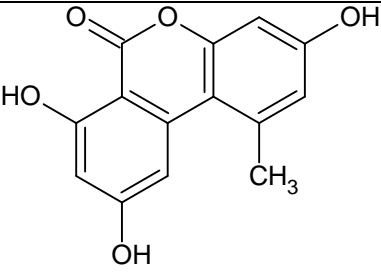
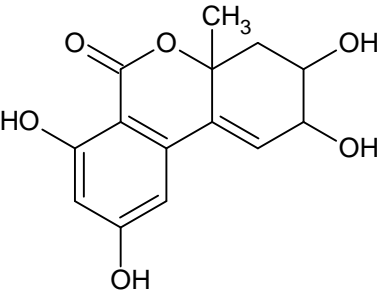
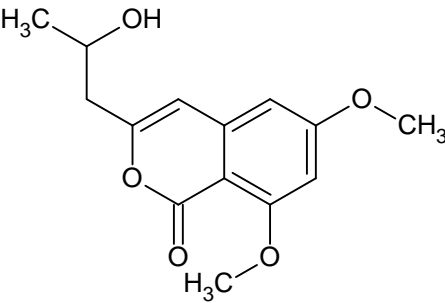
							
21777315	Aspergillicin_A 	Cyclic peptides	741.412	11.384	C ₃₈ H ₅₆ N ₆ O ₉	16.464	Oats MCX 5% *Oats MCX 45% Oats MCX 95%
1550607	Auraptin 	Coumarins	299.145	5.907	C ₁₉ H ₂₂ O ₃	62.838	Oats MCX 45% Rice MCX 5% Rice MCX 45% *Rice MAX 45% Rice MCX 95%

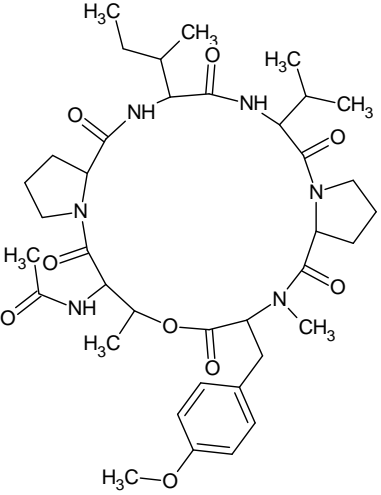
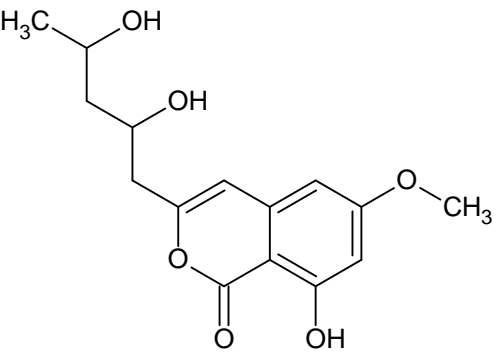
							
54675761	Alpha-Cyclopiazonic acid	Tetramate alkaloids	337.153	13.192	C ₂₀ H ₂₀ N ₂ O ₃	1.448	*Oats MCX 95%
							
2921	Cytochalasin H	Cytochalasin alkaloids	494.284	10.614	C ₃₀ H ₃₉ NO ₅	8.867	MEA MCX 5% MEA MCX 95% Oats MCX 5% Oats MCX 45% Oats MCX 95% Rice MCX 5% *Rice MCX 45% Rice MAX 45% Rice MCX 95%

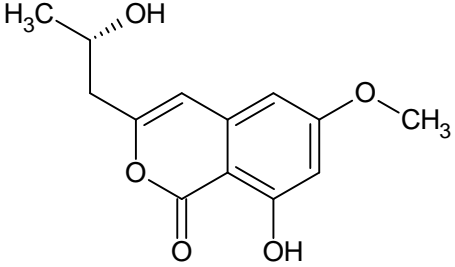
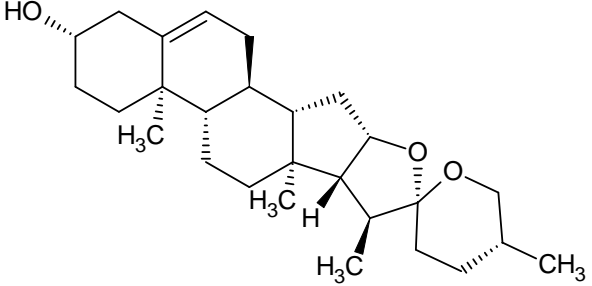
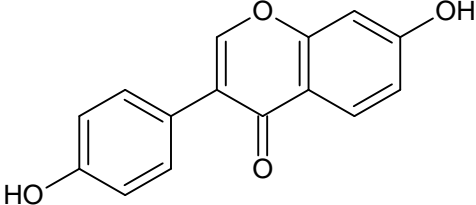
	 <p>The structure shows a complex polycyclic molecule. It features a large ring system with multiple methyl groups (H₃C), hydroxyl groups (OH), and a methylene group (=CH₂). A side chain includes a benzyl group (-CH₂-C₆H₅) and a nitrogen-containing ring system with a carbonyl group (C=O) and a methyl group (H₃C).</p>						
3555370	<p>Cytochalasin J</p>  <p>The structure is similar to the one above but with a different nitrogen-containing ring system, featuring a hydroxyl group (OH) and a hydrogen atom (HN) on the nitrogen.</p>	Cytochalasin alkaloids	452.275	8.839	C ₂₈ H ₃₇ NO ₄	13.697	<p>MEA MCX 5% MEA MCX 95% Oats MCX 5% Oats MCX 45% Oats MCX 95% Rice MCX 5% Rice MCX 45% *Rice MAX 45% Rice MCX 95%</p>
10256	<p>3-Formylindole</p>	Simple indole alkaloids	146.058	5.874	C ₉ H ₇ NO	19.222	<p>*Oats MCX 95% Rice MCX 95%</p>

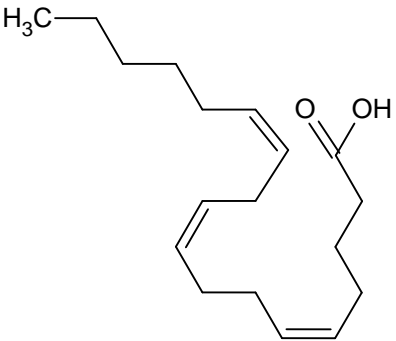
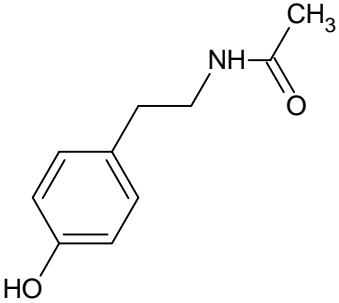
							
6918469	<p>Altenuin (2-(4,5-dihydroxy-2-methylphenyl)-6-hydroxy-4-methoxybenzoic acid")</p> 		291.083	8.240	C ₁₅ H ₁₄ O ₆	12.371	<p>MEA MCX 95%</p> <p>Oats MCX 5%</p> <p>Oats MCX 45%</p> <p>Oats MCX 95%</p> <p>Rice MCX 45%</p> <p>*Rice MCX 95%</p>
34687	<p>Altenuene (2,3,7-Trihydroxy-9-methoxy-4a-methyl-2,3,4,4a-tetrahydro-6H-benzo[c]chromen-6-one)</p>	Isocoumarins	293.099	7.923	C ₁₅ H ₁₆ O ₆	9.163	<p>Oats MCX 5%</p> <p>Oats MCX 45%</p> <p>Oats MCX 95%</p> <p>Rice MCX 45%</p> <p>*Rice MCX 95%</p>

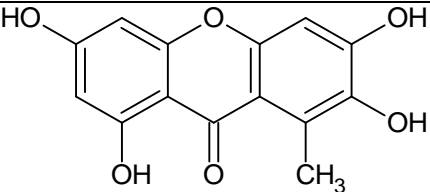
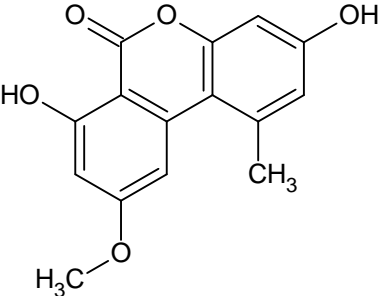
							
296197	<p>(S)-Curvularin (3-Benzoxacyclododecin-2,10(1H)-dione, 4,5,6,7,8,9-hexahydro-11,13-dihydroxy-4-methyl-)</p> 	Zearalenones (Macrolides)	293.135	9.099	C ₁₆ H ₂₀ O ₅	9.890	MEA MCX 95% Oats MCX 5% Oats MCX 45% Oats MCX 95% Rice MCX 5% Rice MCX 45% *Rice MCX 95%
5359485	<p>Alternariol (3,7,9-trihydroxy-1-methylbenzo[c]chromen-6-one)</p>	Isocoumarins	259.058	9.247	C ₁₄ H ₁₀ O ₅	8.835	MEA MCX 95% *Oats MCX 95% Rice MCX 95%

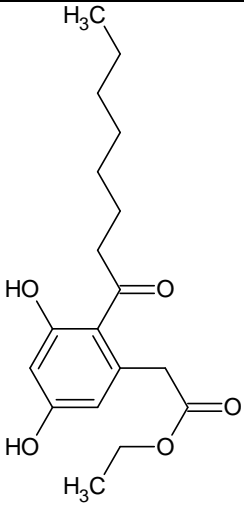
							
45359151	<p>Dibenzo[b,d]pyran-6-one, 2,3,4,4a-tetrahydro-2,3,7,9-tetrahydroxy-4a-methyl-</p> 	Coumarins	279.084	5.560	C ₁₄ H ₁₄ O ₆	8.639	Oats MCX 45% *Oats MCX 95%
14408240	<p>3-(2-Hydroxypropyl)-6,8-dimethoxyisochromen-1-one</p> 	Coumarins	265.105	7.079	C ₁₄ H ₁₆ O ₅	8.288	Oats MCX 5% Oats MCX 45% *Oats MCX 95%

<p>45782841</p>	<p>Acetamide</p> 	<p>Oligopeptides</p>	<p>763.394</p>	<p>11.384</p>	<p>C₃₈H₅₆N₆O₉</p>	<p>8.395</p>	<p>*Oats MCX 5% Oats MCX 95% Rice MCX 45%</p>
<p>75411939</p>	<p>3-(2,4-Dihydroxypentyl)-8-hydroxy-6-methoxyisochromen-1-one</p> 	<p>Coumarins</p>	<p>295.113</p>	<p>8.482</p>	<p>C₁₅H₁₈O₆</p>	<p>11.892</p>	<p>*MEA MCX 95% Oats MCX 95% Rice MCX 95%</p>

5323561	Diaporthin (8-hydroxy-3-[(2S)-2-hydroxypropyl]-6-methoxyisochromen-1-one) 	Isocoumarins	251.089	9.215	C ₁₃ H ₁₄ O ₅	7.171	*Oats MCX 95%
99474	Diosgenin 	Spirostane steroids	397.306	15.242	C ₂₇ H ₄₂ O ₃	9.920	Oats MCX 45% *Oats MCX 95%
5281708	Daidzein 	Isoflavones	255.063	7.210	C ₁₅ H ₁₀ O ₄	12.503	*MEA MCX 95%

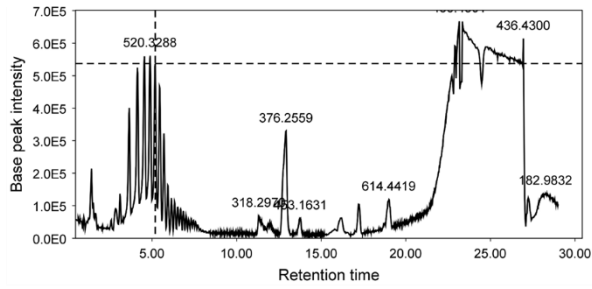
5312495	Pinolenic acid 	Fatty Acids and Conjugates	279.229	16.977	$C_{18}H_{30}O_2$	10.055	MEA MCX 95% *Oats MCX 95%
	N-Acetyltyramine 	Phenylethylamines (Alkaloids)	180.1	4.467	$C_{10}H_{13}N$	8.388	Rice MAX 45% *Oat MCX 45% Oat MCX 95%
10423452	Anomalin A	Methyl xanthenes (Polyketides)	275.052	7.514	$C_{14}H_{10}O_6$	9.764	*Oat MCX 95% Rice MCX 95%

							
5360741	Alternariol Monomethyl ether 	Isocoumarins (Shikimates and Phenylpropanoids)	273.073	8.232	C ₁₅ H ₁₂ O ₅	9.834	MEA MCX 95% Oat MCX 5% Oat MCX 95% Rice MCX 45% *Rice MCX 95%
10687292	Cytosporone B	Polyketides	323.182	15.04754	C ₁₈ H ₂₆ O ₅	10.8592	Oats MCX 95% *Rice MCX 95%

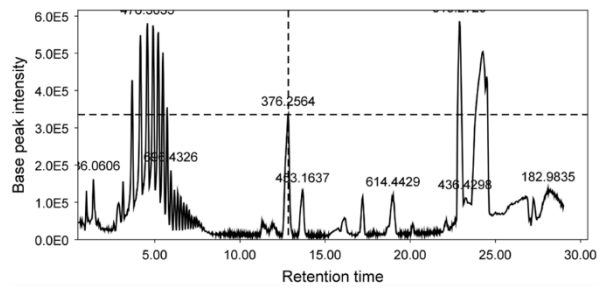
							
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*Mass feature with the highest intensity

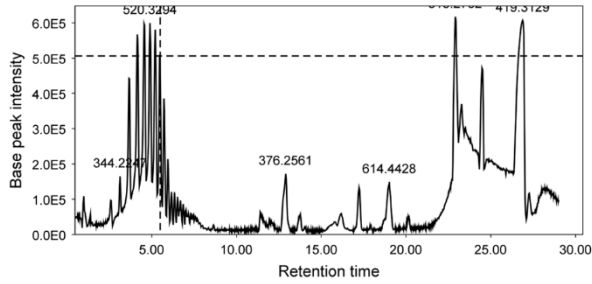
LC-MS Chromatogram for MEA control (media only)



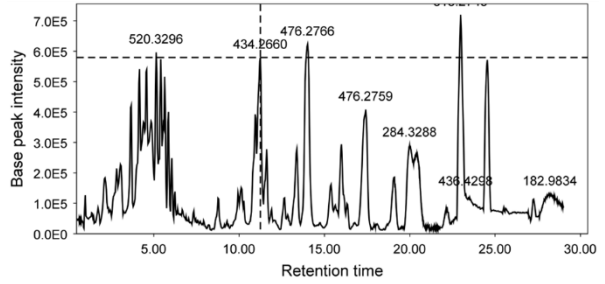
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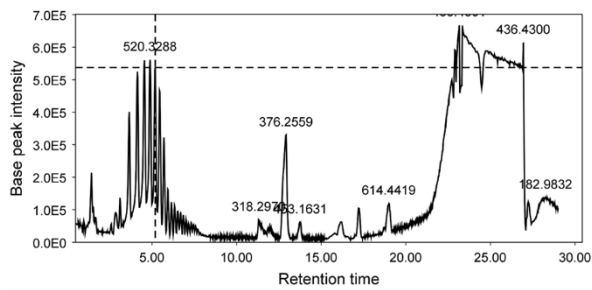
LC-MS Chromatogram for Rice control (media only)



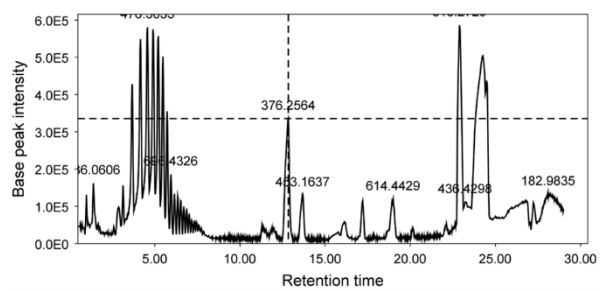
LC-MS Chromatogram for MEA MCX 5%



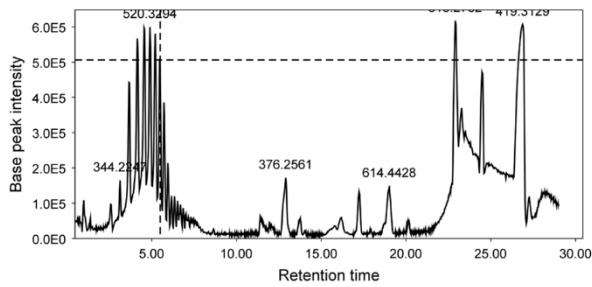
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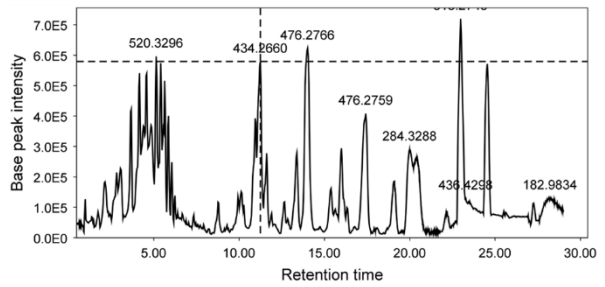
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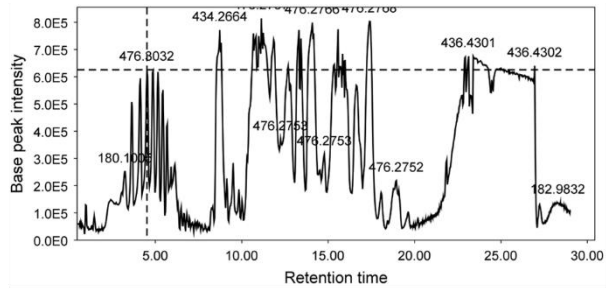
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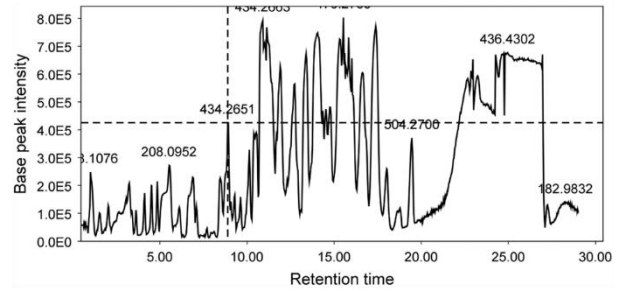
LC-MS Chromatogram for MEA MCX 5%



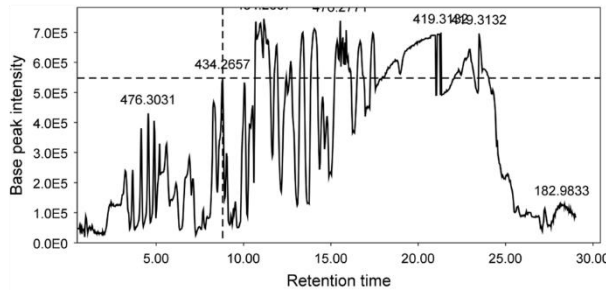
LC-MS Chromatogram for Rice MAX 45%



LC-MS Chromatogram for Rice MCX 5%



LC-MS Chromatogram for Rice MCX 45%



LC-MS Chromatogram for Rice MCX 95%

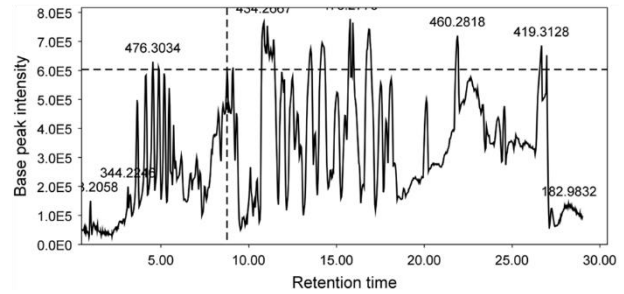
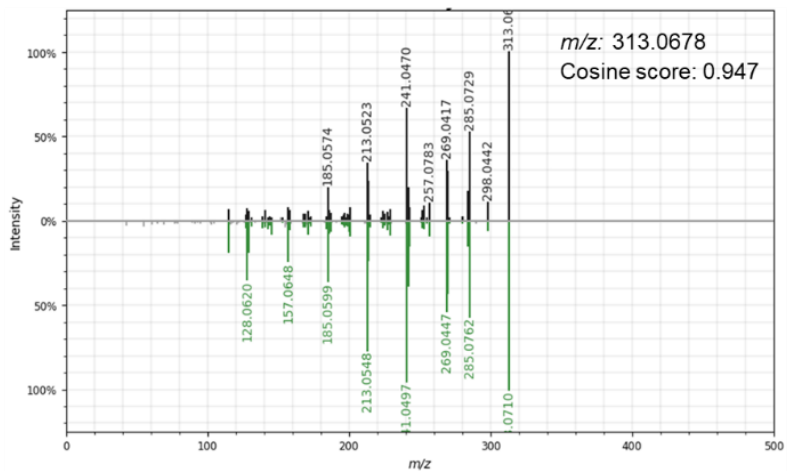
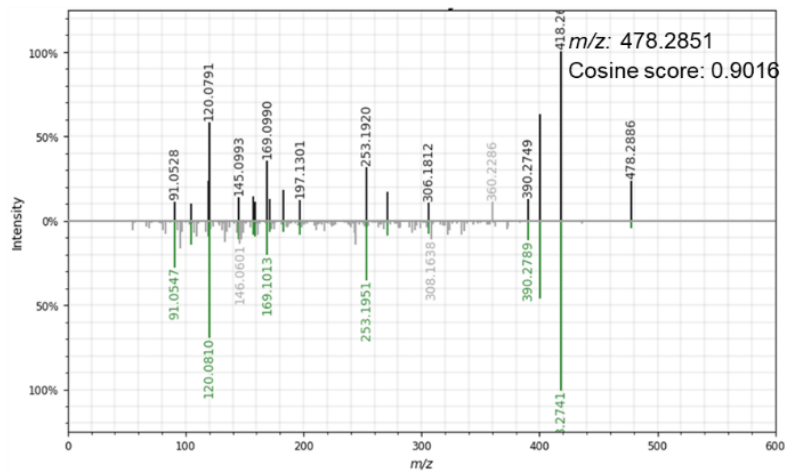


Figure S1: LC-MS chromatograms of *Penicillium chrysogenum* PO3MB3 extract under various media conditions

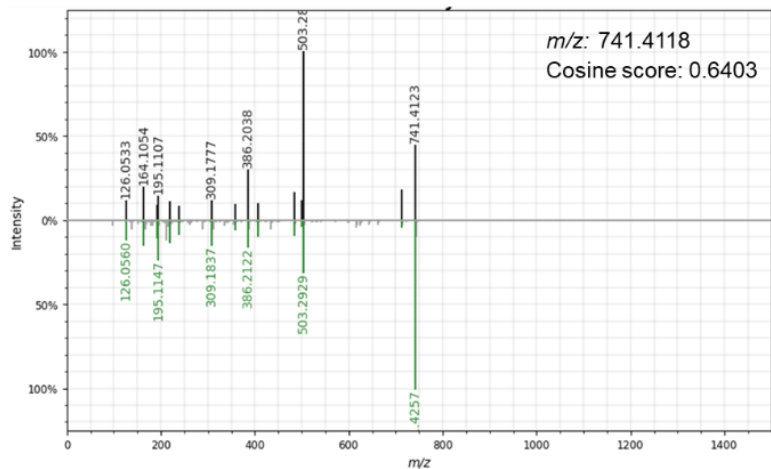
LC-MS/MS Aflatoxin



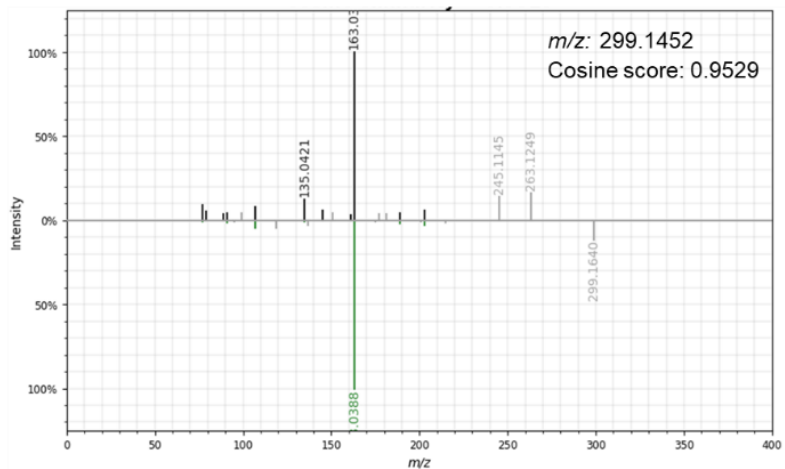
LC-MS/MS Antibiotic L-696,474



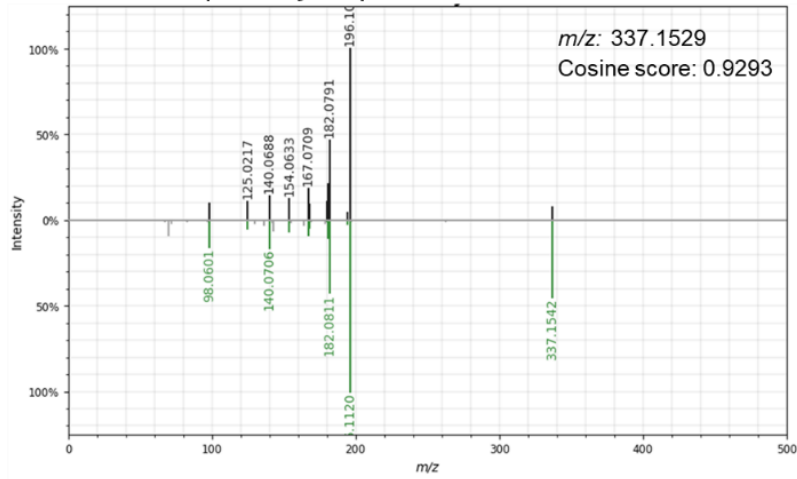
LC-MS/MS Aspergillcin A



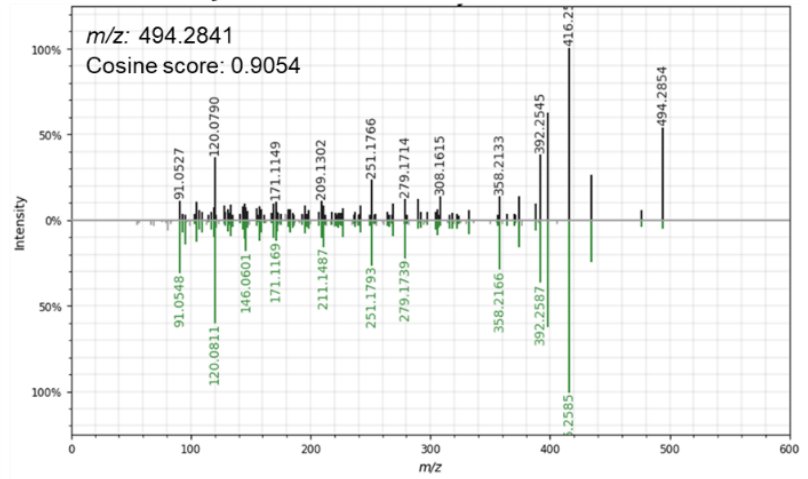
LC-MS/MS Auraptin



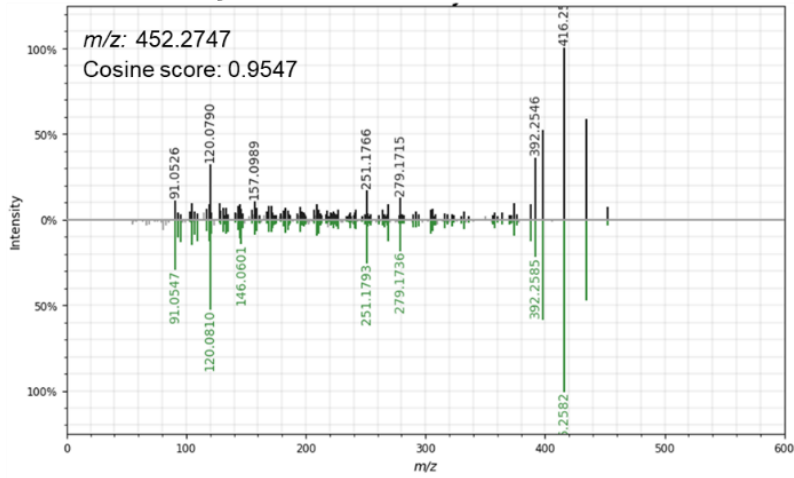
LC-MS/MS Alpha-Cyclopiazonic acid



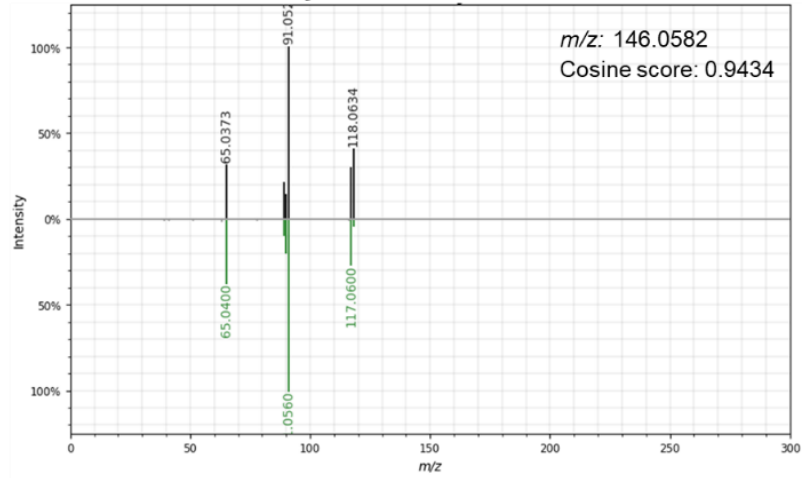
LC-MS/MS Cytochalasin H



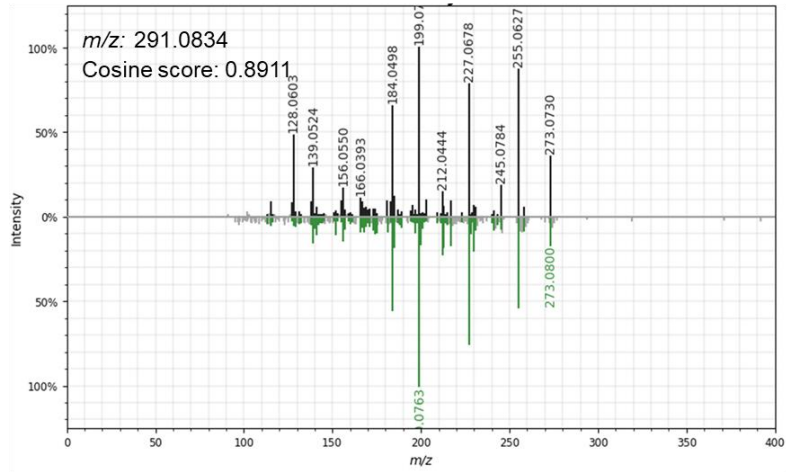
LC-MS/MS Cytochalasin J



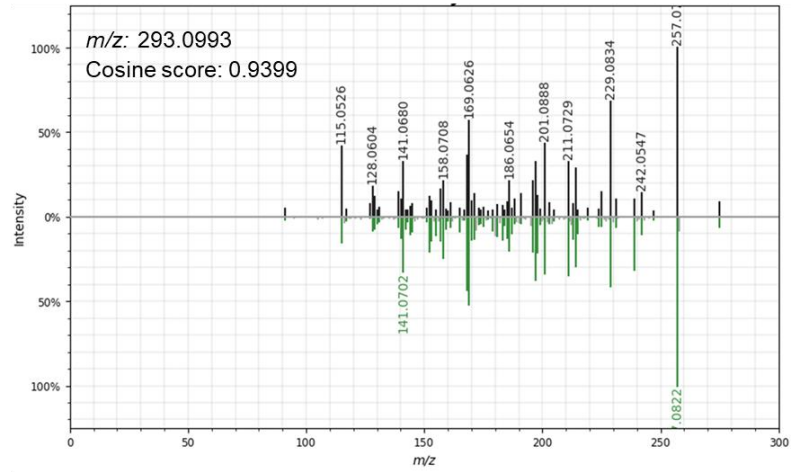
LC-MS/MS 3-Formylindole



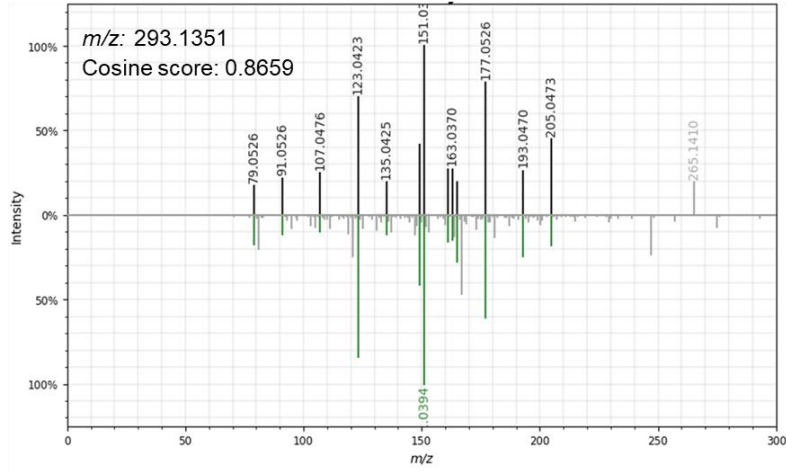
LC-MS/MS Altenusin



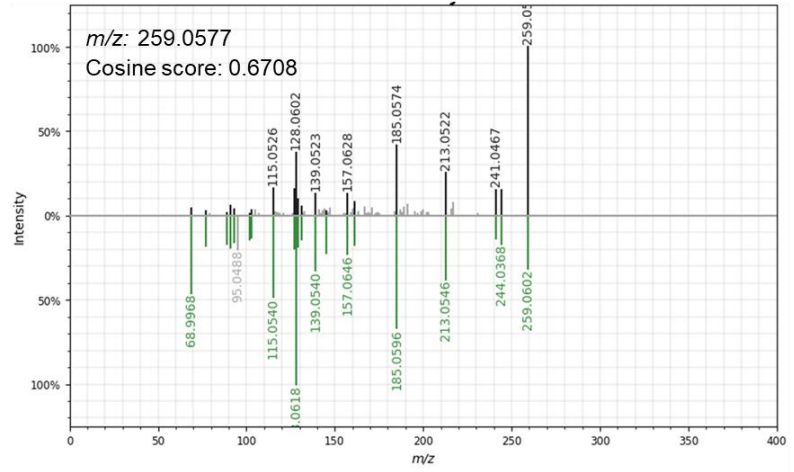
LC-MS/MS Altenuene



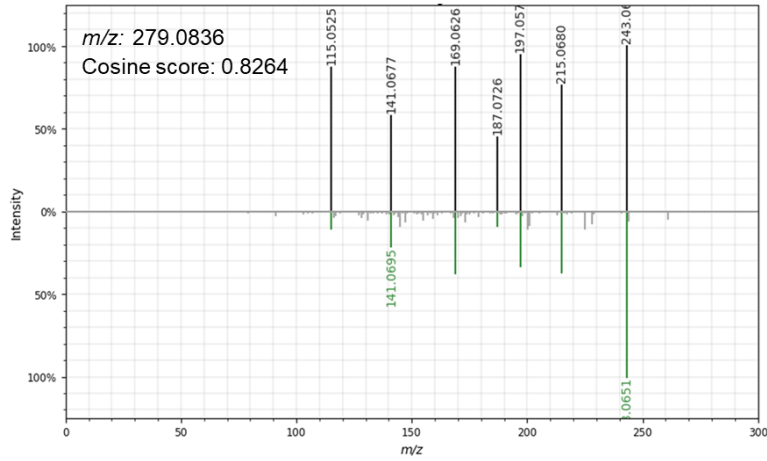
LC-MS/MS Curvularin



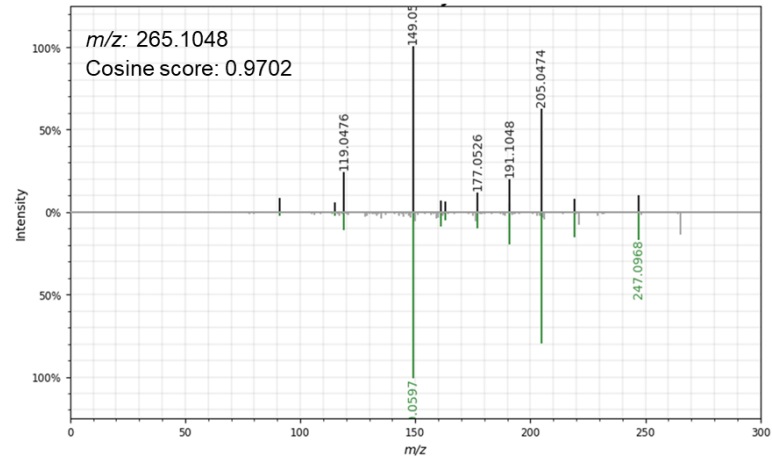
LC-MS/MS Alternariol



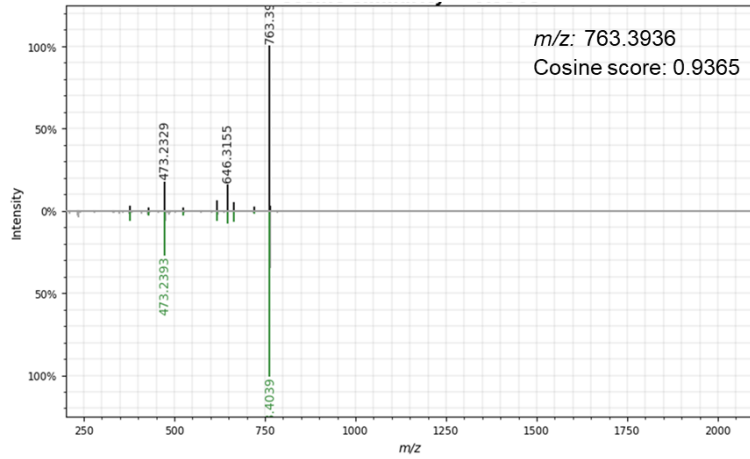
LC-MS/MS Dibenzo[b,d]pyran-6-one, 2,3,4,4a-tetrahydro-2,3,7,9-tetrahydroxy-4a-methyl-



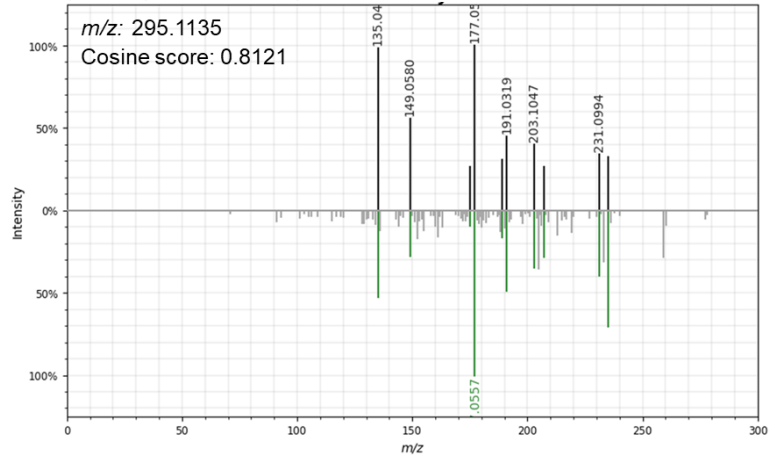
LC-MS/MS 3-(2-Hydroxypropyl)-6,8-dimethoxyisochromen-1-one



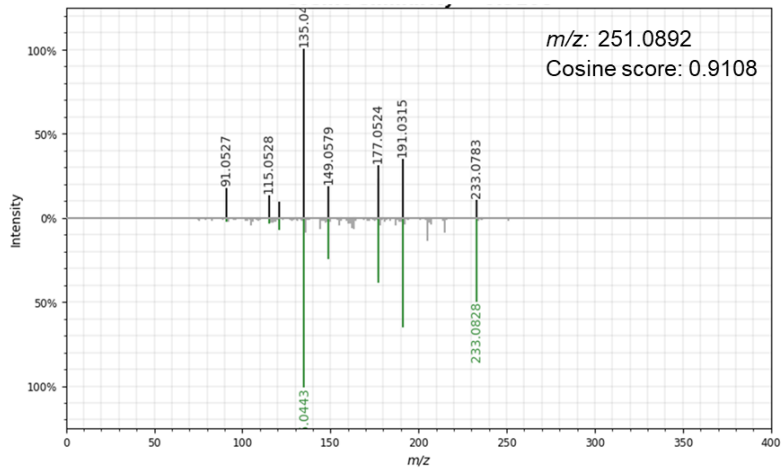
LC-MS/MS Acetamide



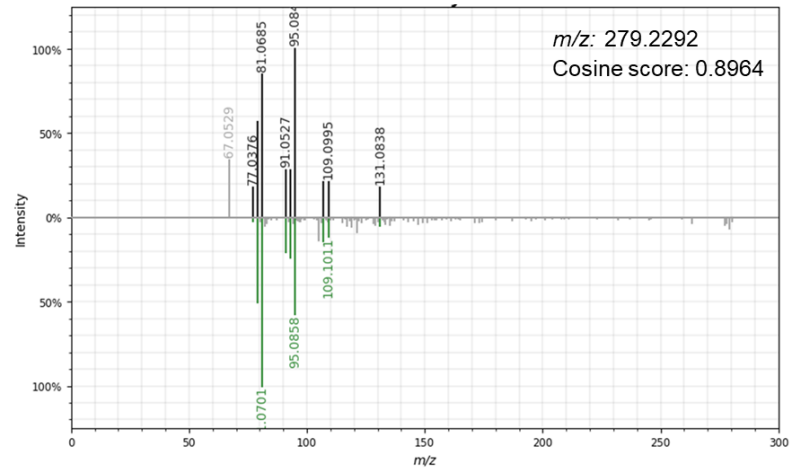
LC-MS/MS 3-(2,4-Dihydroxypropyl)-8-hydroxy-6-methoxyisochromen-1-one



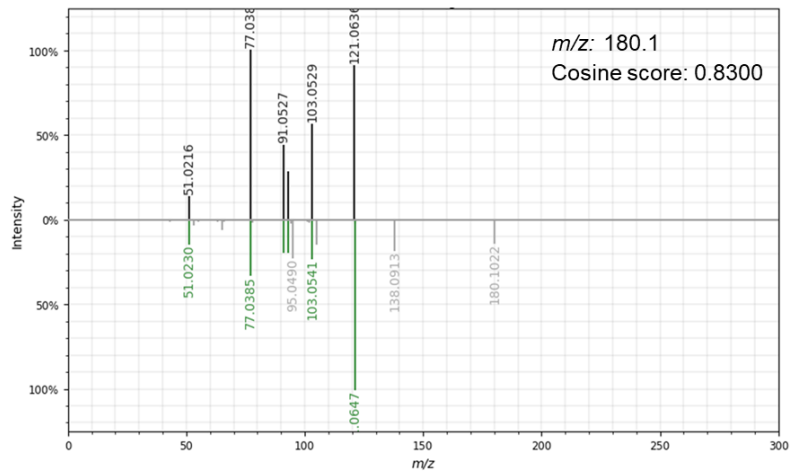
LC-MS/MS Diaporthin



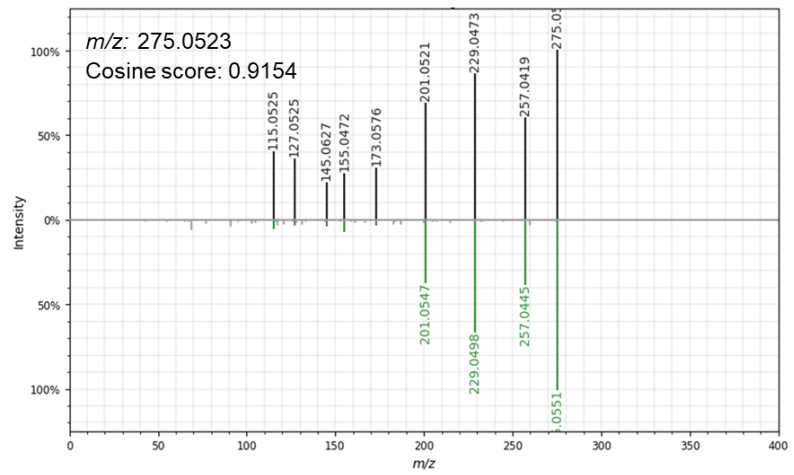
LC-MS/MS Pinolenic acid



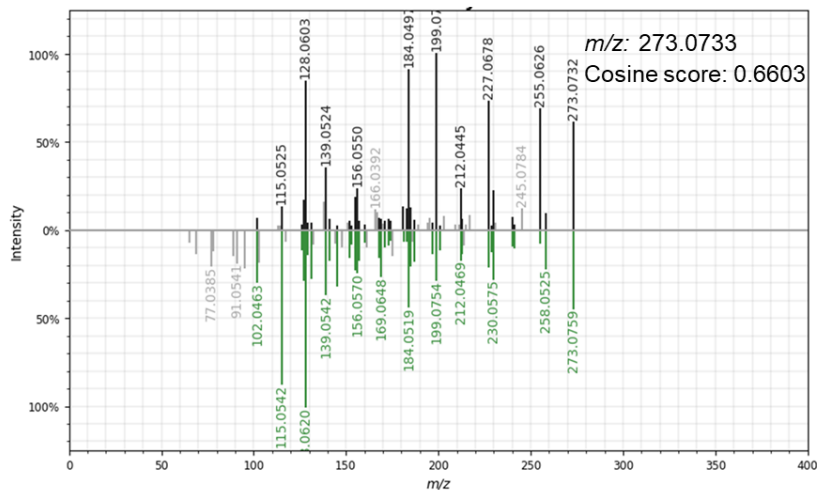
LC-MS/MS N-Acetyltyramine



LC-MS/MS Anomalin



LC-MS/MS Alternariol Monomethyl ether



LC-MS/MS Cytosporone B

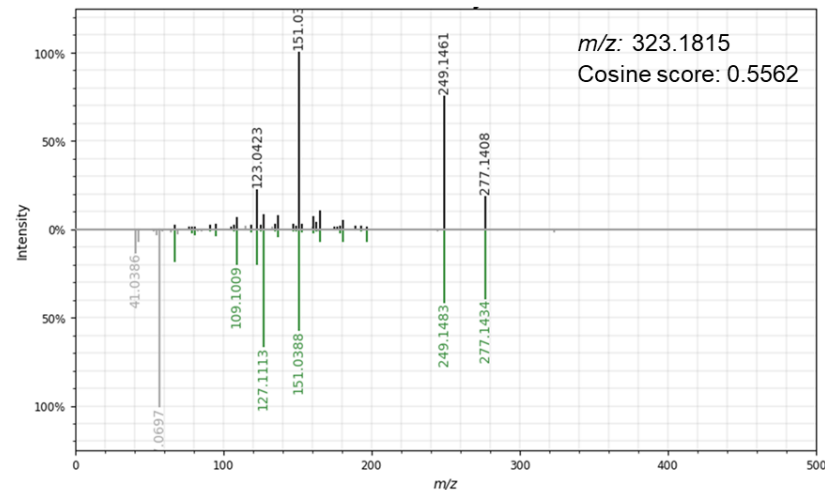


Figure S2: LC-MS/MS mirror plots of secondary metabolites identified from the *P. chrysogenum* P03MB2. The top m/z spectra (black) represent compounds identified in this study. The bottom m/z spectra (green) represent the corresponding GNPS library compounds.