

# Supplementary materials: *Pseudomonas* PA14H7: Identification of 7-hydroxytropolone Iron Complex as a Potential Antagonist against *Dickeya*, the Causal Agent of Blackleg on Potato Plant

## Summary

**Figure S1:** Growth kinetics of *Pseudomonas* PA14H7 in TY medium.

**Table S1:** Detailed composition of Bacto Tryptone (named T)

**Figure S2:** LC-MS (electrospray ionization in the positive ion mode: ESI<sup>+</sup>), base peak intensity (BPI) chromatograms of TY culture medium (A) and CFS-PA14H7 (B)

**Figure S3:** LCMS (ESI<sup>+</sup>) comparison of TY culture medium (left) and CFS-PA14H7 (right) chromatograms of aqueous phases after extraction with chloroform at pH 7 (A), chloroform at pH 2 (B), ethyl acetate pH 7 (C) or ethyl acetate pH 2 (D).

**Figure S4:** LCMS (ESI<sup>+</sup>) comparison of TY culture medium (left) and CFS-PA14H7 (right) chromatograms of organic phases after extraction with chloroform at pH 7 (A), chloroform at pH 2 (B), ethyl acetate pH 7 (C) or ethyl acetate pH 2 (D). Extraction performed on the same batch of TY and PA14H7 filtered supernatant.

**Table S2:** Results of bacteriostatic/bactericidal effect of organic and aqueous phases of CFS-PA14H7 extract with chloroform or ethyl acetate at pH 2 or pH 7.

**Figure S5:** The reconstituted ion current (RIC) of the ions observed between 3.6 and 4.6 min at m/z 139.040 (A), m/z 329.983 (B) and m/z 658.957 (C) in TY culture medium (left) and CFS-PA14H7 (right).

**Table S3:** Summary of markers present in CFS-PA14H7 and absent in TY medium analyzed with marker lynx (Waters, Masslynx V4.2).

**Figure S6:** UV trace corresponding to the maximum of absorbance of the iron complex (B), absent in TY UV trace (A). Corresponding LC-MS (ESI<sup>+</sup>) BPI chromatograms for TY culture medium (C) and CFS-PA14H7 (D) highlighting the presence of the complex at m/z 329.983.

**Figure S7:** Ultra-Violet (UV) at 224 nm (blue) and 320 nm (purple) and Evaporative Light Scattering Diffusion (ELSD) (green) chromatograms of the flash chromatography purification of CFS-PA14H7 using a C18 column. Fraction F1 is highlighted in green, F2 in orange, and F3 in blue.

**Figure S8:** LC-MS (ESI<sup>+</sup>) chromatograms of fraction F2 resulting from CFS-PA14H7 purification by flash chromatography, BPI trace (A), RIC of the ions at m/z 139.040 (B) and m/z 329.983 (C). The purified iron complex is effectively detected between 3.80 and 4.40 min. The corresponding mass spectra are also depicted.

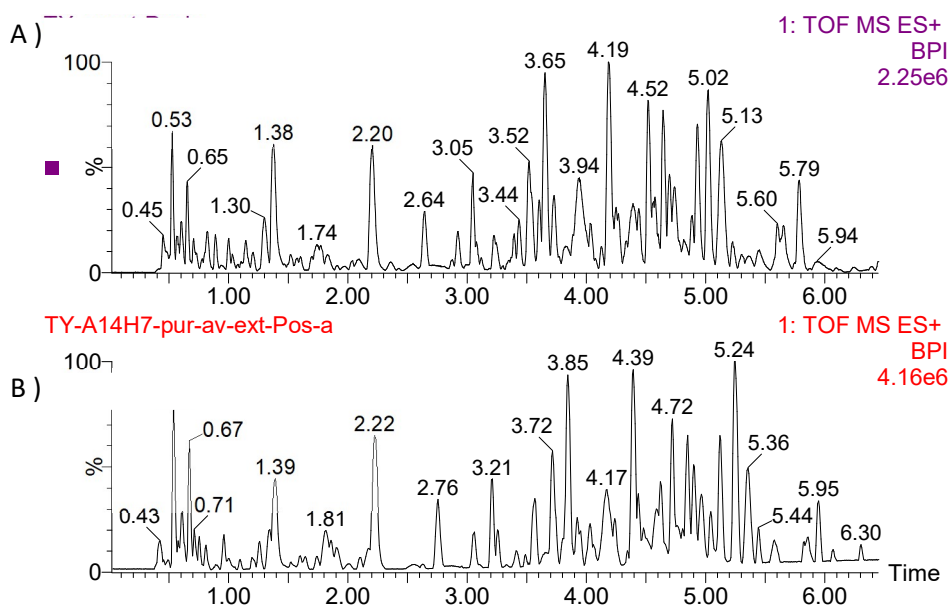
**Figure S9:** EI NIST library spectra of the different hypothesis for the molecular ion observed at m/z 138. 7-HT (A) and 2-ethoxyphenol and isomers (B).

**Figure S10:** Detailed NMR spectra of fraction F2 resulting from CFS-PA14H7 purification by flash chromatography: (A) <sup>1</sup>H NMR (400 MHz) and (B) <sup>13</sup>C NMR (100 MHz).

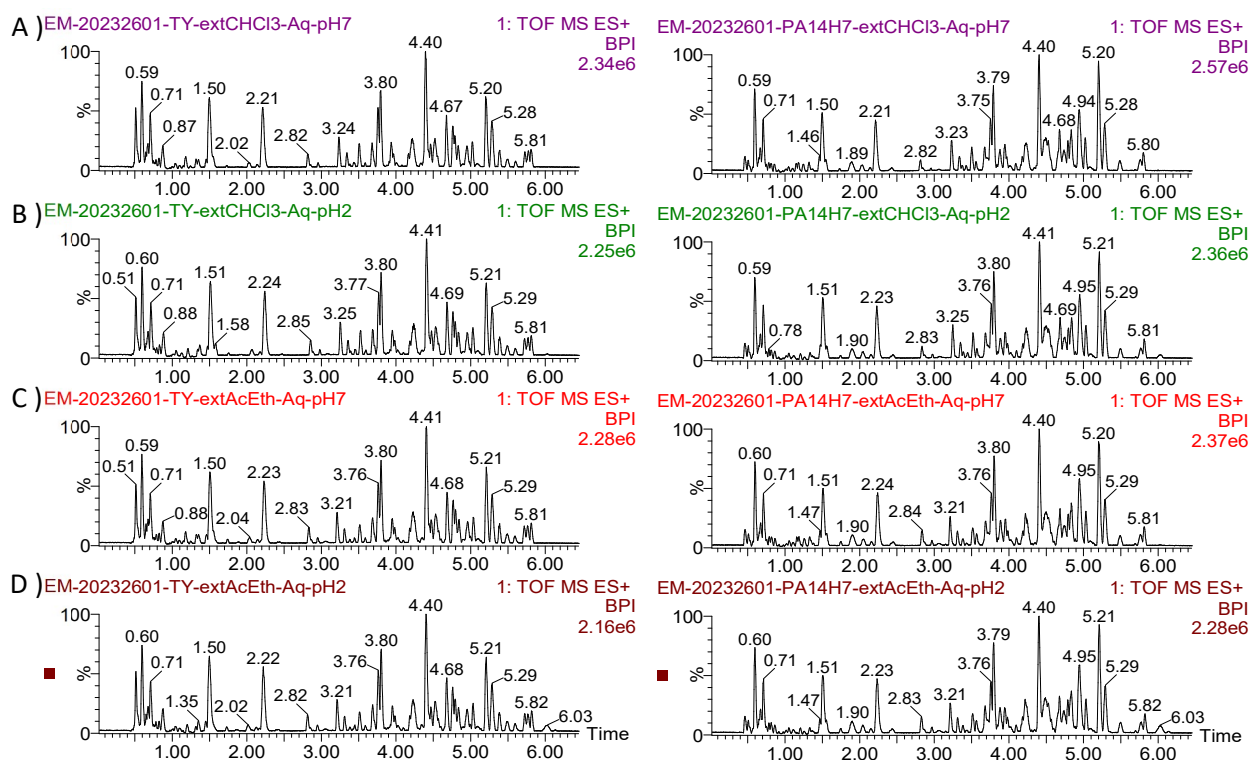
**Figure S11:** Synthesis of 7-hydroxytropolone starting from commercial tropolone.

**Figure S12:** (A) <sup>1</sup>H NMR and (B) <sup>13</sup>C NMR spectra of 7-HT obtained by synthesis and (C) <sup>1</sup>H NMR spectra and (D) <sup>13</sup>C NMR spectra comparison of fraction F2 resulting from CFS-PA14H7 purification by flash chromatography (top) and synthetic 7-HT (bottom) between 6.3 and 9.1 ppm. **Figure S13:** GC-MS chromatograms and spectra of 7-

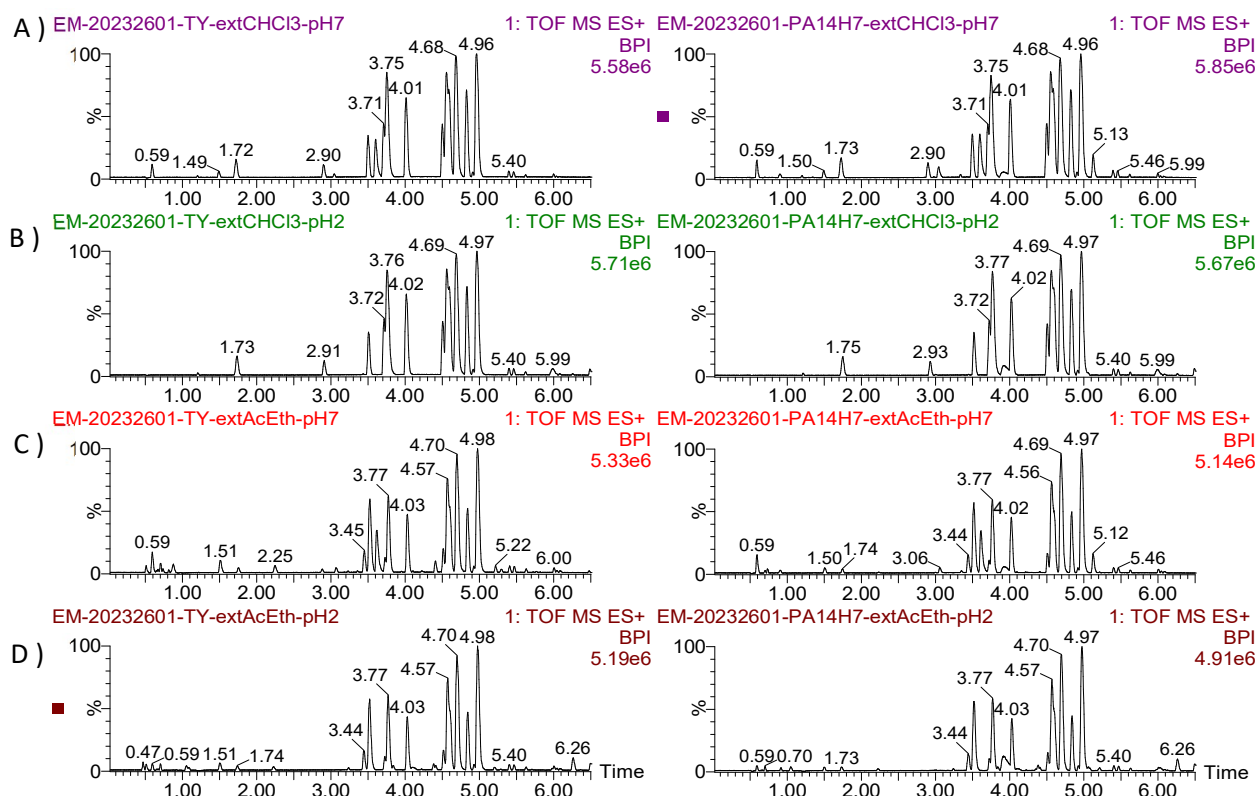




**Figure S2:** LC-MS (electrospray ionization in the positive ion mode: ESI<sup>+</sup>), base peak intensity (BPI) chromatograms of TY culture medium (A) and CFS-PA14H7 (B)



**Figure S3:** LC-MS (ESI<sup>+</sup>) comparison of TY culture medium (left) and CFS-PA14H7 (right) BPI chromatograms for aqueous phases after extraction with chloroform at pH 7 (A), chloroform at pH 2 (B), ethyl acetate pH 7 (C) or ethyl acetate pH 2 (D).



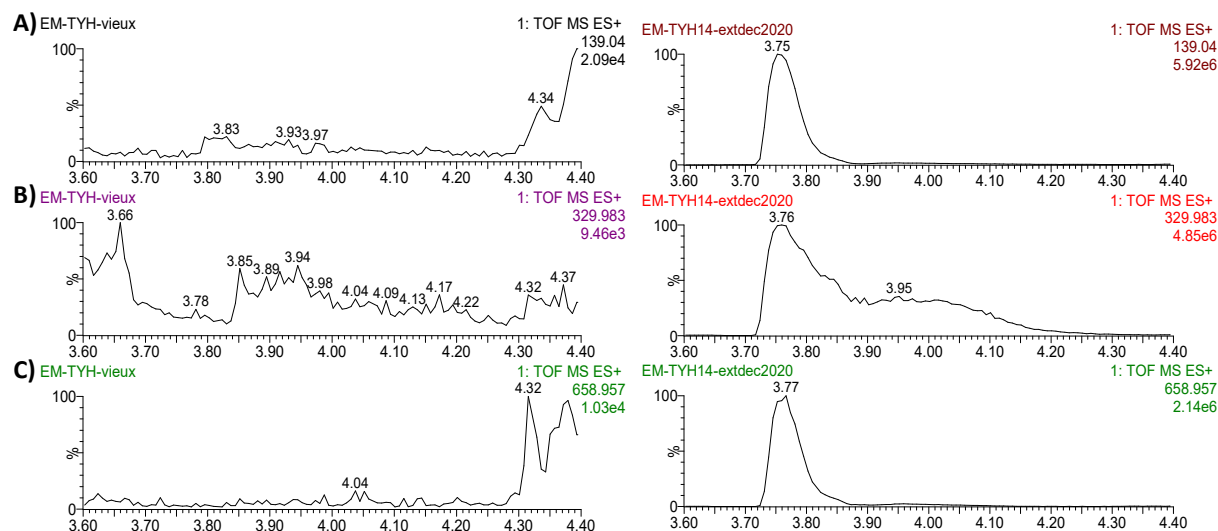
**Figure S4:** LC-MS (ESI<sup>+</sup>) comparison of TY culture medium (left) and CFS-PA14H7 (right) BPI chromatograms of organic phases after extraction with chloroform at pH 7 (A), chloroform at pH 2 (B), ethyl acetate pH 7 (C) or ethyl acetate pH 2 (D). Extraction performed on the same batch of TY and PA14H7 filtered supernatant.

**Table S2:** Optical Density at 600 nm (OD 600nm) measurement of organic and aqueous phases of CFS-PA14H7 extract with chloroform or ethyl acetate at pH 2 or pH 7 after 0 h, 24 h and 48 h of incubation with *D. solani*.

| Extraction method of CFS-PA14H7 |      | dilution | Organic phase |       |       | Aqueous phase |       |       |
|---------------------------------|------|----------|---------------|-------|-------|---------------|-------|-------|
|                                 |      |          | t0            | t24   | t48   | t0            | t24   | t48   |
| Chloroform                      | pH 2 | control  | 0,043         | 0,044 | 0,044 | 0,040         | 0,040 | 0,040 |
|                                 |      | pur      | 0,058         | 0,060 | 0,071 | 0,043         | 0,042 | 0,043 |
|                                 |      | 1/2      | 0,052         | 0,055 | 0,055 | 0,041         | 0,041 | 0,041 |
|                                 |      | 1/4      | 0,044         | 0,044 | 0,045 | 0,041         | 0,040 | 0,040 |
|                                 |      | 1/10     | 0,045         | 0,044 | 0,045 | 0,040         | 0,041 | 0,041 |
|                                 |      | 1/20     | 0,042         | 0,042 | 0,042 | 0,041         | 0,042 | 0,041 |
|                                 |      | 1/100    | 0,042         | 0,131 | 0,189 | 0,041         | 0,174 | 0,198 |
|                                 |      | 1/200    | 0,041         | 0,144 | 0,213 | 0,040         | 0,176 | 0,178 |
|                                 |      | 1/1000   | 0,042         | 0,131 | 0,211 | 0,041         | 0,120 | 0,201 |
|                                 | pH 7 | control  | 0,044         | 0,044 | 0,044 | 0,052         | 0,041 | 0,041 |
|                                 |      | pur      | 0,070         | 0,066 | 0,061 | 0,047         | 0,213 | 0,277 |
|                                 |      | 1/2      | 0,053         | 0,050 | 0,049 | 0,042         | 0,170 | 0,222 |
|                                 |      | 1/4      | 0,046         | 0,045 | 0,045 | 0,040         | 0,147 | 0,218 |
|                                 |      | 1/10     | 0,045         | 0,043 | 0,043 | 0,042         | 0,147 | 0,209 |
|                                 |      | 1/20     | 0,043         | 0,042 | 0,044 | 0,041         | 0,171 | 0,185 |
|                                 |      | 1/100    | 0,041         | 0,137 | 0,178 | 0,041         | 0,185 | 0,180 |
|                                 |      | 1/200    | 0,041         | 0,188 | 0,193 | 0,040         | 0,189 | 0,182 |
| Ethyl acetate                   | pH 2 | 1/1000   | 0,041         | 0,126 | 0,206 | 0,041         | 0,133 | 0,198 |
|                                 |      | control  | 0,041         | 0,042 | 0,041 | 0,040         | 0,041 | 0,041 |
|                                 |      | pur      | 0,053         | 0,053 | 0,060 | 0,043         | 0,287 | 0,269 |
|                                 |      | 1/2      | 0,046         | 0,047 | 0,047 | 0,042         | 0,163 | 0,245 |
|                                 |      | 1/4      | 0,046         | 0,046 | 0,046 | 0,041         | 0,154 | 0,238 |
|                                 |      | 1/10     | 0,043         | 0,043 | 0,043 | 0,042         | 0,153 | 0,231 |
|                                 |      | 1/20     | 0,043         | 0,043 | 0,044 | 0,044         | 0,162 | 0,222 |
|                                 |      | 1/100    | 0,042         | 0,094 | 0,166 | 0,041         | 0,157 | 0,214 |
|                                 | pH 7 | 1/200    | 0,041         | 0,146 | 0,203 | 0,041         | 0,157 | 0,198 |
|                                 |      | 1/1000   | 0,042         | 0,161 | 0,202 | 0,042         | 0,164 | 0,206 |
|                                 |      | control  | 0,043         | 0,043 | 0,045 | 0,039         | 0,040 | 0,040 |
|                                 |      | pur      | 0,045         | 0,045 | 0,045 | 0,042         | 0,167 | 0,386 |
|                                 |      | 1/2      | 0,042         | 0,043 | 0,043 | 0,040         | 0,139 | 0,282 |
|                                 |      | 1/4      | 0,041         | 0,041 | 0,041 | 0,039         | 0,141 | 0,226 |
|                                 |      | 1/10     | 0,042         | 0,041 | 0,041 | 0,039         | 0,129 | 0,209 |
|                                 |      | 1/20     | 0,041         | 0,042 | 0,042 | 0,039         | 0,128 | 0,208 |
|                                 |      | 1/100    | 0,040         | 0,119 | 0,182 | 0,041         | 0,156 | 0,215 |
|                                 |      | 1/200    | 0,040         | 0,129 | 0,213 | 0,039         | 0,172 | 0,215 |
|                                 |      | 1/1000   | 0,040         | 0,133 | 0,202 | 0,041         | 0,149 | 0,198 |

**Legend:**  
no growth in liquid medium  
growth in liquid medium

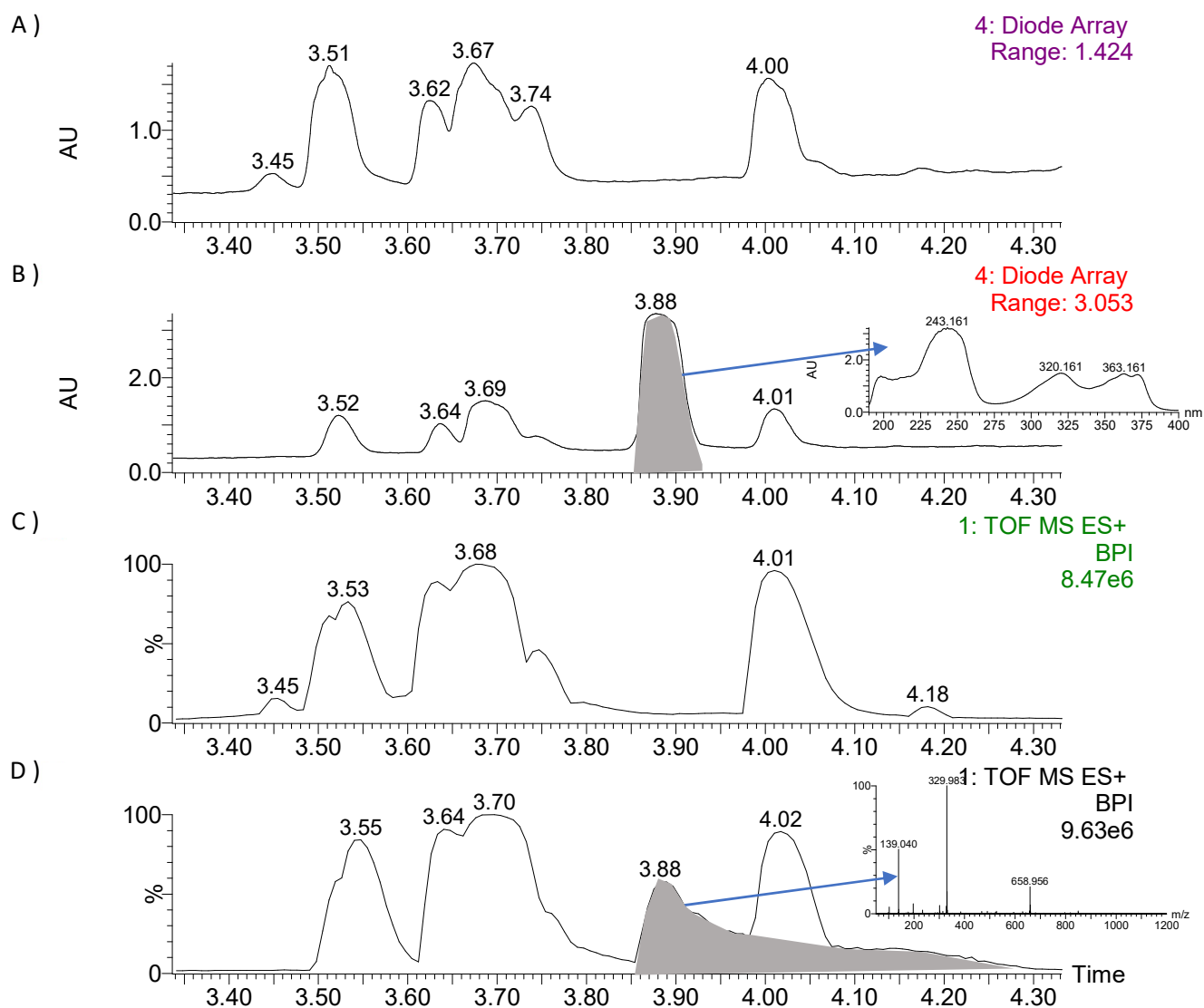
Negative controls are used to verify the absence of contamination of extracts and medium used.  
The combination where no bacterial growth is observed after 48 of incubation (green) are enumerated on Petri dish for the determination of bactericidal/bacteriostatic effect.



**Figure S5:** The reconstituted ion current (RIC) of the ions observed between 3.6 and 4.6 min at m/z 139.040 (A), m/z 329.983 (B) and m/z 658.957 (C) in TY culture medium (left) and CFS-PA14H7 (right).

**Table S3:** Summary of markers present in CFS-PA14H7 and absent in TY medium analyzed with marker lynx (Waters, Masslynx V4.2).

| Ret. Time | m/z      | Included | Saturated | EM-081221-MeOH-a | EM-081221-extTY- | EM-081221-extA14H7-CHCl3-a | EM-081221-MeOH-b | EM-081221-extTY- | EM-081221-extA14H7-CHCl3-b | EM-081221-MeOH-c | EM-081221-extTY- | EM-081221-extA14H7-CHCl3-c | MOY-MeOH | MOY-extTY | MOY-ext CFS-PA14H7 |
|-----------|----------|----------|-----------|------------------|------------------|----------------------------|------------------|------------------|----------------------------|------------------|------------------|----------------------------|----------|-----------|--------------------|
| 3.5814    | 329.9821 | Yes      | No        | 0                | 0                | 64,2733                    | 0                | 0                | 74,0706                    | 0                | 0                | 67,0571                    | 0,0000   | 0,0000    | 68,4670            |
| 3.5631    | 139.0393 | Yes      | No        | 0                | 0                | 26,5547                    | 0                | 0                | 28,0006                    | 0                | 0                | 29,36                      | 0,0000   | 0,0000    | 27,9718            |
| 2.6099    | 107.0497 | Yes      | No        | 0                | 0                | 0                          | 0                | 0                | 25,5209                    | 0                | 0                | 26,8061                    | 0,0000   | 0,0000    | 17,4423            |
| 5.4185    | 473.2756 | Yes      | No        | 0                | 0                | 15,6818                    | 0                | 0                | 16,2169                    | 0                | 0                | 16,4323                    | 0,0000   | 0,0000    | 16,1103            |
| 3.6385    | 197.1290 | Yes      | No        | 0                | 0                | 0                          | 0                | 0                | 26,6137                    | 0                | 0                | 21,5049                    | 0,0000   | 0,0000    | 16,0395            |
| 4.8521    | 430.7162 | Yes      | No        | 0                | 0                | 12,8648                    | 0                | 0                | 13,6696                    | 0                | 0                | 13,8827                    | 0,0000   | 0,0000    | 13,4724            |
| 4.6698    | 860.4260 | Yes      | No        | 0                | 0                | 14,2627                    | 0                | 0                | 9,6497                     | 0                | 0                | 14,3276                    | 0,0000   | 0,0000    | 12,7467            |
| 4.8537    | 609.3388 | Yes      | No        | 0                | 0                | 12,3265                    | 0                | 0                | 12,219                     | 0                | 0                | 12,0869                    | 0,0000   | 0,0000    | 12,2108            |
| 4.2119    | 540.3383 | Yes      | No        | 0                | 0                | 11,8303                    | 0                | 0                | 11,9083                    | 0                | 0                | 11,9421                    | 0,0000   | 0,0000    | 11,8936            |
| 4.8505    | 860.4267 | Yes      | No        | 0                | 0                | 10,9442                    | 0                | 0                | 11,7755                    | 0                | 0                | 12,3206                    | 0,0000   | 0,0000    | 11,6801            |
| 4.9946    | 746.3832 | Yes      | No        | 0                | 0                | 10,7787                    | 0                | 0                | 11,7357                    | 0                | 0                | 11,8424                    | 0,0000   | 0,0000    | 11,4523            |
| 5.2181    | 657.3956 | Yes      | No        | 0                | 0                | 10,4574                    | 0                | 0                | 10,4247                    | 0                | 0                | 10,4402                    | 0,0000   | 0,0000    | 10,4408            |



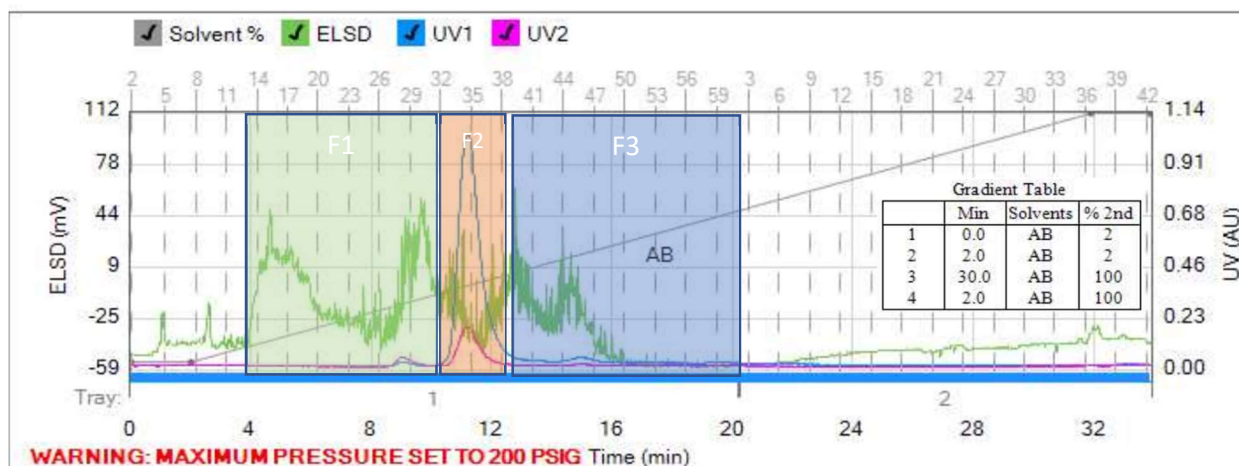
**Figure S6:** UV trace corresponding to the maximum of absorbance of the iron complex (B), absent in TY UV trace (A). Corresponding LC-MS (ESI<sup>+</sup>) BPI chromatograms for TY culture medium (C) and CFS-PA14H7 (D) highlighting the presence of the complex at m/z 329.983.

Column: FP ECOFLEX C18 12g  
Flow Rate: 30 mL/min  
Equilibration: 4.8 min  
Run Length: 34.0 min  
Mode: Flash Liquid

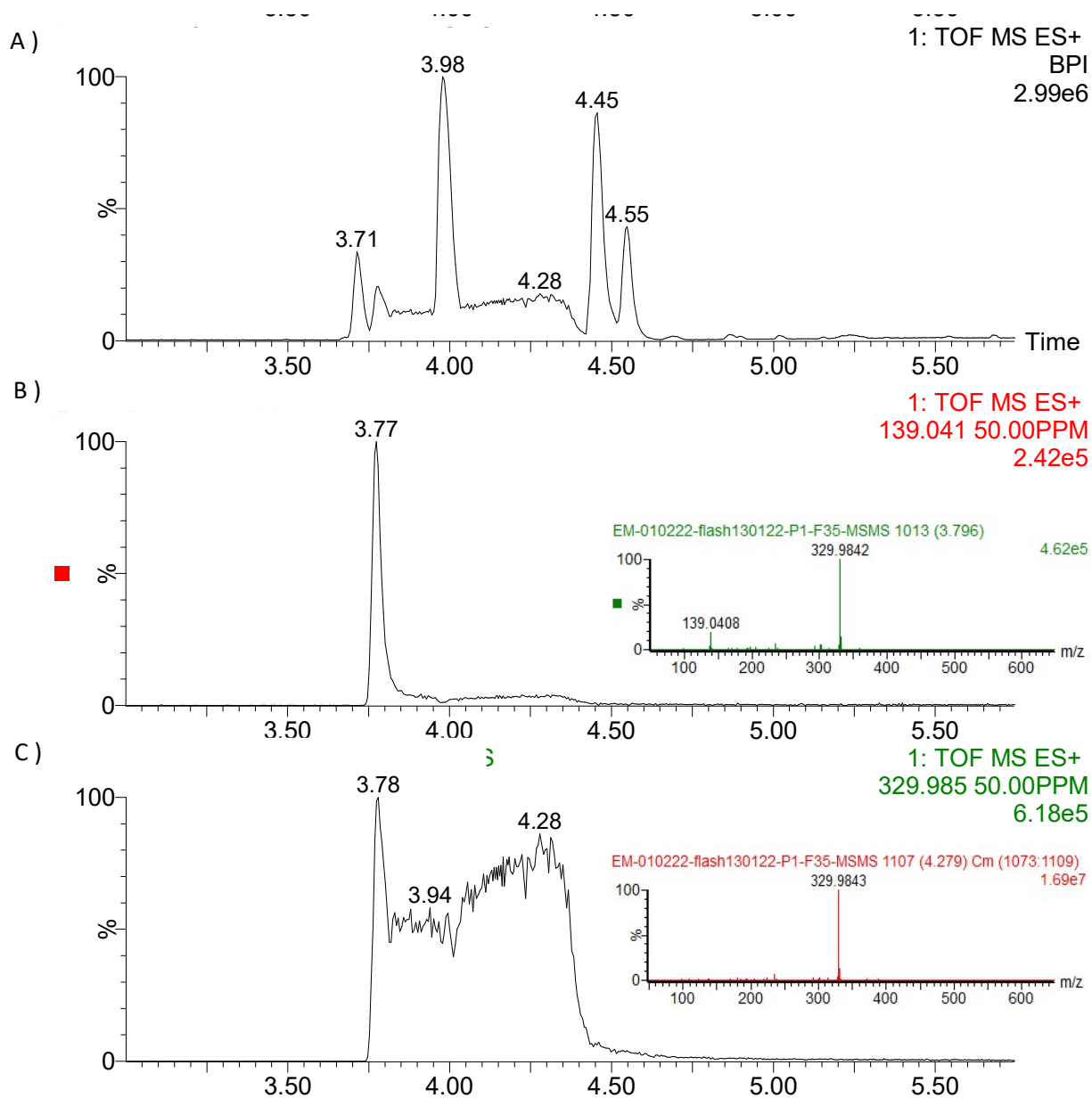
Solvent A: Water  
Solvent B: Methanol  
Solvent C: Empty  
Solvent D: Empty  
Slope Detection: Off

UV Threshold: N/A  
UV Sensitivity: Low  
UV1 Wavelength: 244 nm  
UV2 Wavelength: 320 nm  
UV3 Wavelength: N/A

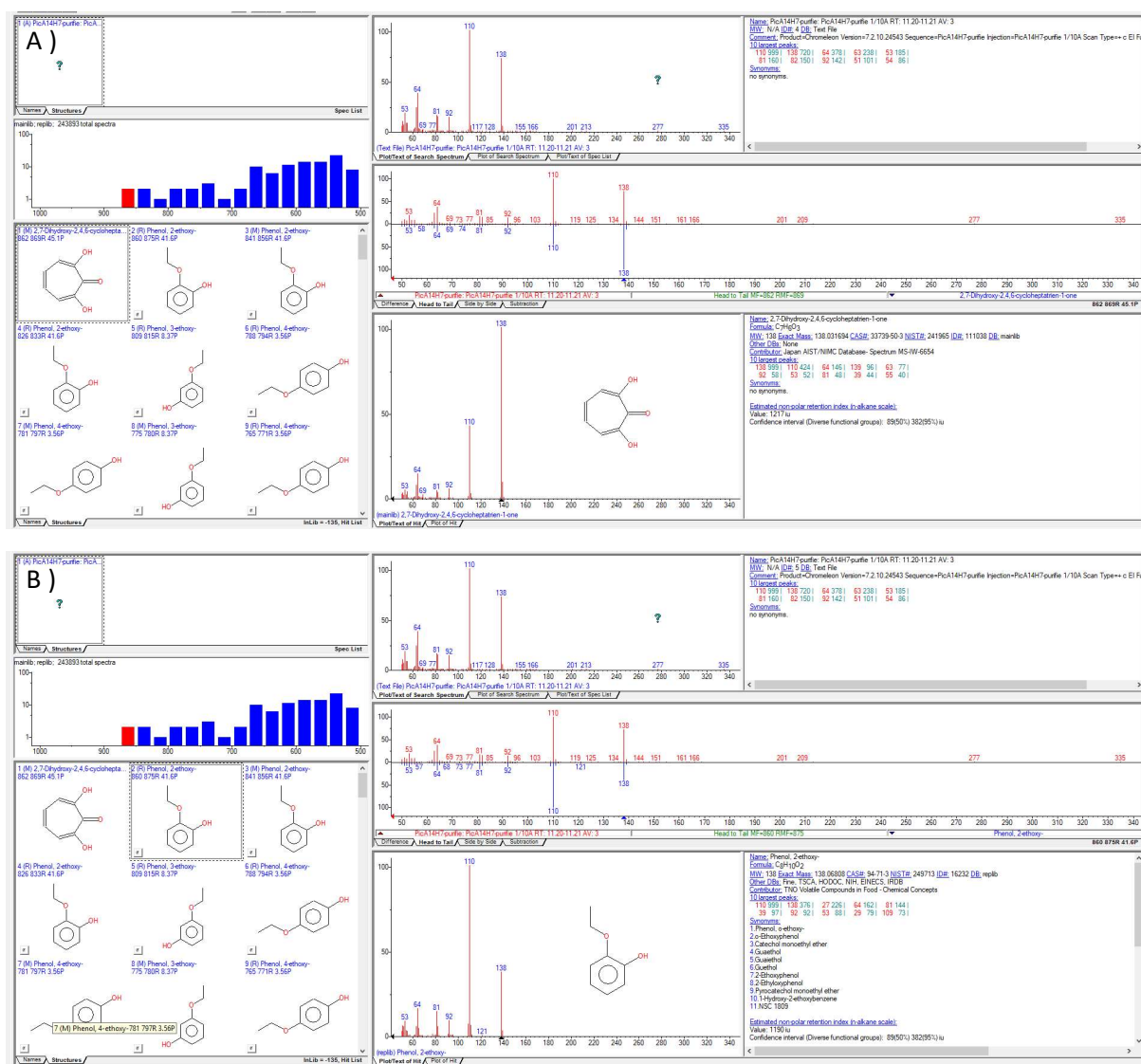
ELSD Threshold: N/A  
ELSD Sensitivity: Low  
Collection: Collect All  
Per-Vial Volume: 10 mL  
Non-Peak Volume: 10 mL



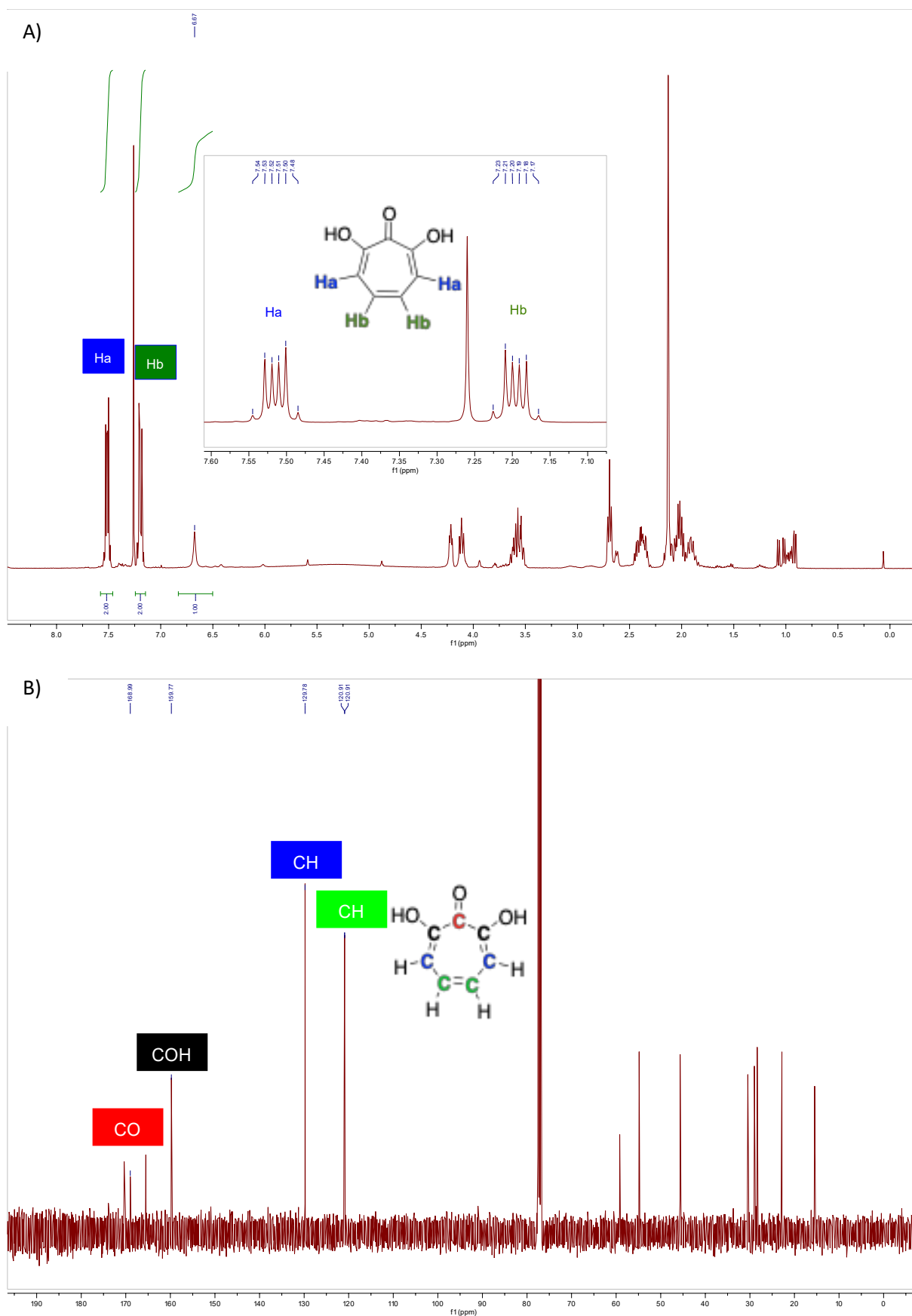
**Figure S7:** Ultra-Violet (UV) at 224 nm (blue) and 320 nm (purple) and Evaporative Light Scattering Diffusion (ELSD) (green) chromatograms of the flash chromatography purification of CFS-PA14H7 using a C18 column. Fraction F1 is highlighted in green, F2 in orange, and F3 in blue.

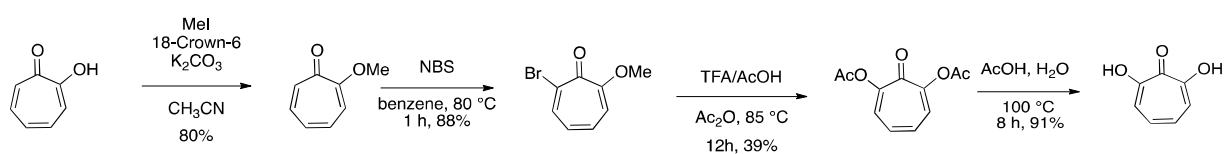


**Figure S8:** LC-MS (ESI+) chromatograms of fraction F2 resulting from CFS-PA14H7 purification by flash chromatography, BPI trace (A), RIC of the ions at  $m/z$  139.040 (B) and  $m/z$  329.983 (C). The purified iron complex is effectively detected between 3.80 and 4.40 min. The corresponding mass spectra are also depicted.

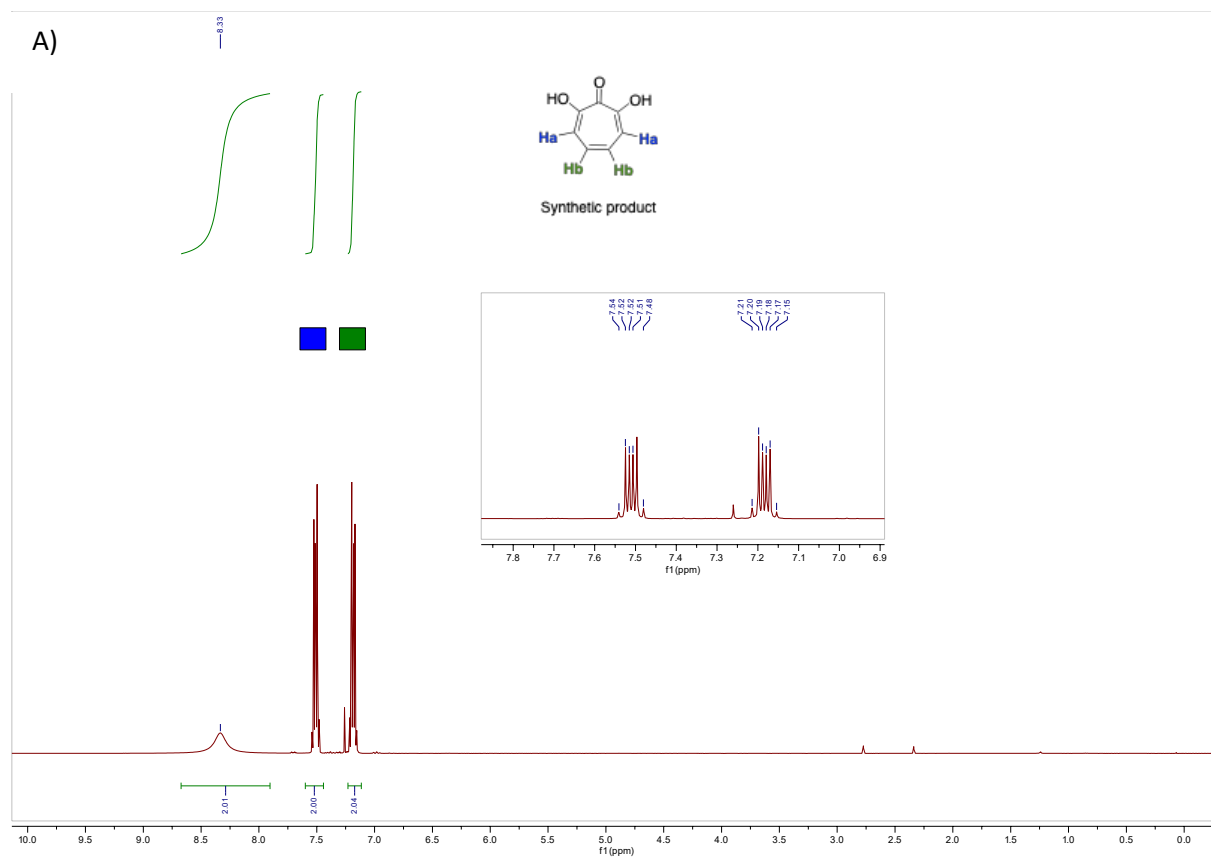


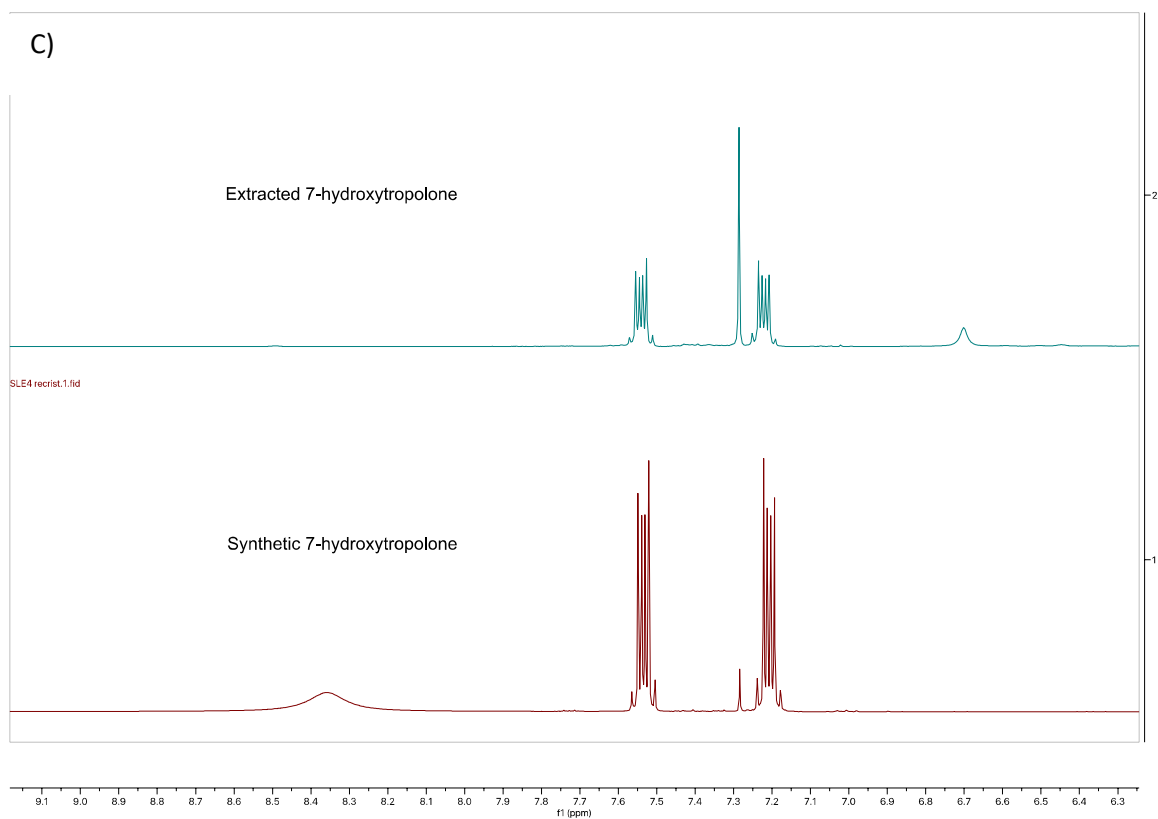
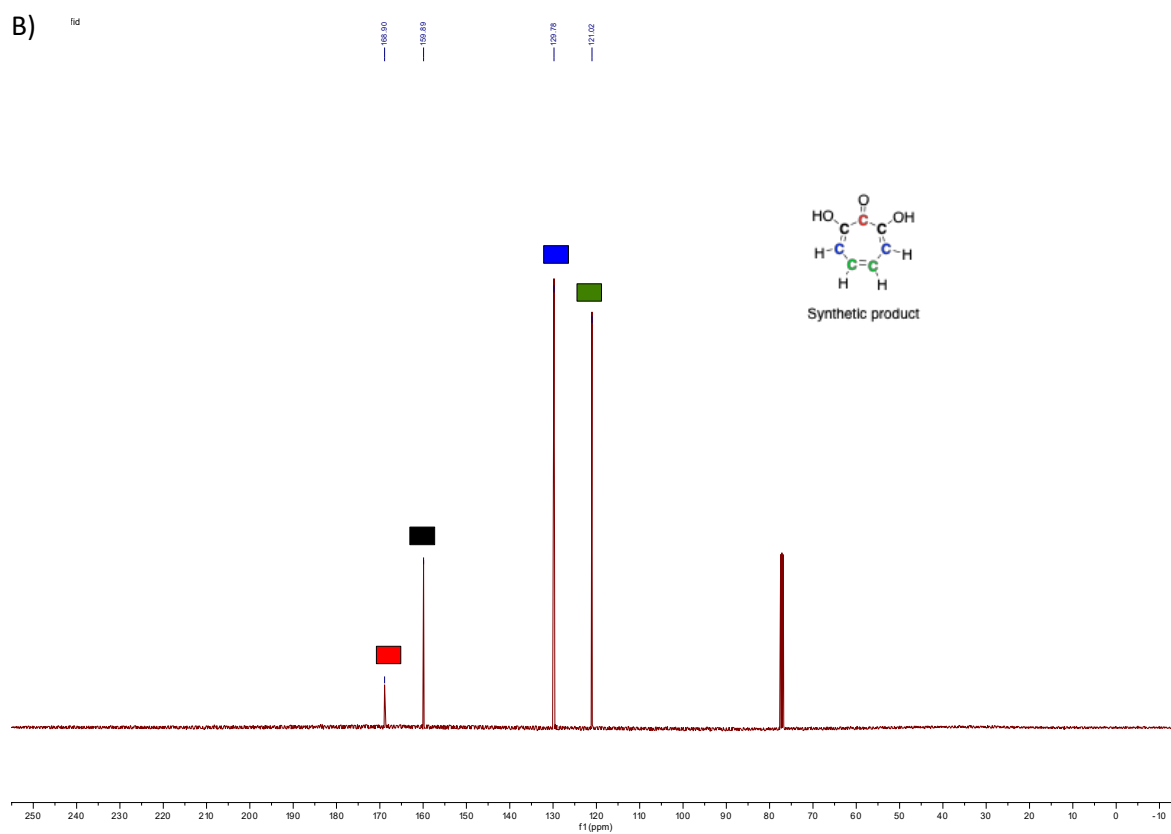
**Figure S9:** EI NIST library spectra of the different hypothesis for the molecular ion observed at  $m/z$  138. 7-HT (A) and 2-ethoxyphenol and isomers (B).

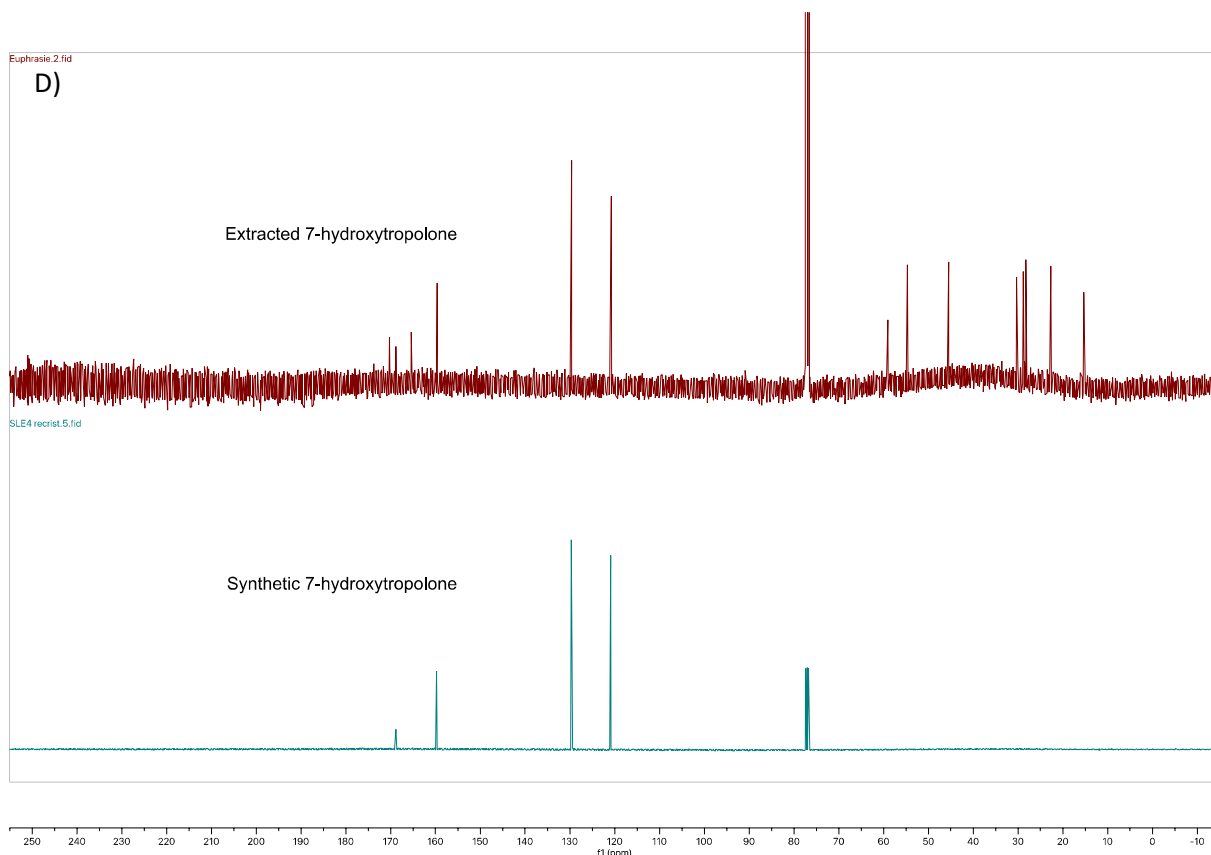




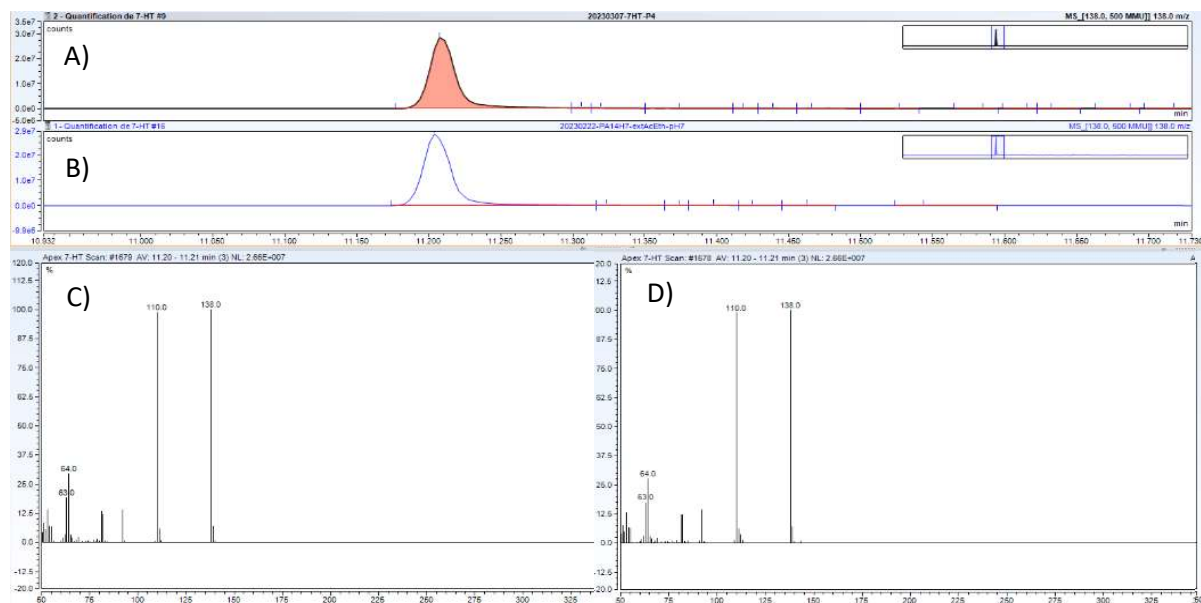
**Figure S11:** Synthesis of 7-hydroxytropolone starting from commercial tropolone. From Winter, N.; Trauner ; D. *J. Am. Chem. Soc.* **2017**, 139, 11706-11709 and Takeshita, H.; Mori, A. *Synthesis* **1985**, 578-579. The final product was recrystallized in toluene.



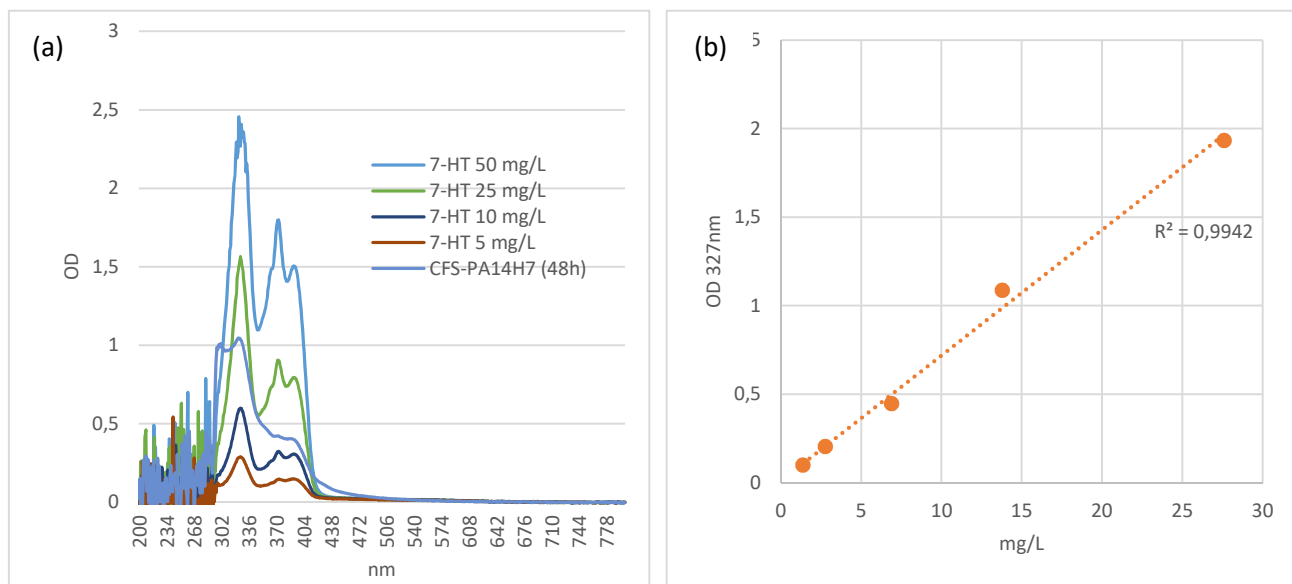
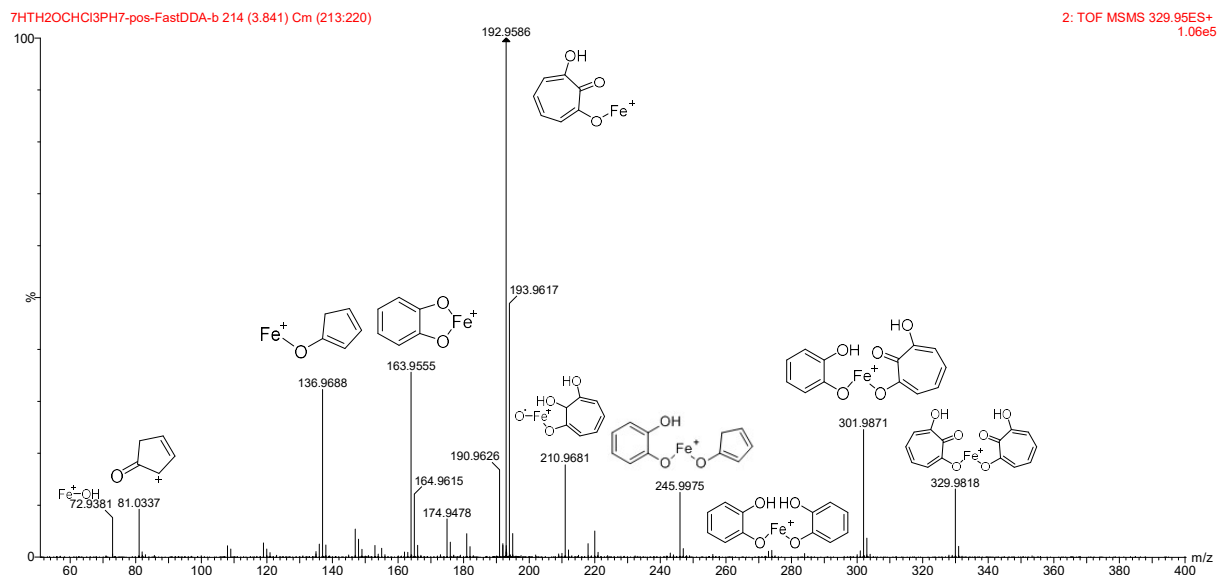


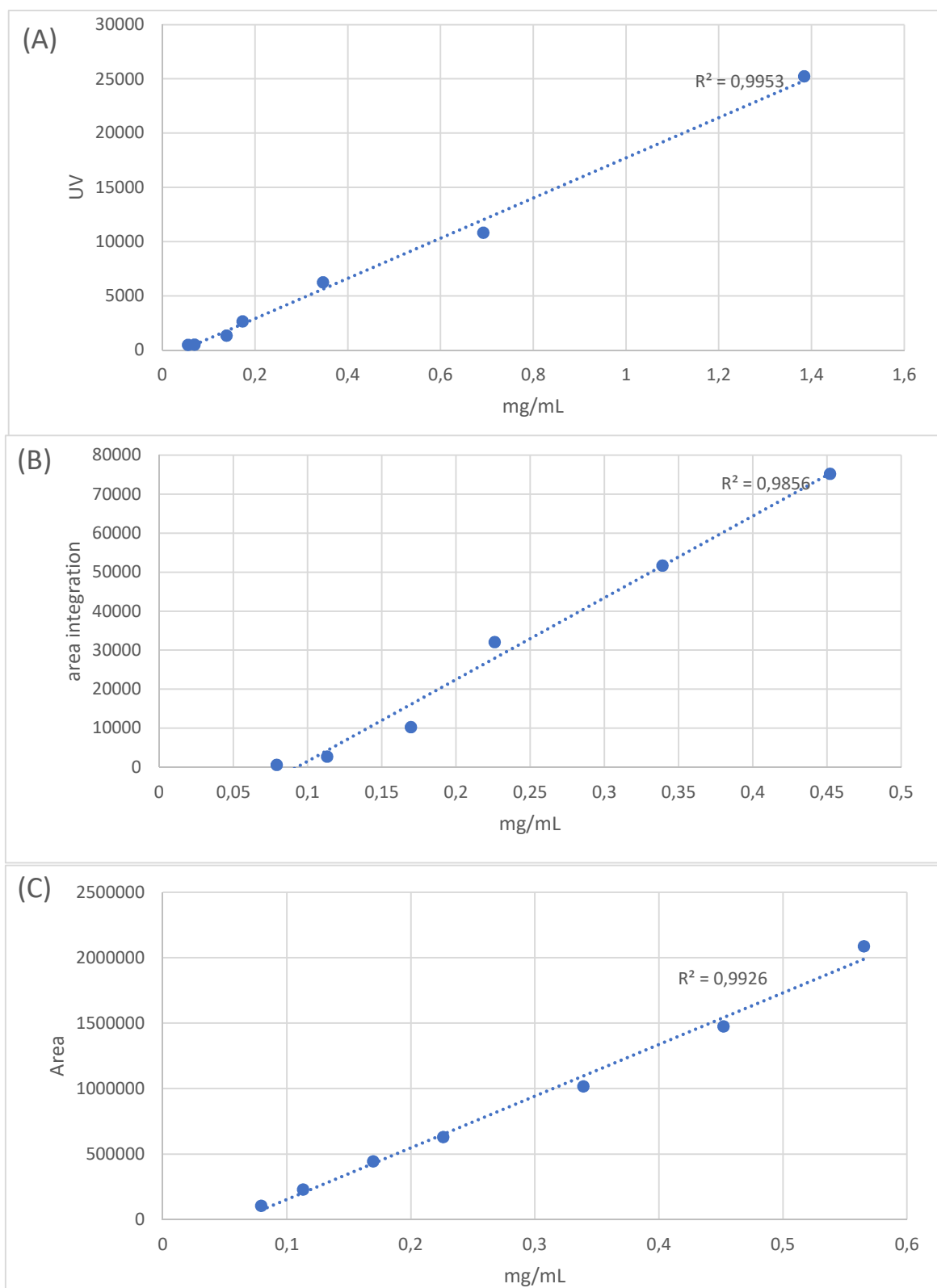


**Figure S12:** (A)  $^1\text{H}$  NMR and (B)  $^{13}\text{C}$  NMR spectra of 7-HT obtained by synthesis and (C)  $^1\text{H}$  NMR spectra and (D)  $^{13}\text{C}$  NMR spectra comparison of fraction F2 resulting from CFS-PA14H7 purification by flash chromatography (top) and synthetic 7-HT (bottom) between 6.3 and 9.1 ppm.



**Figure S13:** GC-MS chromatograms and spectra of 7-HT obtained by synthesis (A, C) and of fraction F2 resulting from CFS-PA14H7 purification by flash chromatography (B, D).





**Figure S16:** Standard curve performed with 7-HT synthetic molecule range from 0.07 mg/mL and 0.56 mg/mL according to LC-UV (320nm) (A), LC-MS (m/z 329.983) (B) and GC-MS (m/z 138) analysis (C).