

SUPPORTING INFORMATION

Metabolism of Benzalkonium Chlorides by Human Hepatic Cytochromes P450

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Table of Contents:

Page S-3:	Figure S1. MS/MS fragmentation of parent BAC compounds.
Page S-4:	Figure S2. MS/MS fragmentation of major +1O BAC microsomal metabolites.
Page S-5:	Figure S3. MS/MS fragmentation of minor +1O BAC microsomal metabolites.
Page S-6:	Figure S4. MS/MS fragmentation of major +2O BAC microsomal metabolites.
Page S-7:	Figure S5. MS/MS fragmentation of major +1O, -2H BAC microsomal metabolites.
Page S-8:	Figure S6. MS/MS fragmentation of major +1O, -2H BAC microsomal metabolites.
Page S-9:	Figure S7. MS/MS fragmentation of authentic C ₁₀ -BAC metabolite standards.
Page S-10:	Figure S8. LC-MS chromatograms of authentic C ₁₀ -BAC metabolite standards.
Page S-11:	Figure S9. LC-MS chromatograms of +1O BAC microsomal metabolites.
Page S-12:	Figure S10. LC-MS chromatograms of +2O BAC microsomal metabolites.
Page S-13:	Figure S11. LC-MS chromatograms of +1O, -2H BAC microsomal metabolites.
Page S-14:	Figure S12. LC-MS chromatograms of +2O, -2H BAC microsomal metabolites.
Page S-15:	Figure S13. LC-MS chromatograms of C ₁₀ -BAC metabolites in recombinant CYP2D6.
Page S-16:	Figure S14. LC-MS chromatograms of C ₁₀ -BAC metabolites in recombinant CYP4F12.
Page S-17:	Figure S15. LC-MS chromatograms of C ₁₆ -BAC metabolites in recombinant CYP2D6.
Page S-18:	Figure S16. LC-MS chromatograms of C ₁₆ -BAC metabolites in recombinant CYP4F12.
Page S-19:	Figure S17. LC-MS chromatograms of C ₁₆ -BAC metabolites in recombinant CYP4F2.
Page S-20:	Figure S18. ¹ H-NMR of ω-hydroxy C ₁₀ -BAC in CDCl ₃ .

- Page S-21:** **Figure S19.** $^1\text{H-NMR}$ of ω -alkene C_{10} -BAC in CDCl_3 .
- Page S-22:** **Figure S20.** $^1\text{H-NMR}$ of (ω -1)-hydroxy C_{10} -BAC in CDCl_3 .
- Page S-23:** **Figure S21.** $^1\text{H-NMR}$ of (ω , ω -1)-dihydroxy C_{10} -BAC in CDCl_3 .
- Page S-24:** **Figure S22.** $^1\text{H-NMR}$ of (ω -1)-ketone C_{10} -BAC in CDCl_3 .
- Page S-25:** **Figure S23.** $^1\text{H-NMR}$ of ω -carboxylic acid C_{10} -BAC in CDCl_3 .
- Page S-26:** **Figure S24.** $^1\text{H-NMR}$ of ω -carboxylic acid C_{10} -BAC in d_6 -DMSO.

Figure S1. Representative MS/MS fragmentation spectra (25 eV collision energy) of (A) C₁₀-BAC, (B) C₁₂-BAC, (C) C₁₄-BAC, and (D) C₁₆-BAC are shown to demonstrate consistent loss of and detection of the unmodified benzyl cation fragment (theoretical: 91.0548 *m/z*). Parent BAC structures and retention times (*t_R*) are provided.

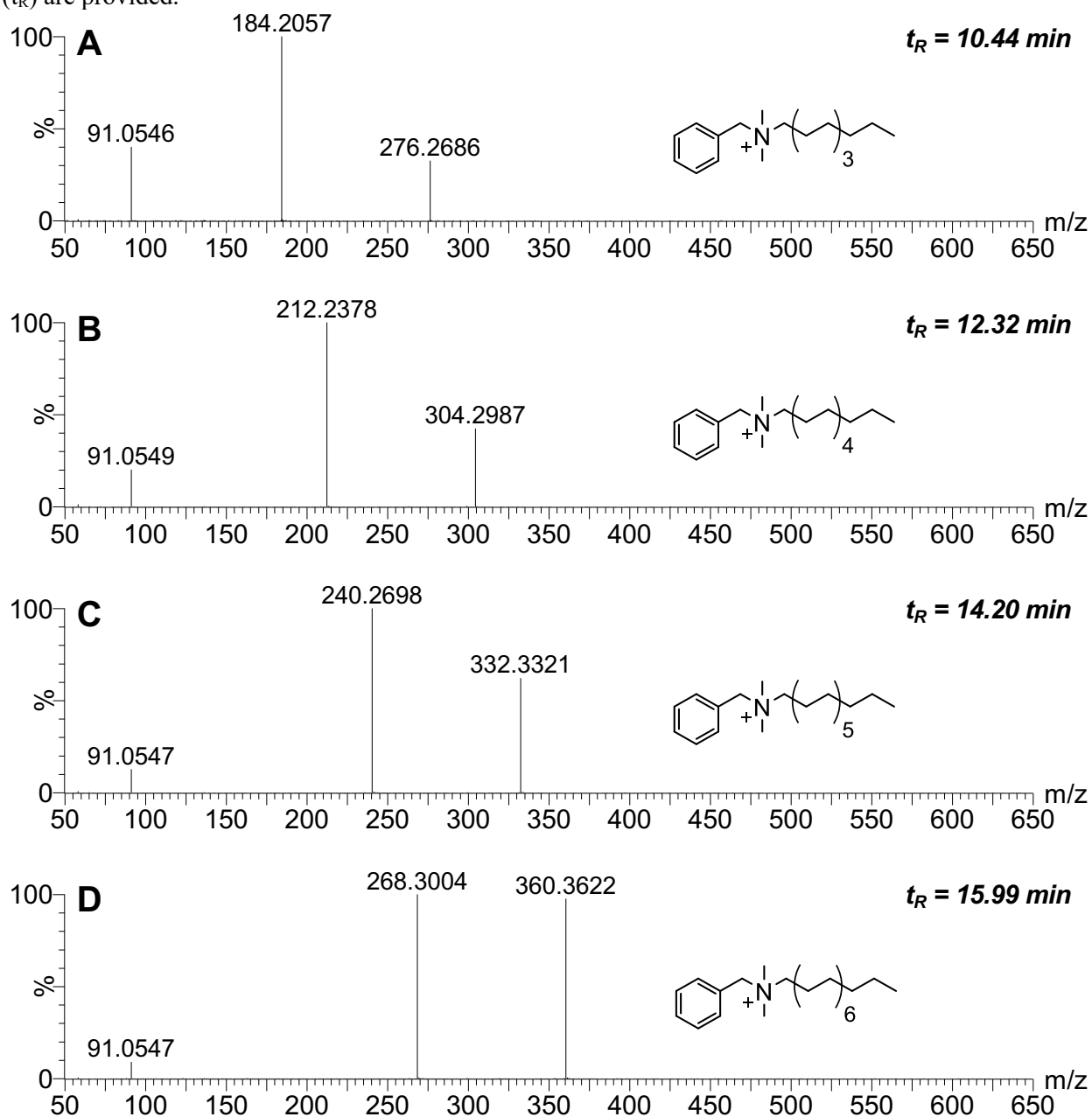


Figure S2. Representative MS/MS fragmentation spectra (25 eV collision energy) of the major +1O BAC metabolites (A) C₁₀-BAC+1O, (B) C₁₂-BAC+1O, (C) C₁₄-BAC+1O, and (D) C₁₆-BAC+1O produced by NADPH-dependent metabolism in HLM are shown to demonstrate consistent loss of and detection of the unmodified benzyl cation fragment (theoretical: 91.0548 *m/z*). Analyte retention times (*t_R*) and proposed metabolite structures are provided.

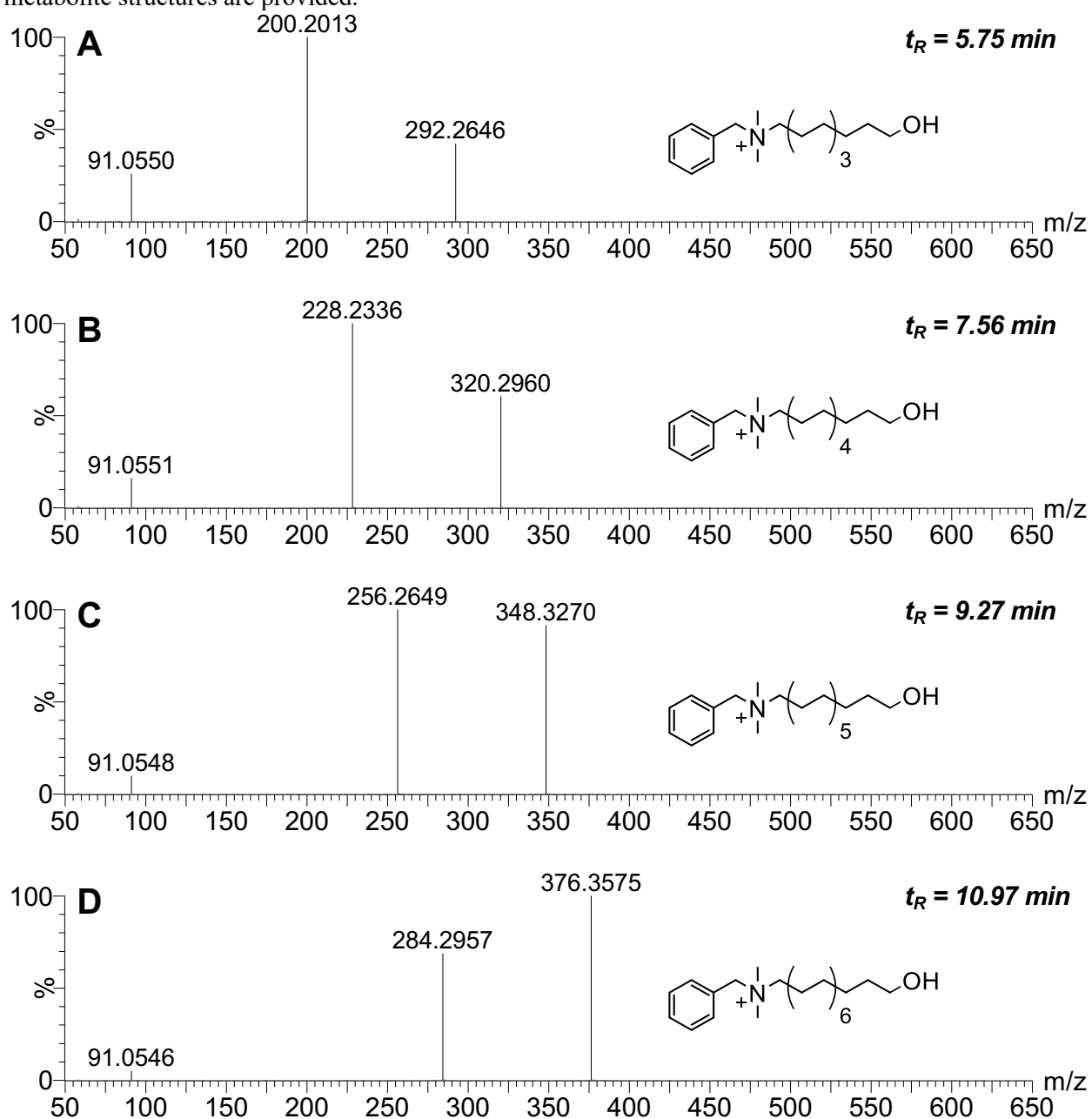


Figure S3. Representative MS/MS fragmentation spectra (25 eV collision energy) of the minor +1O BAC metabolites (A) C₁₀-BAC+1O, (B) C₁₂-BAC+1O, (C) C₁₄-BAC+1O, and (D) C₁₆-BAC+1O produced by NADPH-dependent metabolism in HLM are shown to demonstrate consistent loss of and detection of the unmodified benzyl cation fragment (theoretical: 91.0548 *m/z*). Analyte retention times (*t_R*) and proposed metabolite structures are provided.

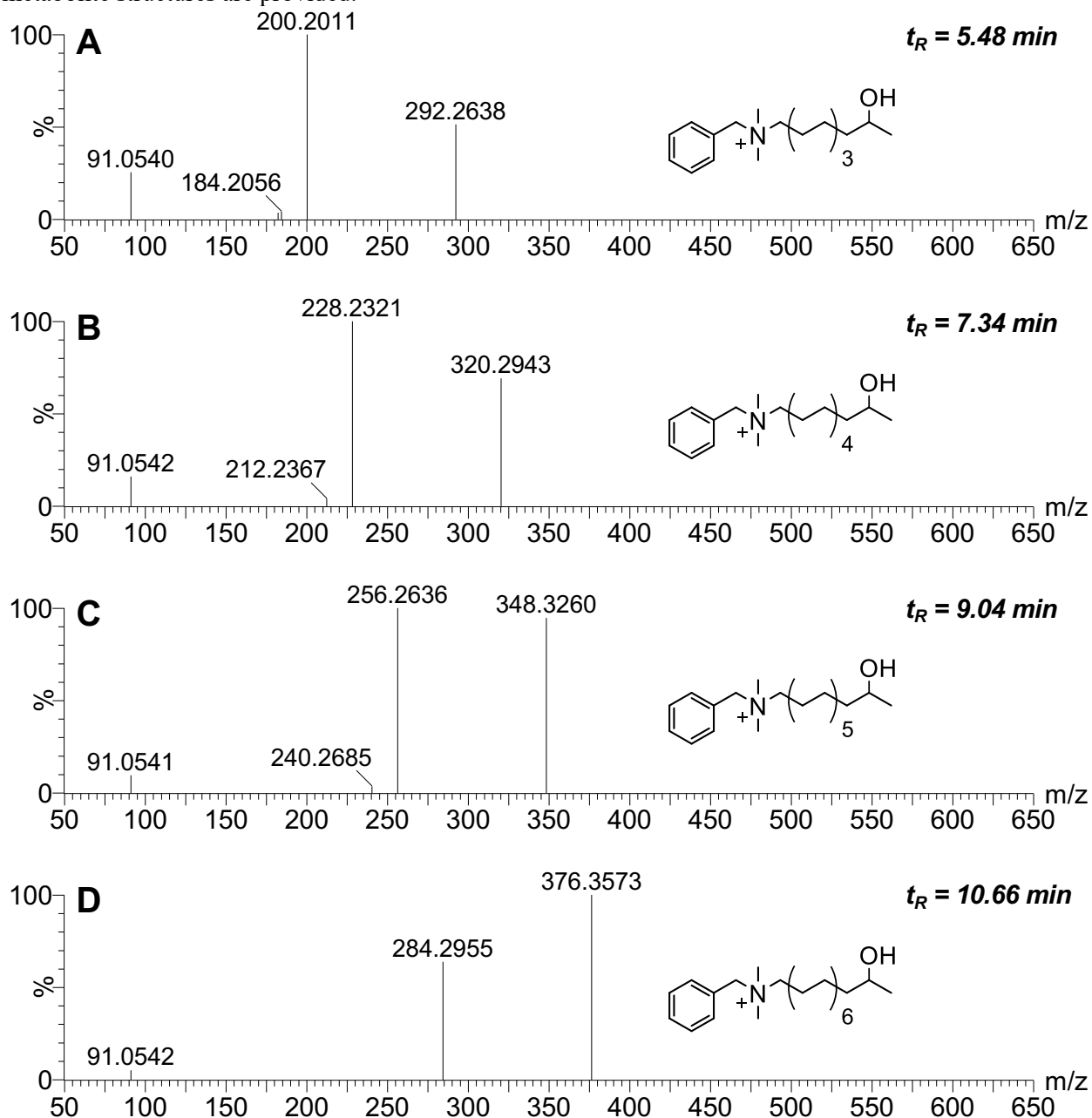


Figure S4. Representative MS/MS fragmentation spectra (25 eV collision energy) of the major +2O BAC metabolites (A) C₁₀-BAC+2O, (B) C₁₂-BAC+2O, (C) C₁₄-BAC+2O, and (D) C₁₆-BAC+2O produced by NADPH-dependent metabolism in HLM are shown to demonstrate consistent loss of and detection of the unmodified benzyl cation fragment (theoretical: 91.0548 *m/z*). Analyte retention times (*t_R*) and proposed metabolite structures are provided.

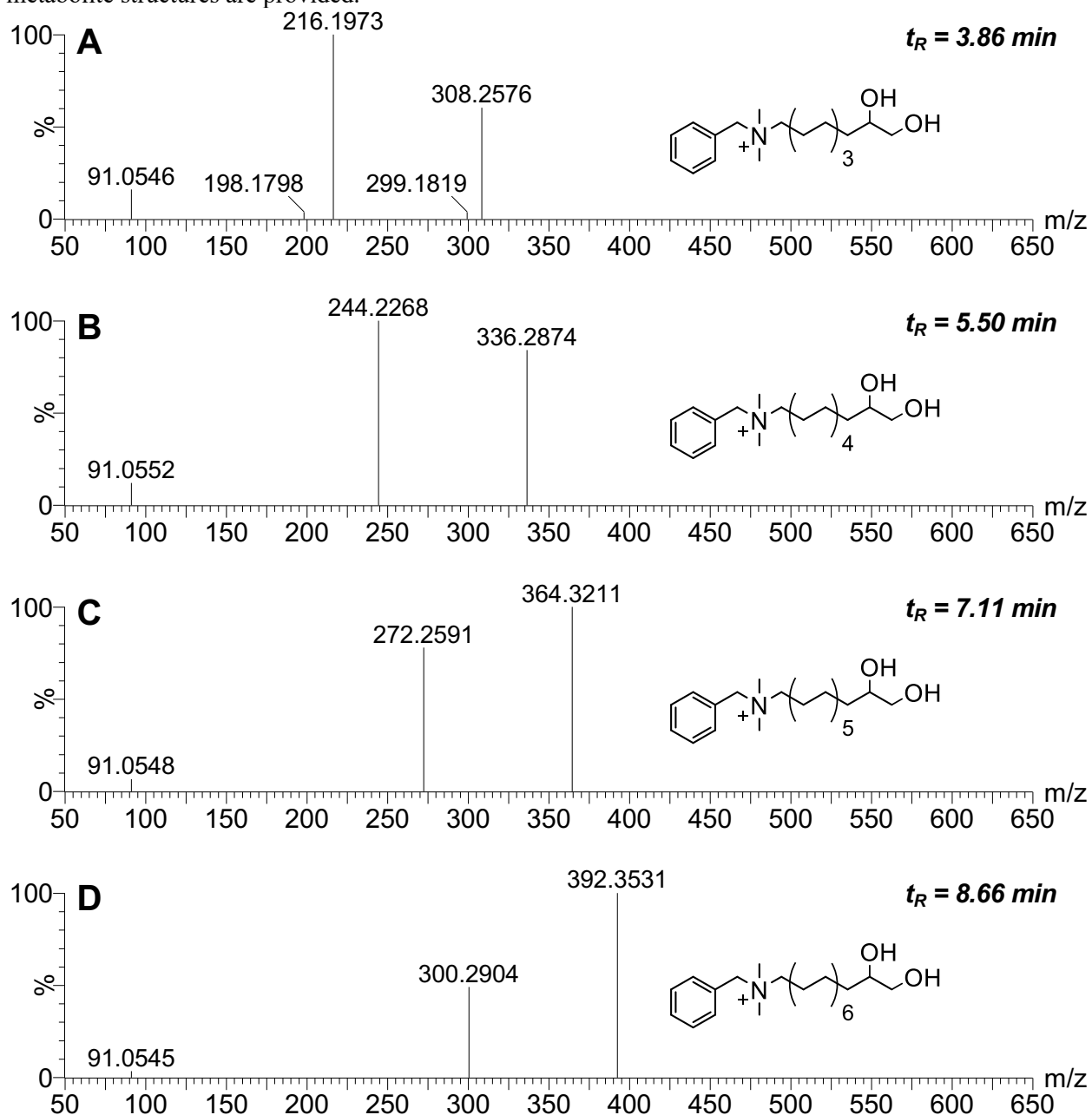


Figure S5. Representative MS/MS fragmentation spectra (25 eV collision energy) of the major +1O, -2H BAC metabolites (A) C₁₀-BAC+1O-2H, (B) C₁₂-BAC+1O-2H, (C) C₁₄-BAC+1O-2H, and (D) C₁₆-BAC+1O-2H produced by NADPH-dependent metabolism in HLM are shown to demonstrate consistent loss of and detection of the unmodified benzyl cation fragment (theoretical: 91.0548 *m/z*). Analyte retention times (*t_R*) and proposed metabolite structures are provided.

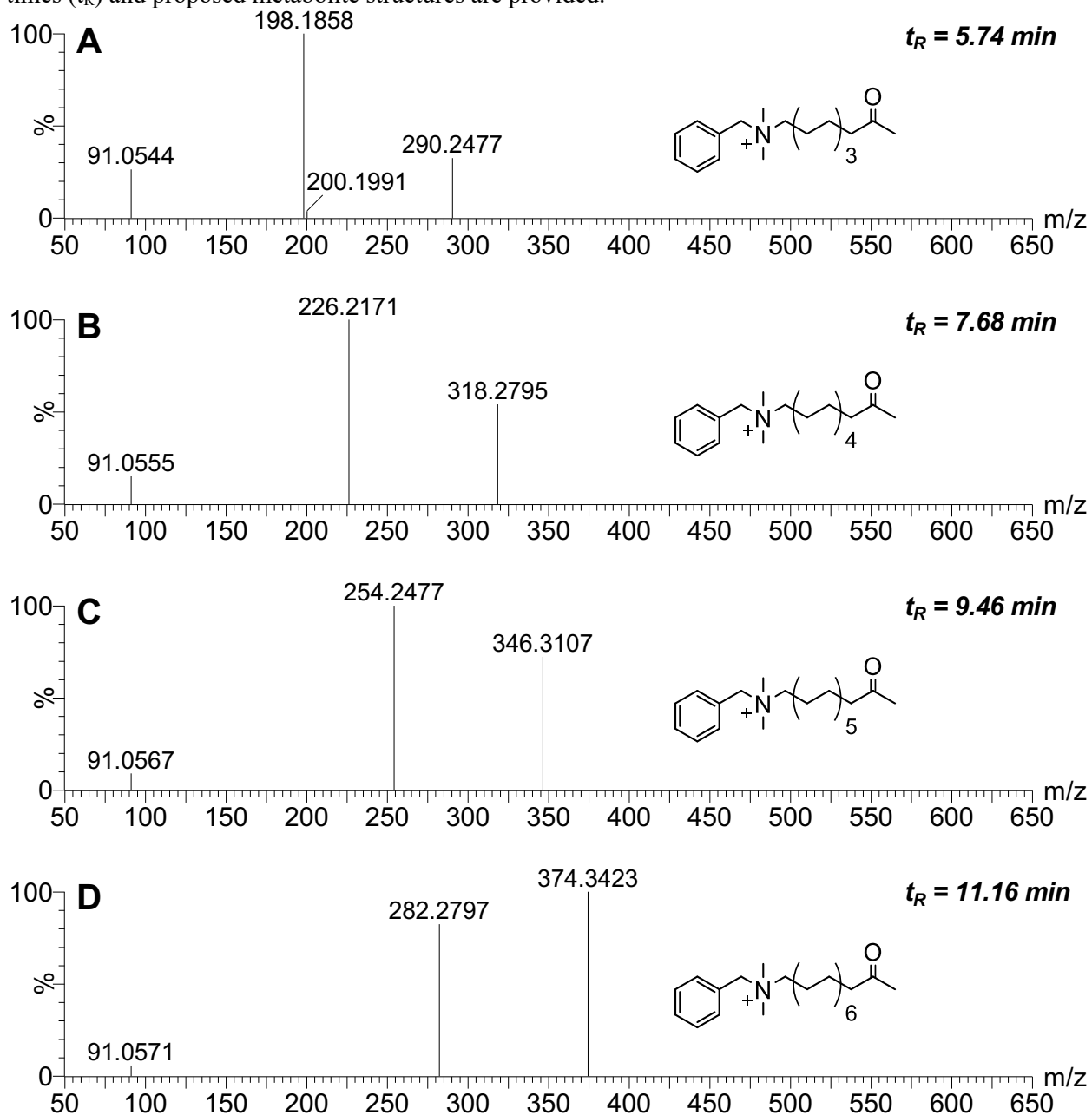


Figure S6. Representative MS/MS fragmentation spectra (25 eV collision energy) of the major +2O, -2H BAC metabolites (A) C₁₀-BAC+2O-2H, (B) C₁₂-BAC+2O-2H, (C) C₁₄-BAC+2O-2H, and (D) C₁₆-BAC+2O-2H produced by NADPH-dependent metabolism in HLM are shown to demonstrate consistent loss of and detection of the unmodified benzyl cation fragment (theoretical: 91.0548 *m/z*). Analyte retention times (*t_R*) and proposed metabolite structures are provided.

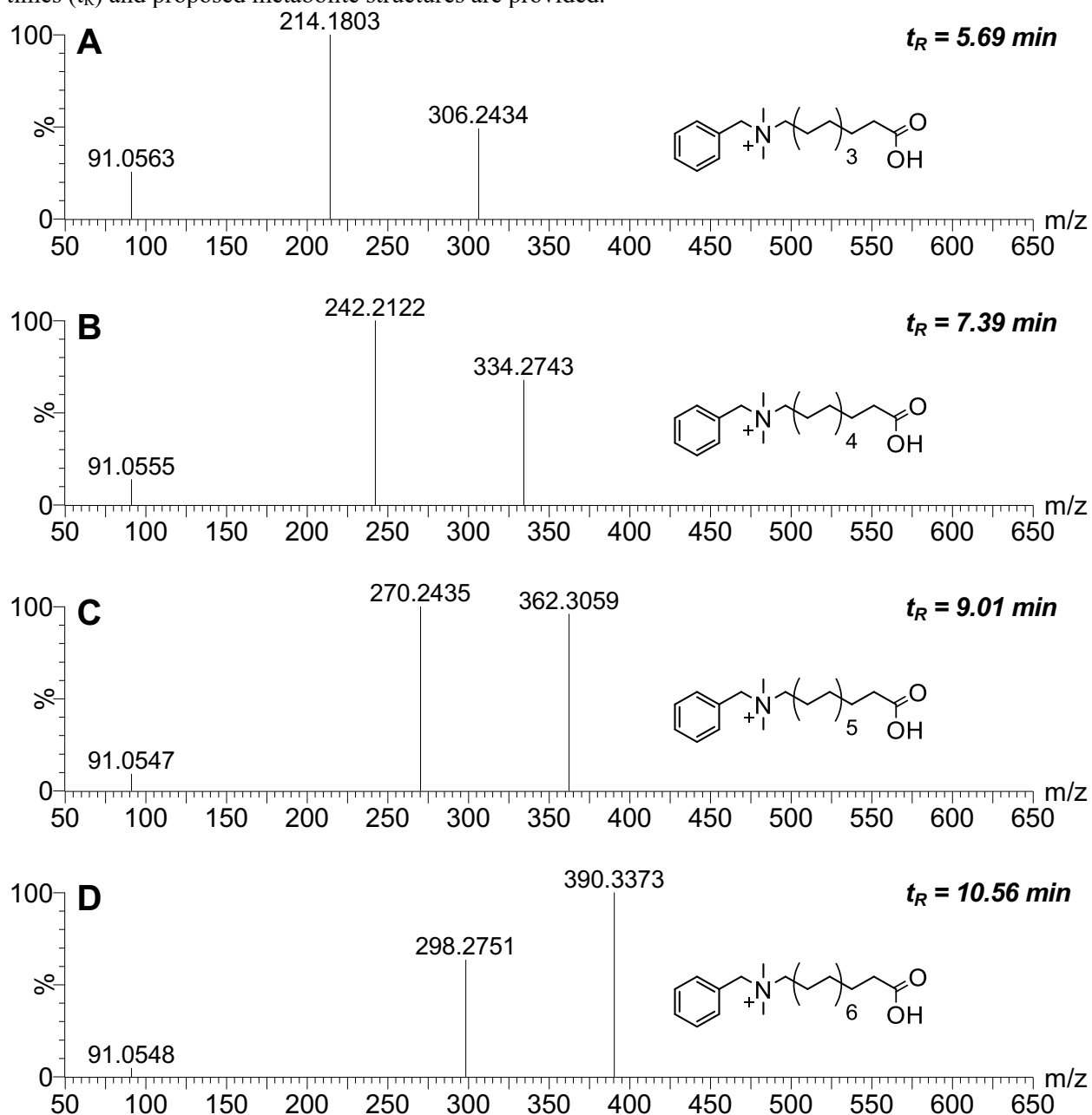


Figure S7. Representative MS/MS fragmentation spectra (25 eV collision energy) of authentic C₁₀-BAC metabolite standards synthesized in-house (A) (ω-1)-hydroxy C₁₀-BAC, (B) ω-hydroxy C₁₀-BAC, (C) (ω, ω-1)-dihydroxy C₁₀-BAC, (D) (ω-1)-ketone C₁₀-BAC, and (E) ω-carboxylic acid C₁₀-BAC are shown to demonstrate consistent loss of and detection of the unmodified benzyl cation fragment (theoretical: 91.0548 m/z). Analyte retention times (*t_R*) and the chemical structures (confirmed by ¹H-NMR) are provided.

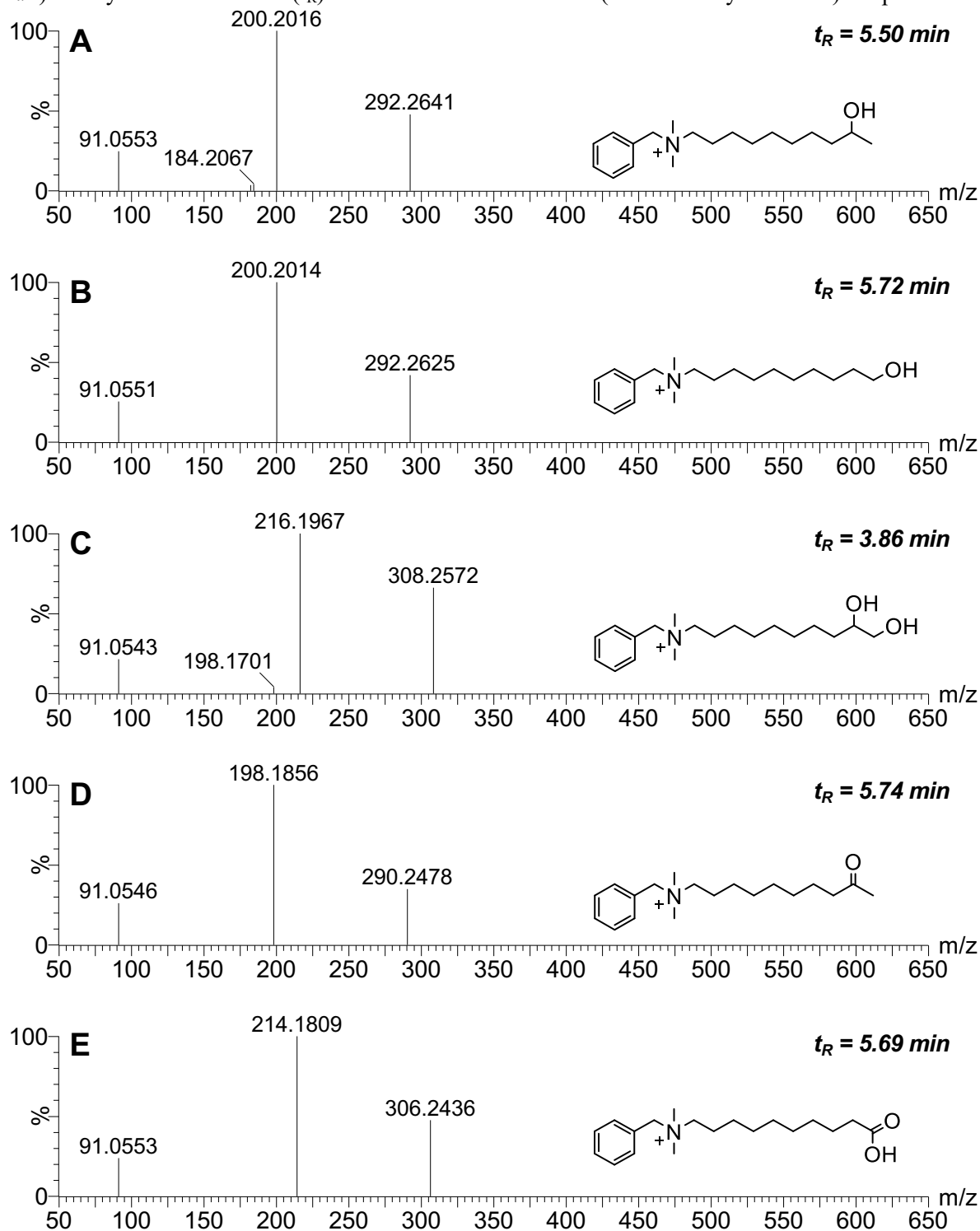


Figure S8. LC-MS chromatograms of authentic C₁₀-BAC metabolite standards synthesized in-house (A) (ω-1)-hydroxy C₁₀-BAC, (B) ω-hydroxy C₁₀-BAC, (C) (ω, ω-1)-dihydroxy C₁₀-BAC, (D) (ω-1)-ketone C₁₀-BAC, and (E) ω-carboxylic acid C₁₀-BAC are shown. Analyte peaks are labeled with retention times. The mass (*m/z*) filtered (± 0.005 *m/z*) and maximum peak height are also provided. In Panels A and D, respectively, (ω-2)-hydroxy C₁₀-BAC and (ω-2)-ketone C₁₀-BAC are noted as minor impurities.

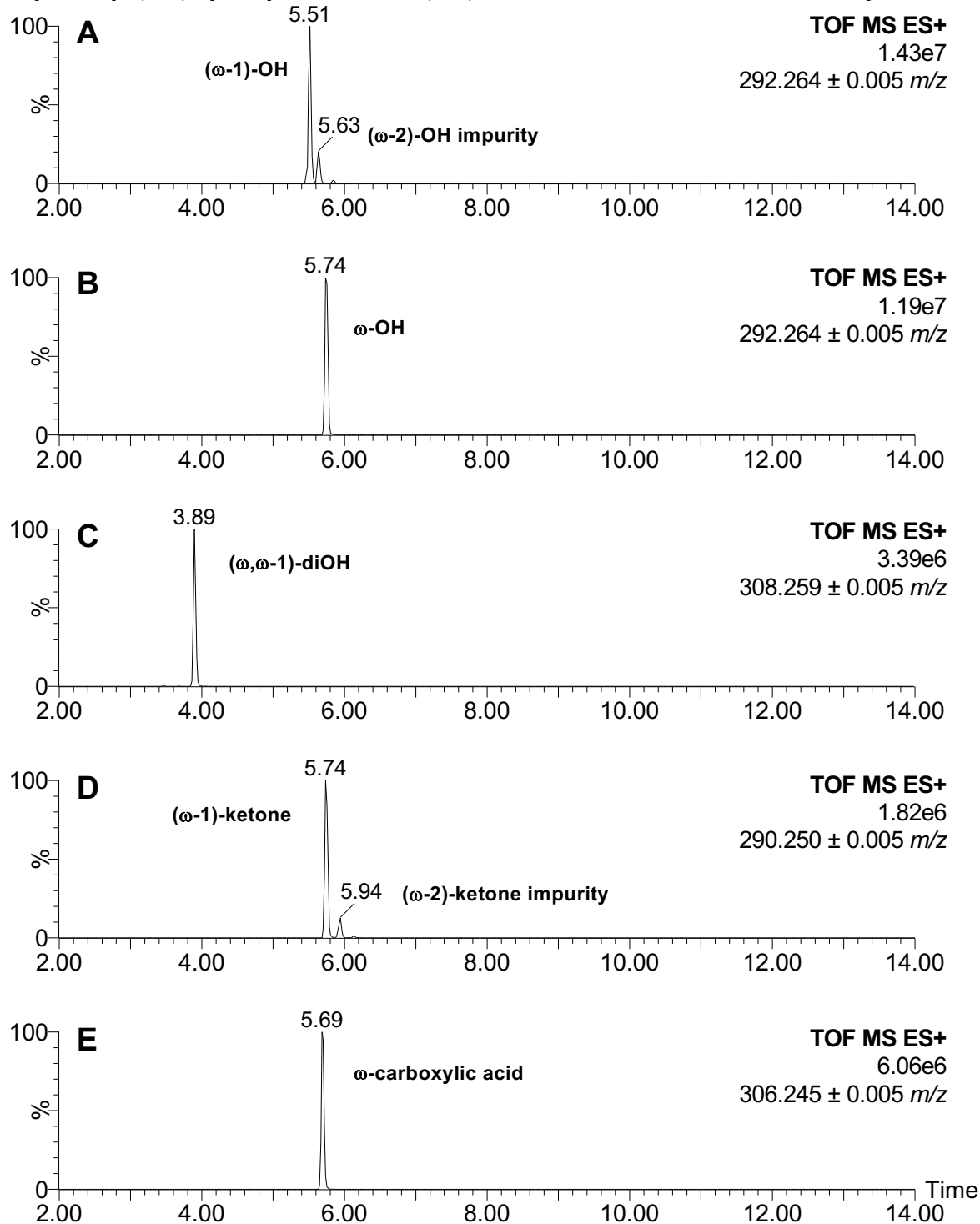


Figure S9. LC-MS chromatograms displaying +10 BAC metabolites of the substrates (A) C₁₀-BAC, (B) C₁₂-BAC, (C) C₁₄-BAC, and (D) C₁₆-BAC produced by NADPH-dependent metabolism in HLM are shown. Analyte peaks are labeled with retention times. The mass (*m/z*) filtered (± 0.005 *m/z*) and maximum peak height are also provided.

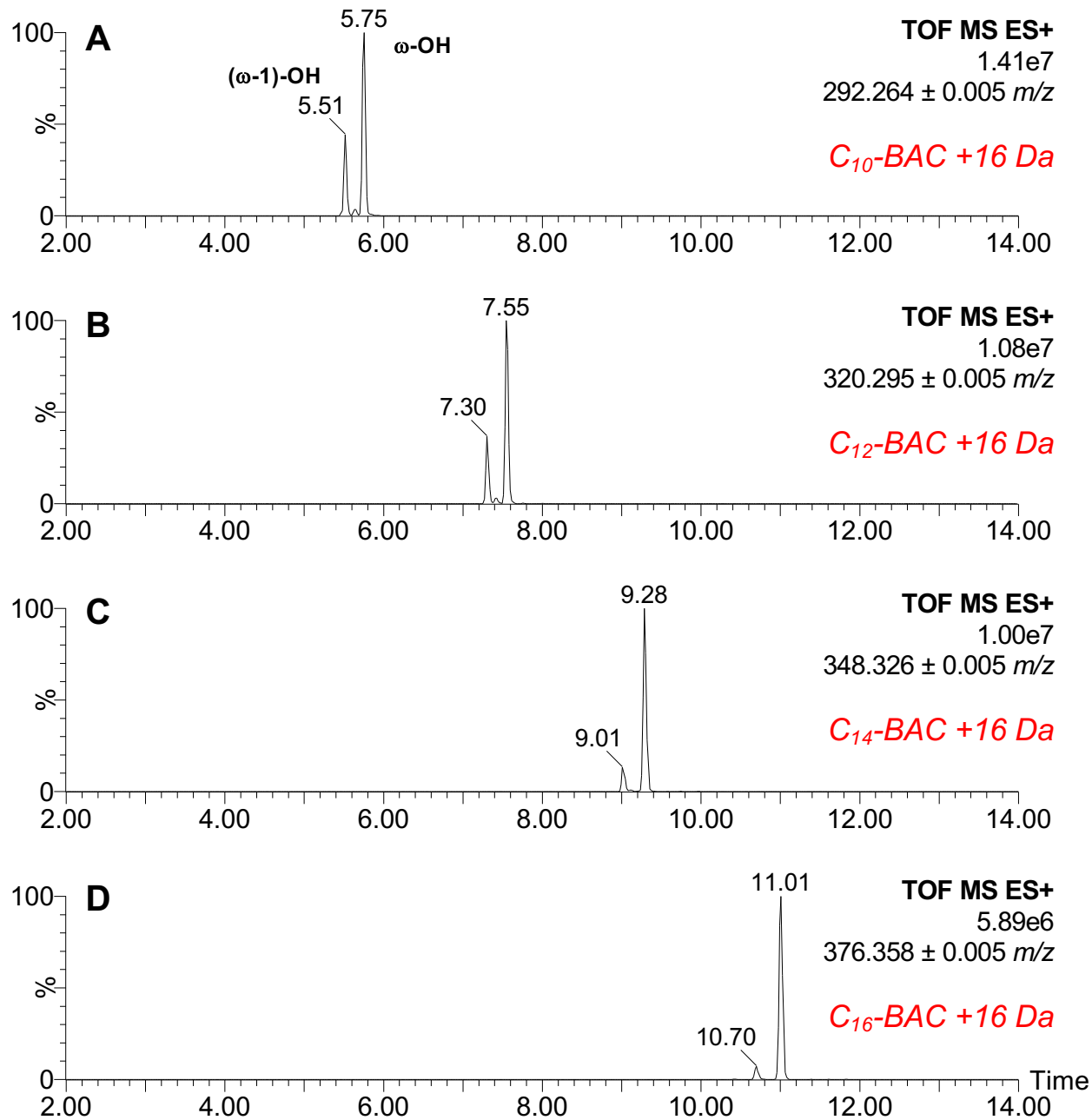


Figure S10. LC-MS chromatograms displaying +20 BAC metabolites of the substrates (A) C₁₀-BAC, (B) C₁₂-BAC, (C) C₁₄-BAC, and (D) C₁₆-BAC produced by NADPH-dependent metabolism in HLM are shown. Analyte peaks are labeled with retention times. The mass (*m/z*) filtered (± 0.005 *m/z*) and maximum peak height are also provided.

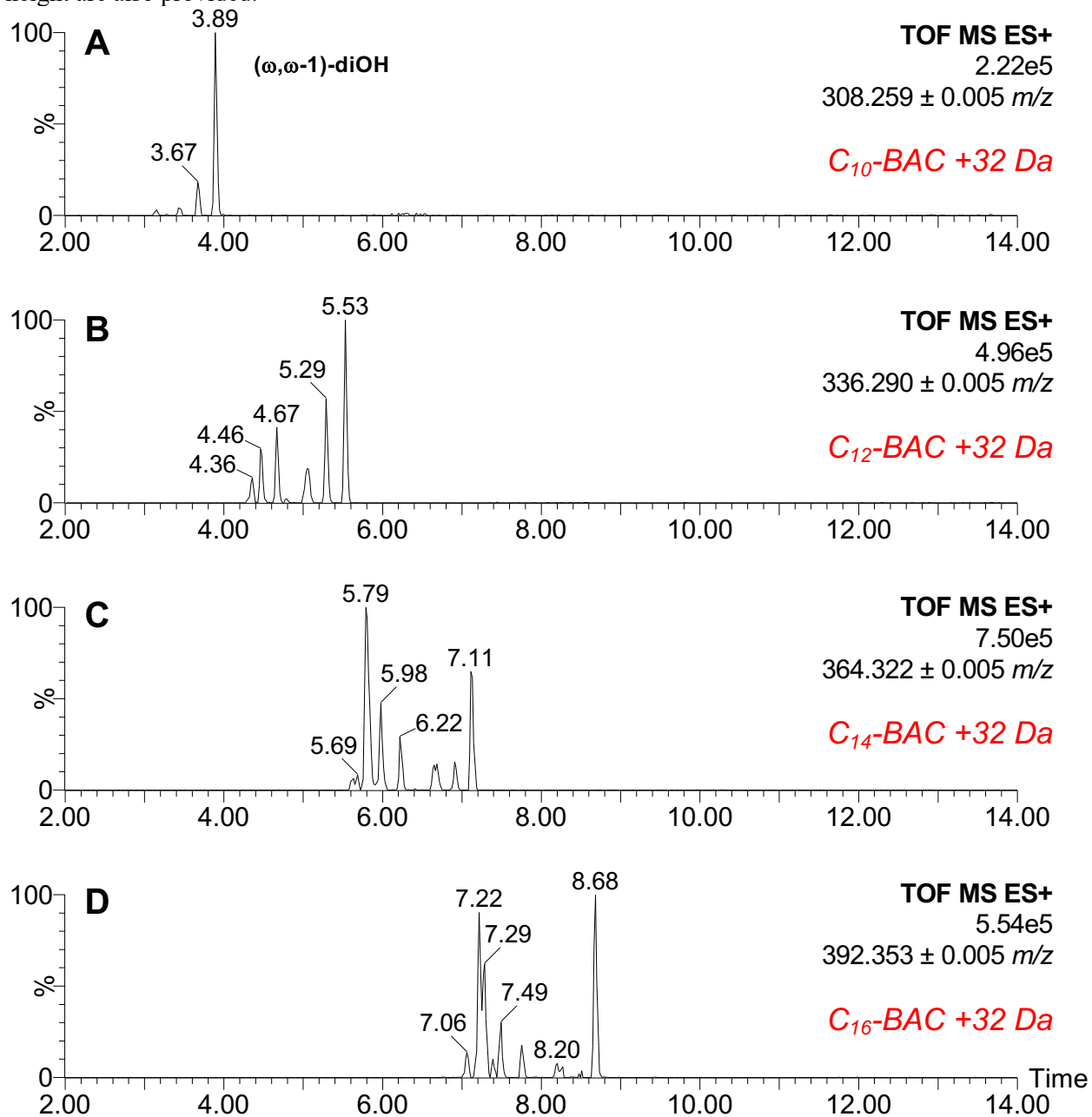


Figure S11. LC-MS chromatograms displaying +1O, -2H BAC metabolites of the substrates (A) C₁₀-BAC, (B) C₁₂-BAC, (C) C₁₄-BAC, and (D) C₁₆-BAC produced by NADPH-dependent metabolism in HLM are shown. Analyte peaks are labeled with retention times. The mass (*m/z*) filtered (± 0.005 *m/z*) and maximum peak height are also provided.

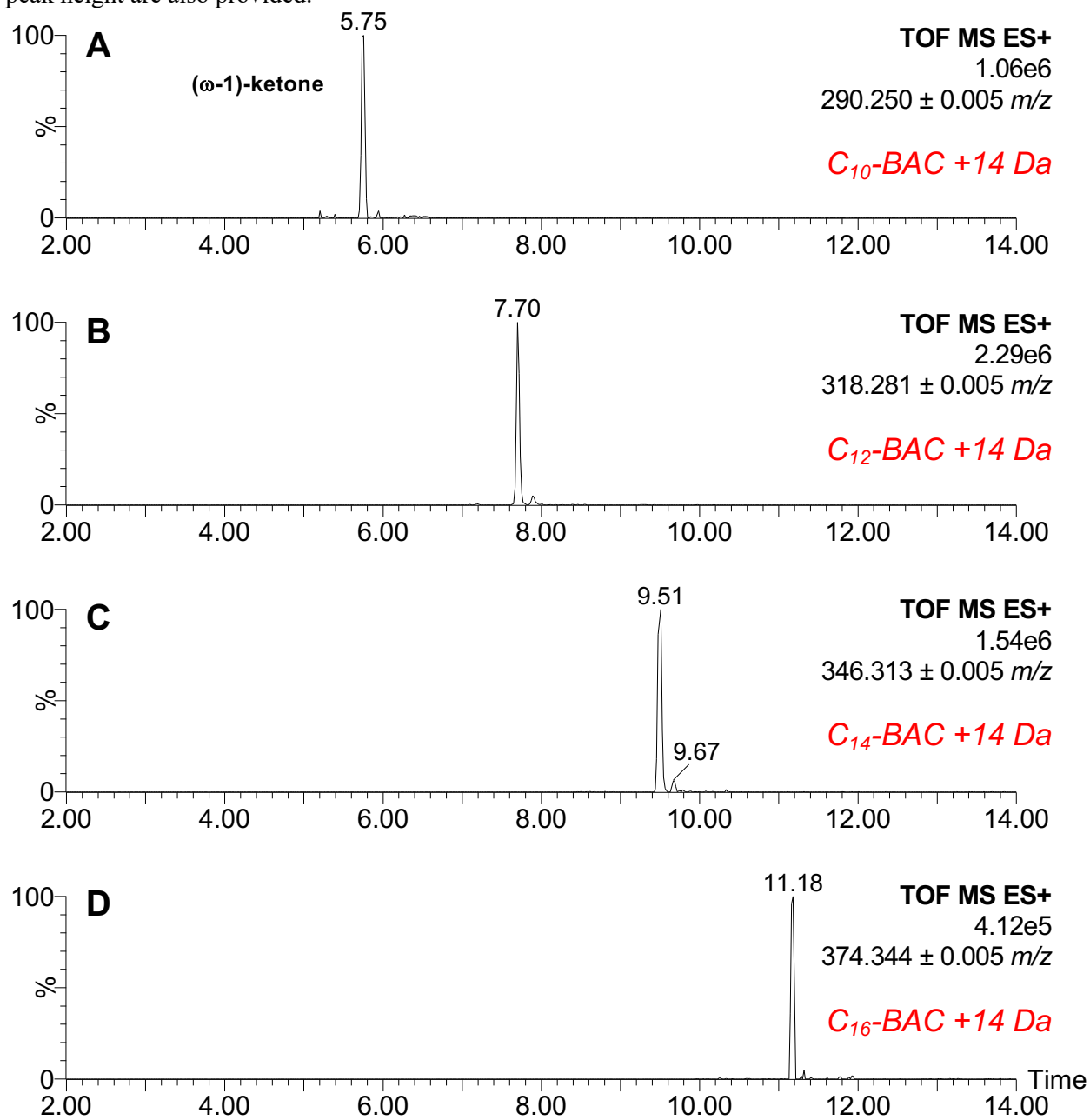


Figure S12. LC-MS chromatograms displaying +2O, -2H BAC metabolites of the substrates (A) C₁₀-BAC, (B) C₁₂-BAC, (C) C₁₄-BAC, and (D) C₁₆-BAC produced by NADPH-dependent metabolism in HLM are shown. Analyte peaks are labeled with retention times. The mass (*m/z*) filtered (± 0.005 *m/z*) and maximum peak height are also provided.

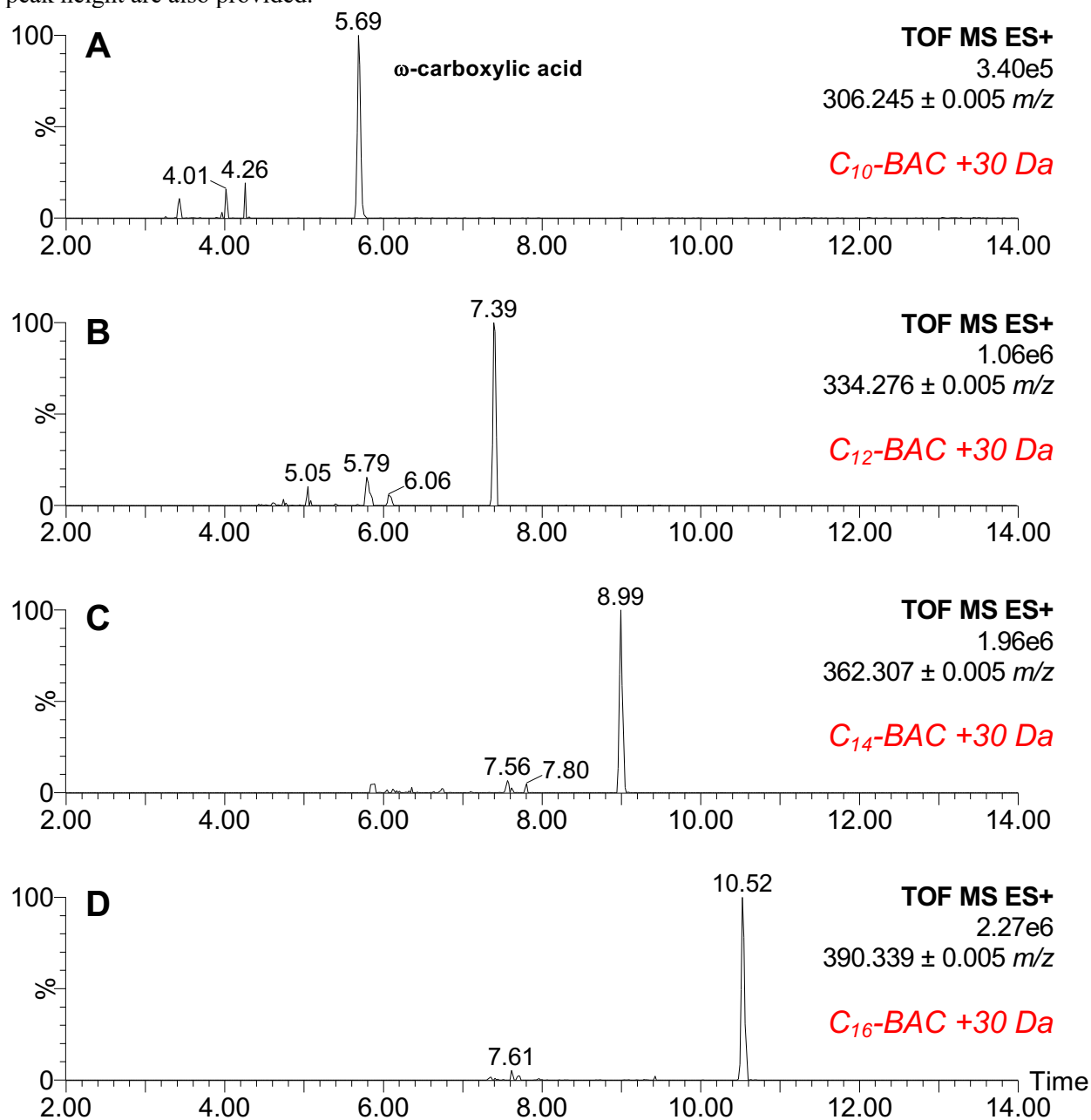


Figure S13. LC-MS chromatograms displaying (A) +1O (B) +2O (C) +1O, -2H, and (D) +2O, -2H metabolites of C₁₀-BAC produced by NADPH-dependent metabolism in recombinant CYP2D6 are shown. Analyte peaks are labeled with retention times. The mass (*m/z*) filtered (± 0.005 *m/z*) and maximum peak height are also provided.

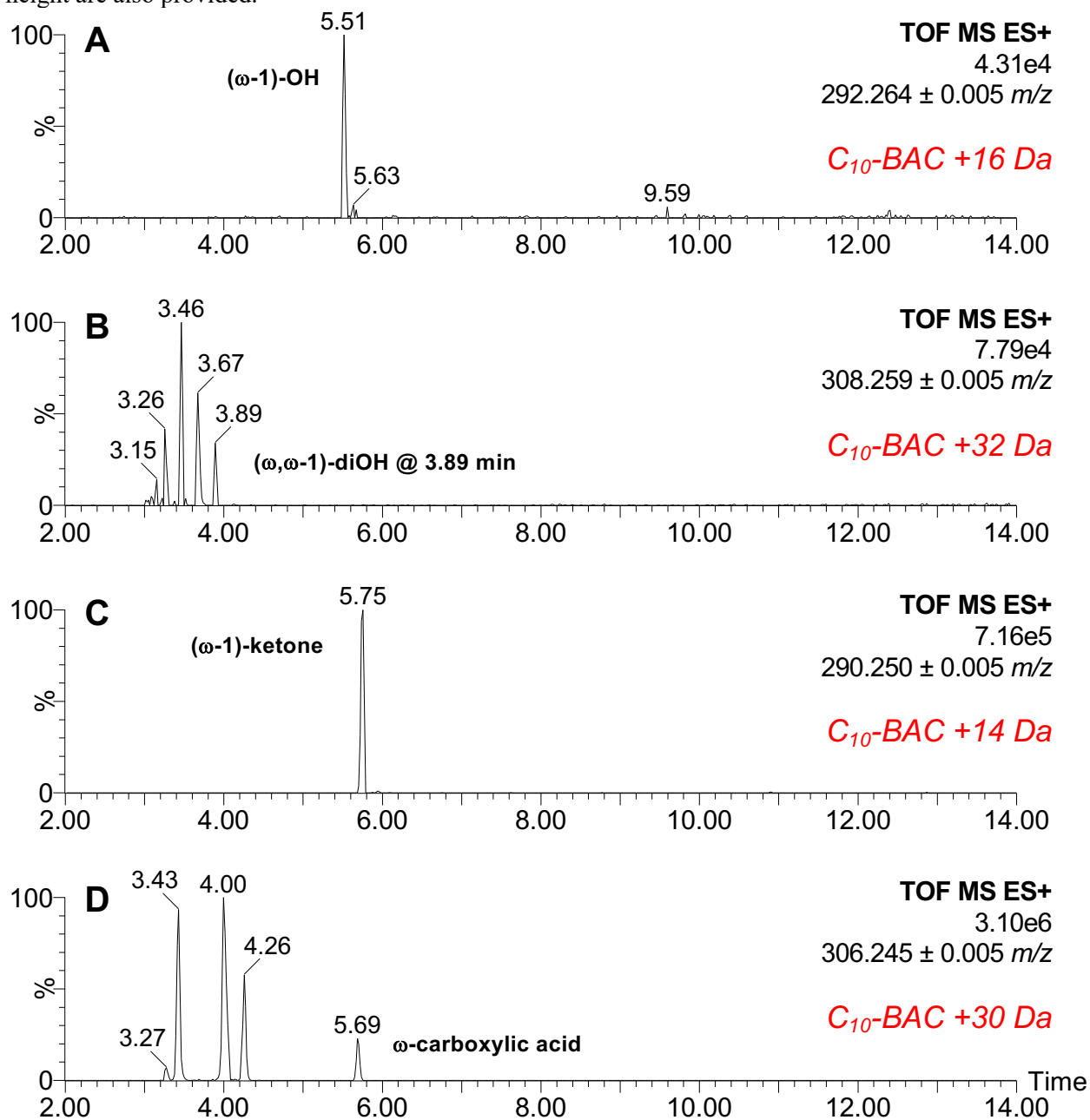


Figure S14. LC-MS chromatograms displaying (A) +1O (B) +2O (C) +1O, -2H, and (D) +2O, -2H metabolites of C₁₀-BAC produced by NADPH-dependent metabolism in recombinant CYP4F12 are shown. Analyte peaks are labeled with retention times. The mass (*m/z*) filtered (± 0.005 *m/z*) and maximum peak height are also provided.

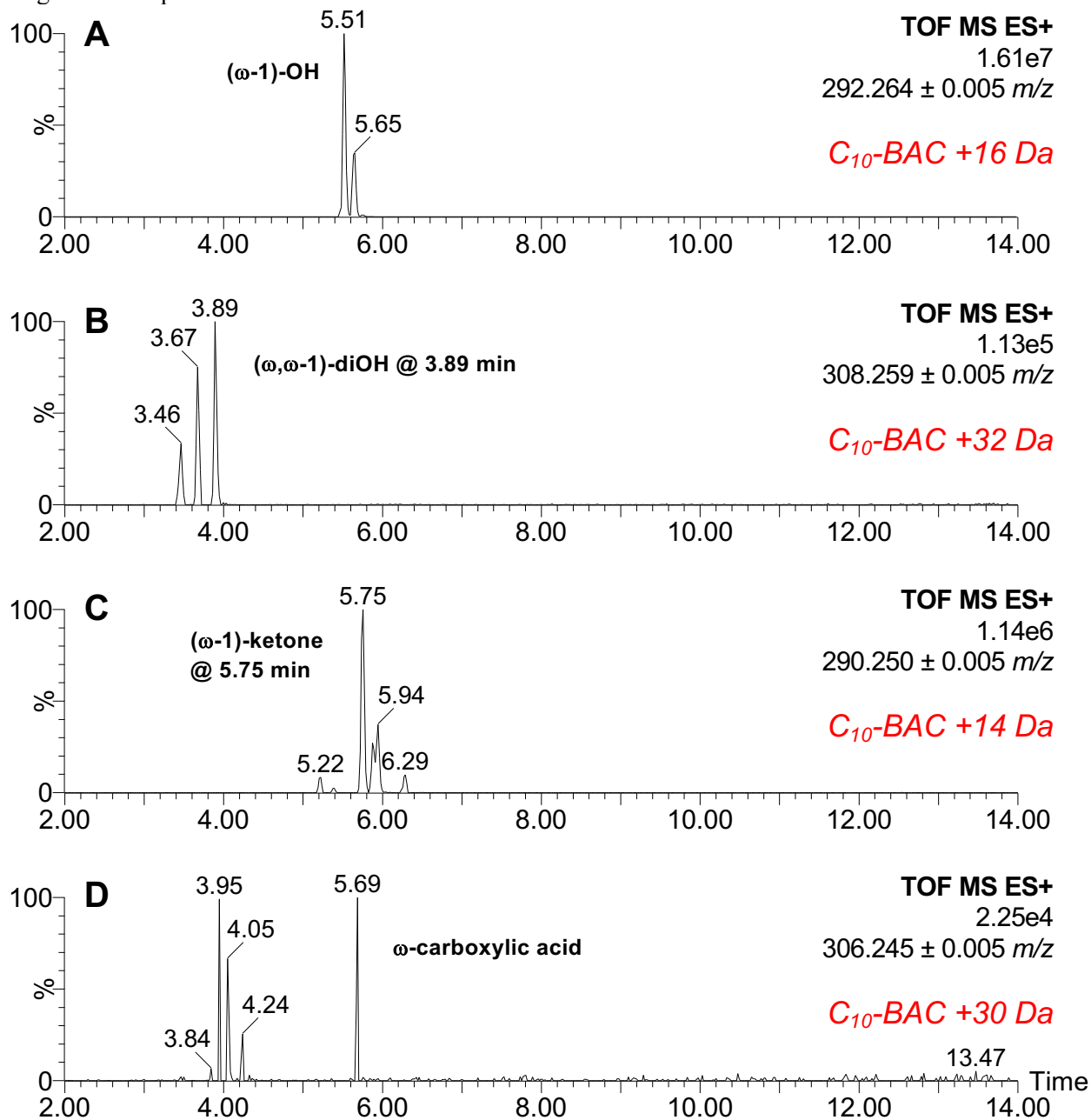


Figure S15. LC-MS chromatograms displaying (A) +1O (B) +2O (C) +1O, -2H, and (D) +2O, -2H metabolites of C₁₆-BAC produced by NADPH-dependent metabolism in recombinant CYP2D6 are shown. Analyte peaks are labeled with retention times. The mass (*m/z*) filtered (± 0.005 *m/z*) and maximum peak height are also provided.

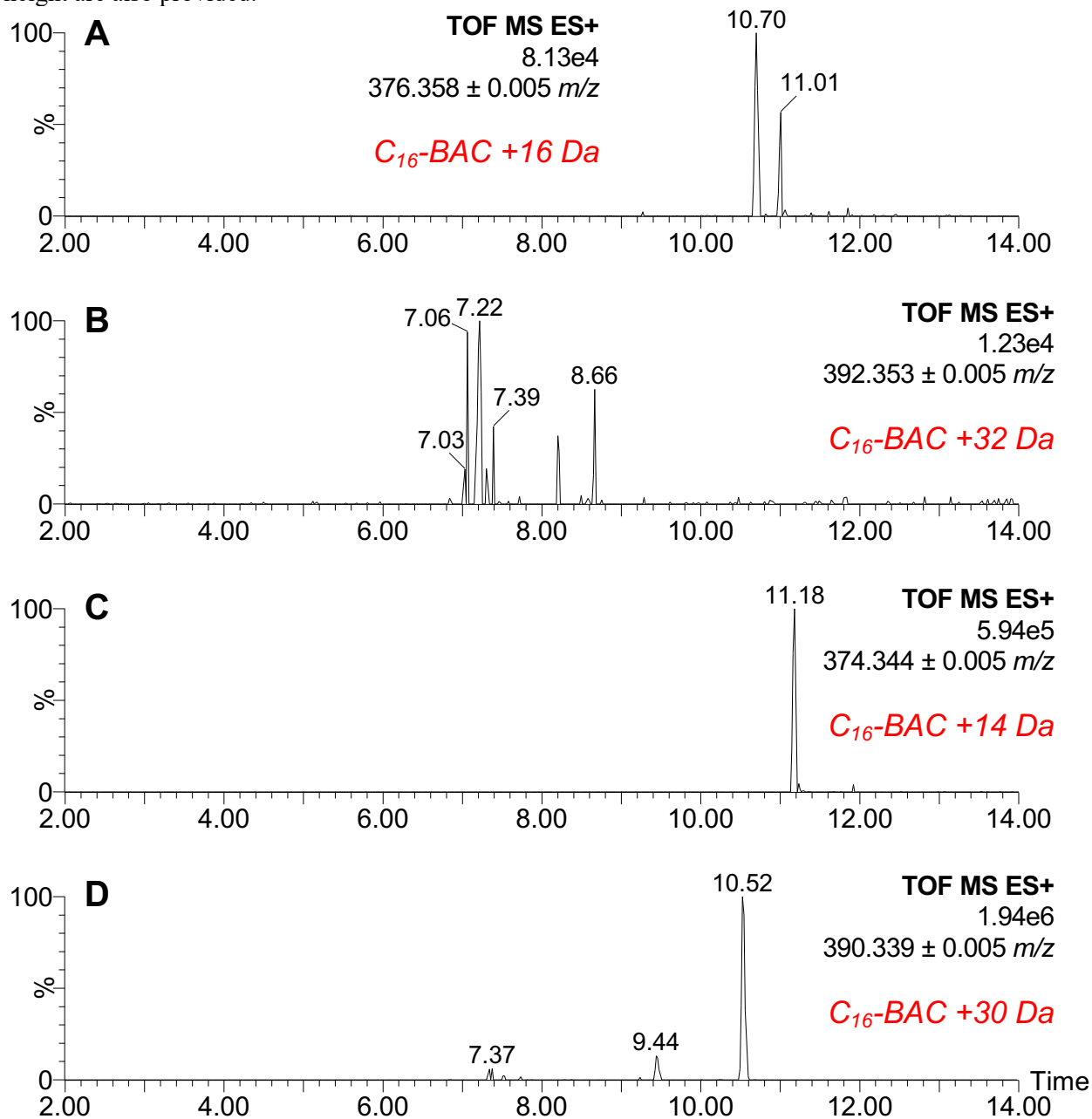


Figure S16. LC-MS chromatograms displaying (A) +1O (B) +2O (C) +1O, -2H, and (D) +2O, -2H metabolites of C₁₆-BAC produced by NADPH-dependent metabolism in recombinant CYP4F12 are shown. Analyte peaks are labeled with retention times. The mass (*m/z*) filtered (± 0.005 *m/z*) and maximum peak height are also provided.

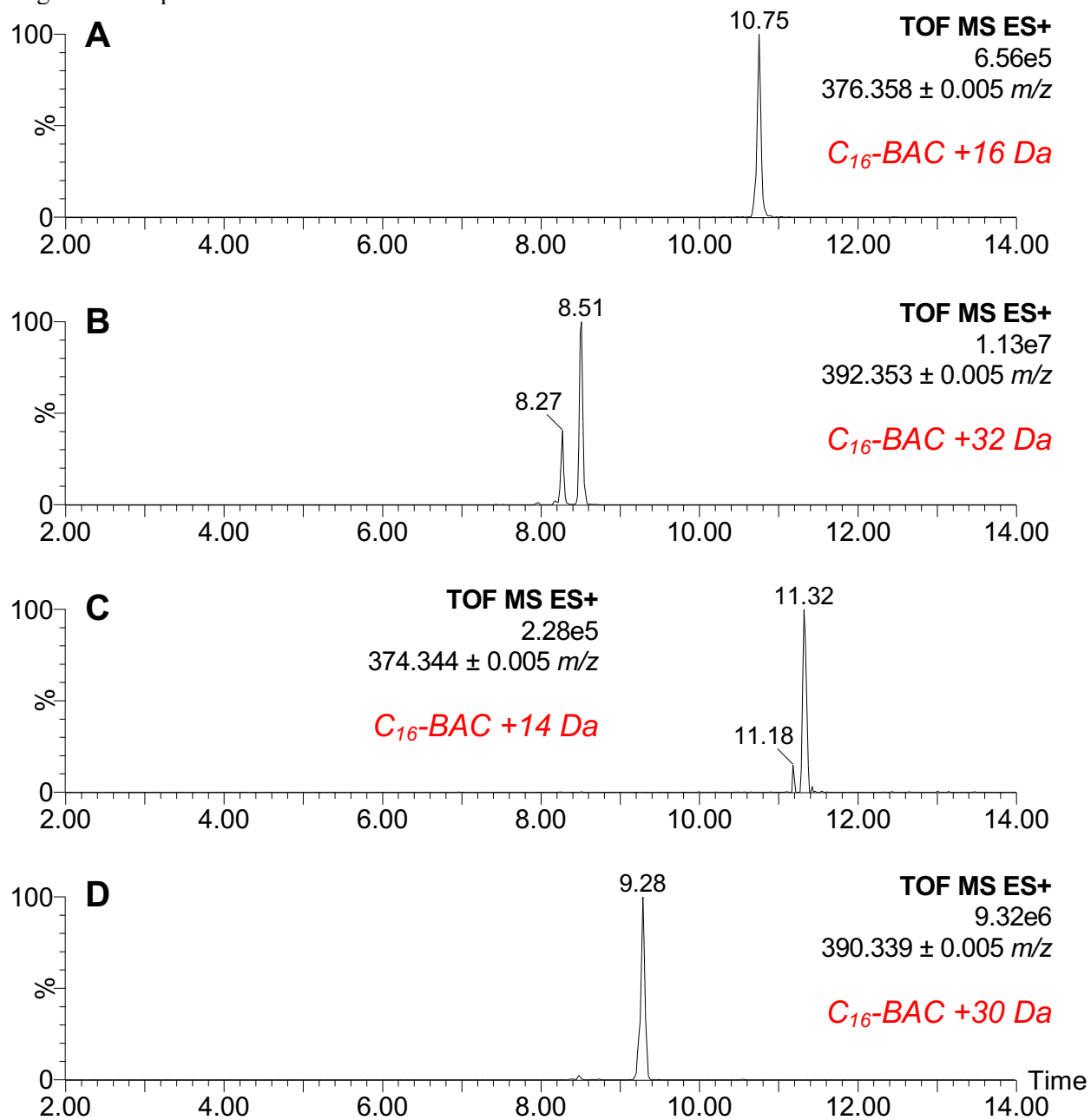


Figure S17. LC-MS chromatograms displaying (A) +1O (B) +2O (C) +1O, -2H, and (D) +2O, -2H metabolites of C₁₆-BAC produced by NADPH-dependent metabolism in recombinant CYP4F2 are shown. Analyte peaks are labeled with retention times. The mass (*m/z*) filtered (± 0.005 *m/z*) and maximum peak height are also provided.

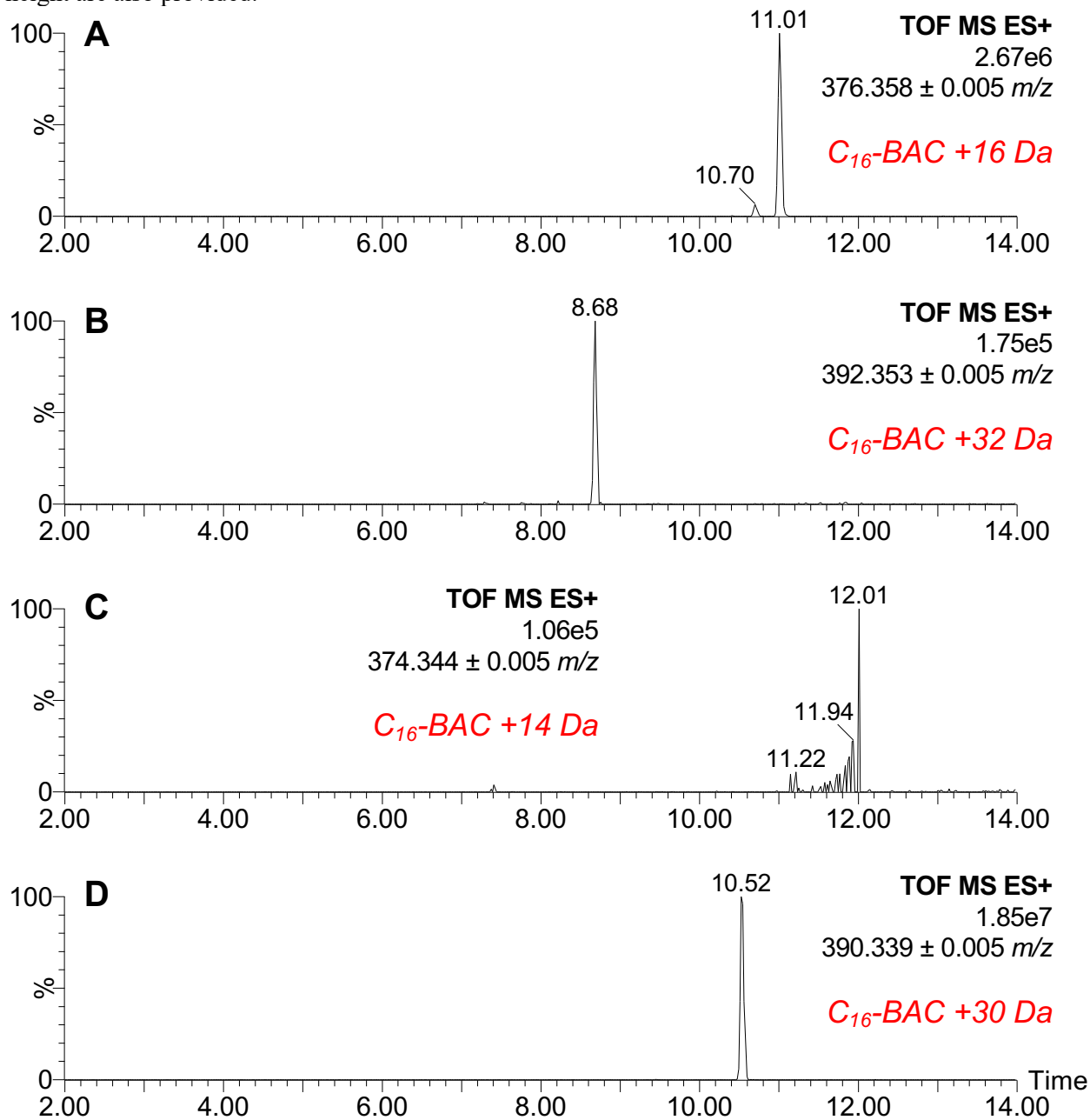


Figure S18. $^1\text{H-NMR}$ spectrum of ω -hydroxy- C_{10} -BAC in CDCl_3 .

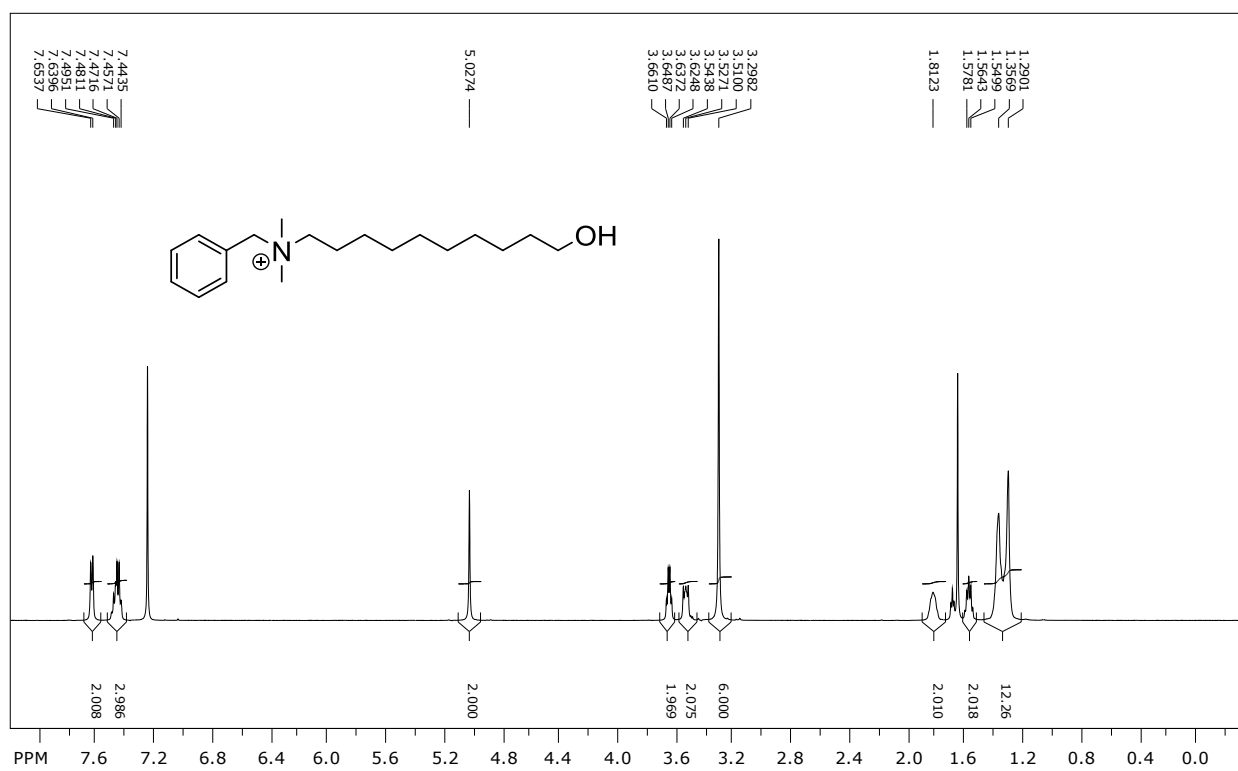


Figure S19. ¹H-NMR spectrum of ω-alkene-C₁₀-BAC in CDCl₃.

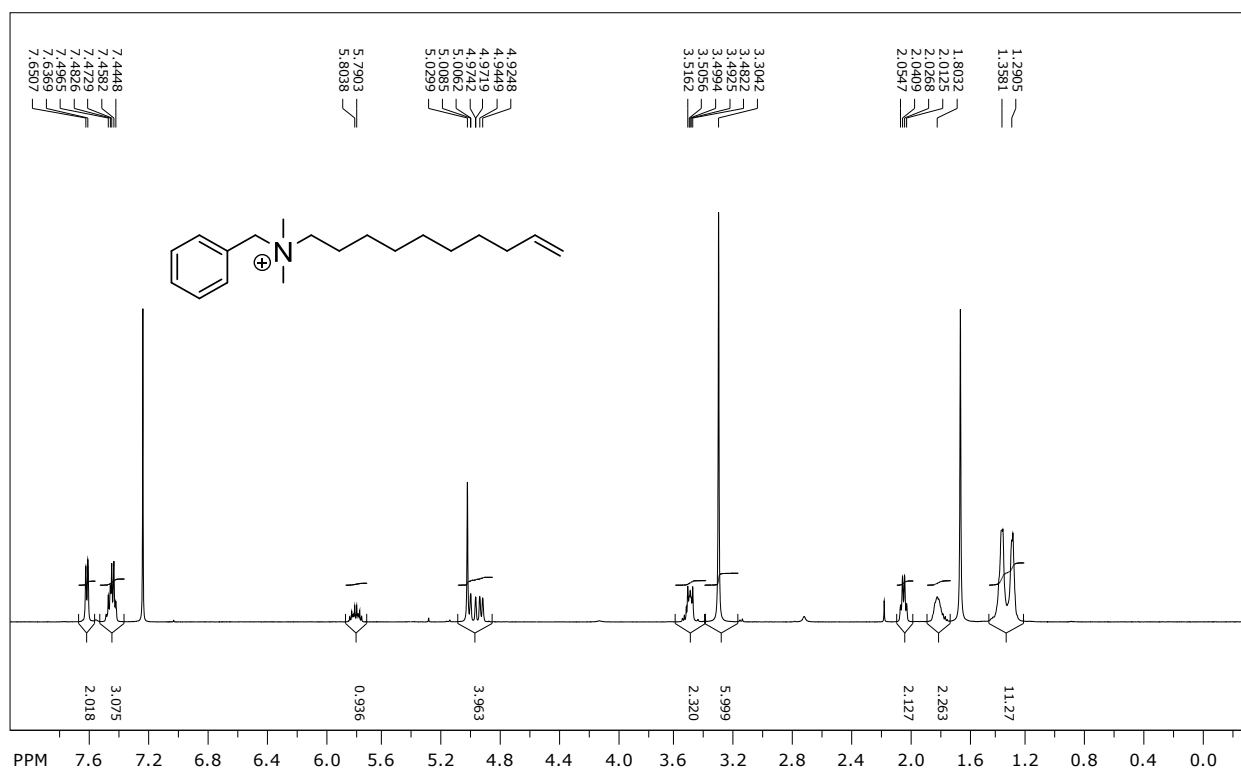


Figure S20. $^1\text{H-NMR}$ spectrum of (ω -1)-hydroxy- C_{10} -BAC in CDCl_3 . The small triplet at 0.94 ppm is due to the presence of (ω -2)-hydroxy- C_{10} -BAC as a minor impurity.

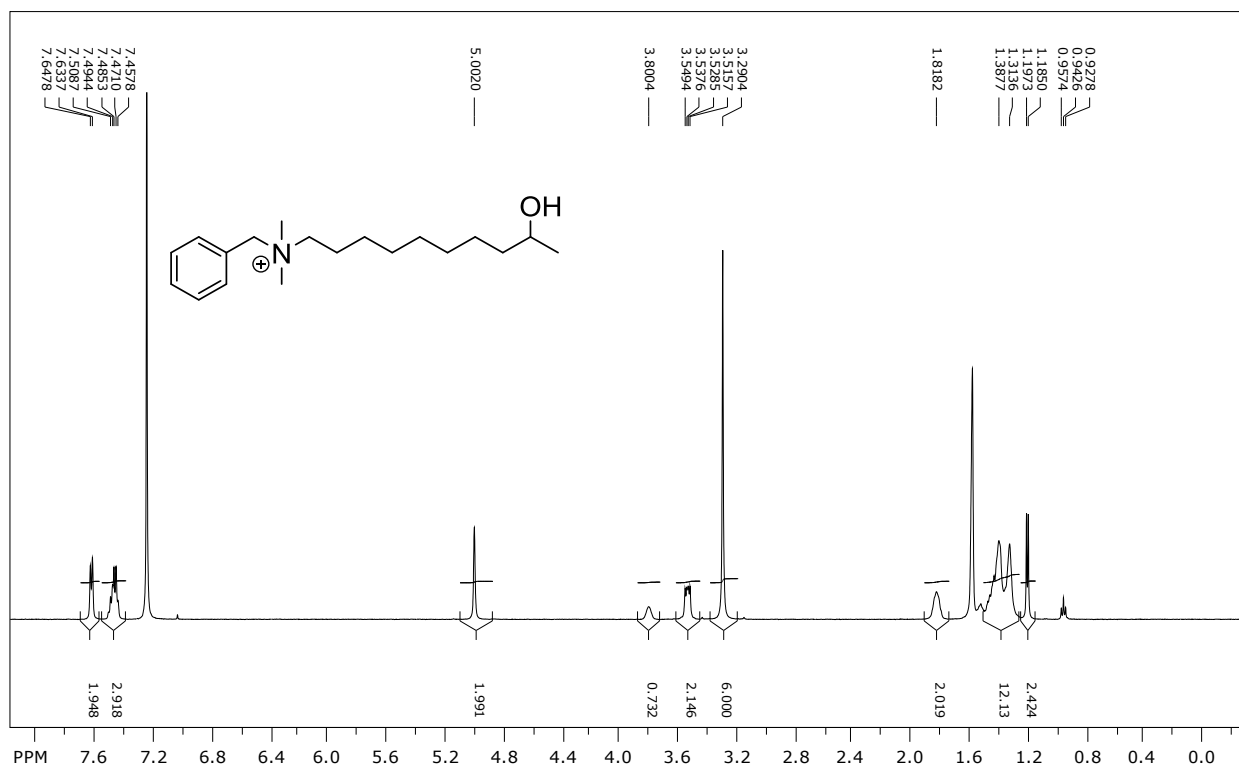


Figure S21. ¹H-NMR spectrum of (ω, ω-1)-dihydroxy-C₁₀-BAC in CDCl₃.

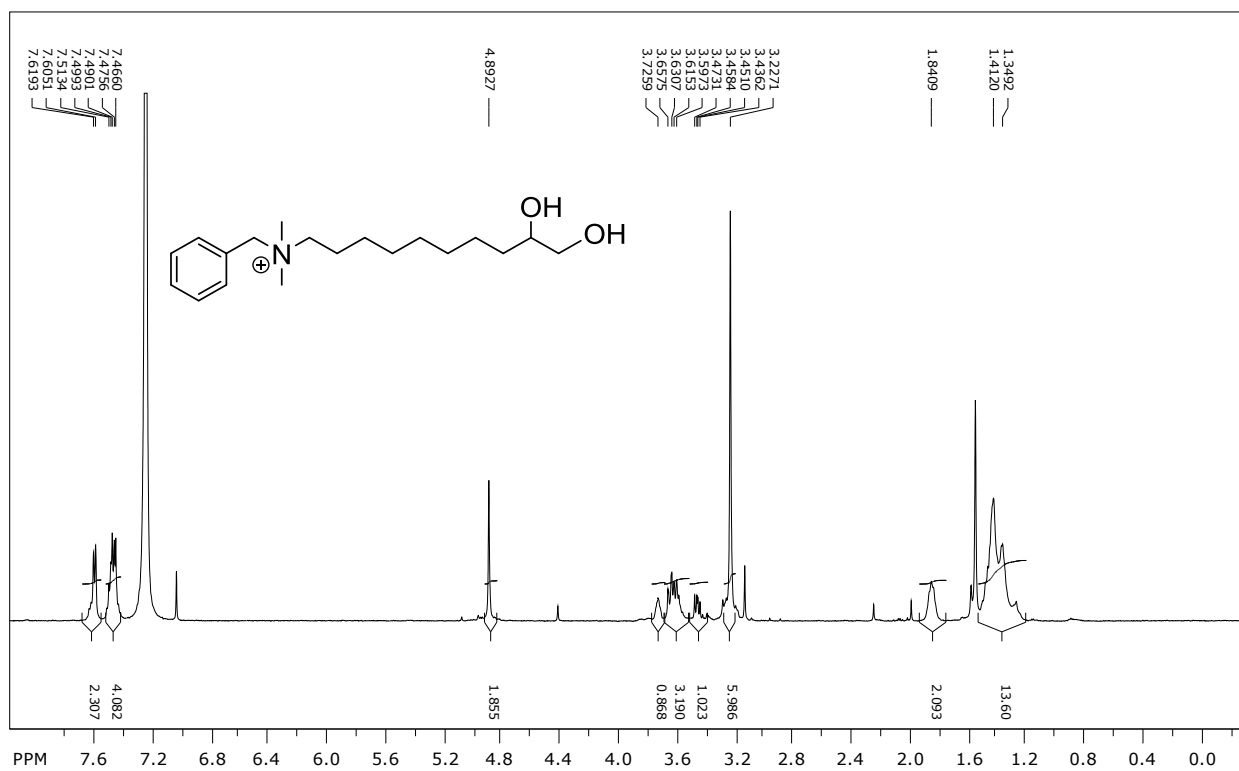


Figure S22. $^1\text{H-NMR}$ spectrum of (ω -1)-ketone- C_{10} -BAC in CDCl_3 . The triplet at 1.04 ppm is due to the presence of (ω -2)-ketone- C_{10} -BAC as a minor impurity.

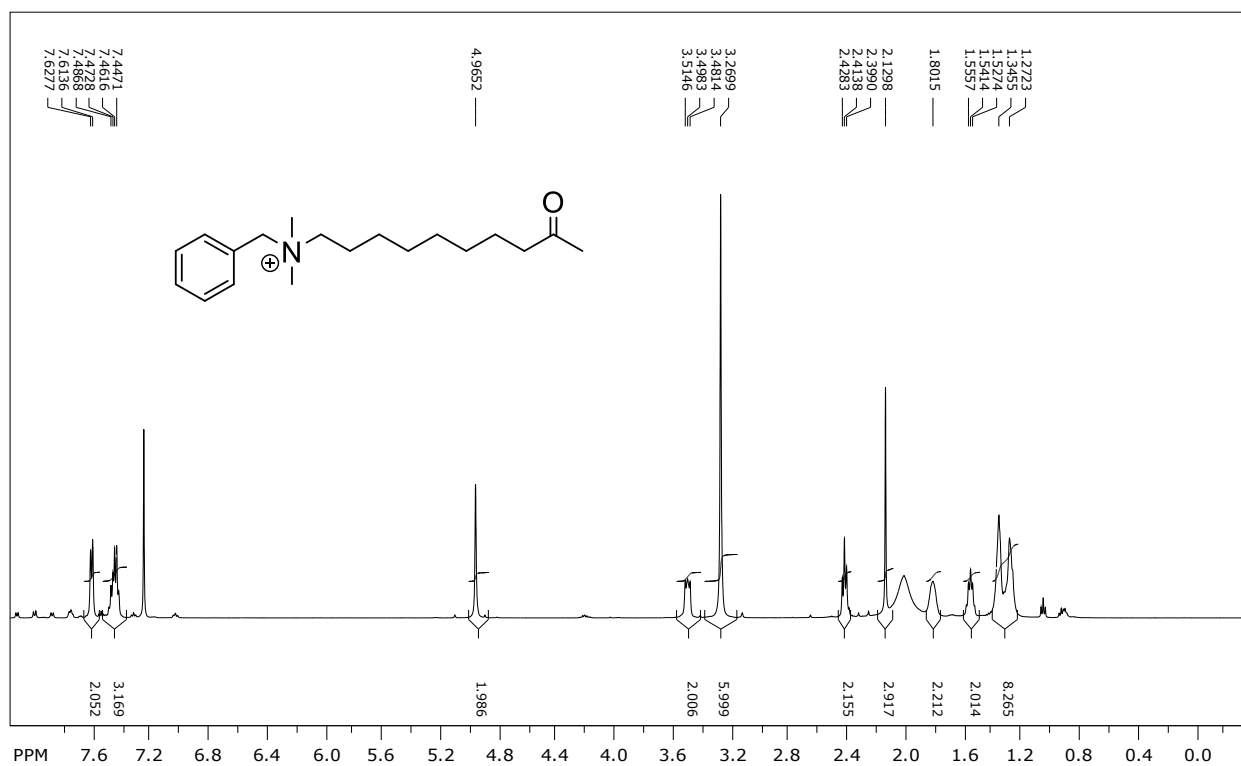


Figure S23. ¹H-NMR spectrum of ω-carboxylic acid-C₁₀-BAC in CDCl₃.

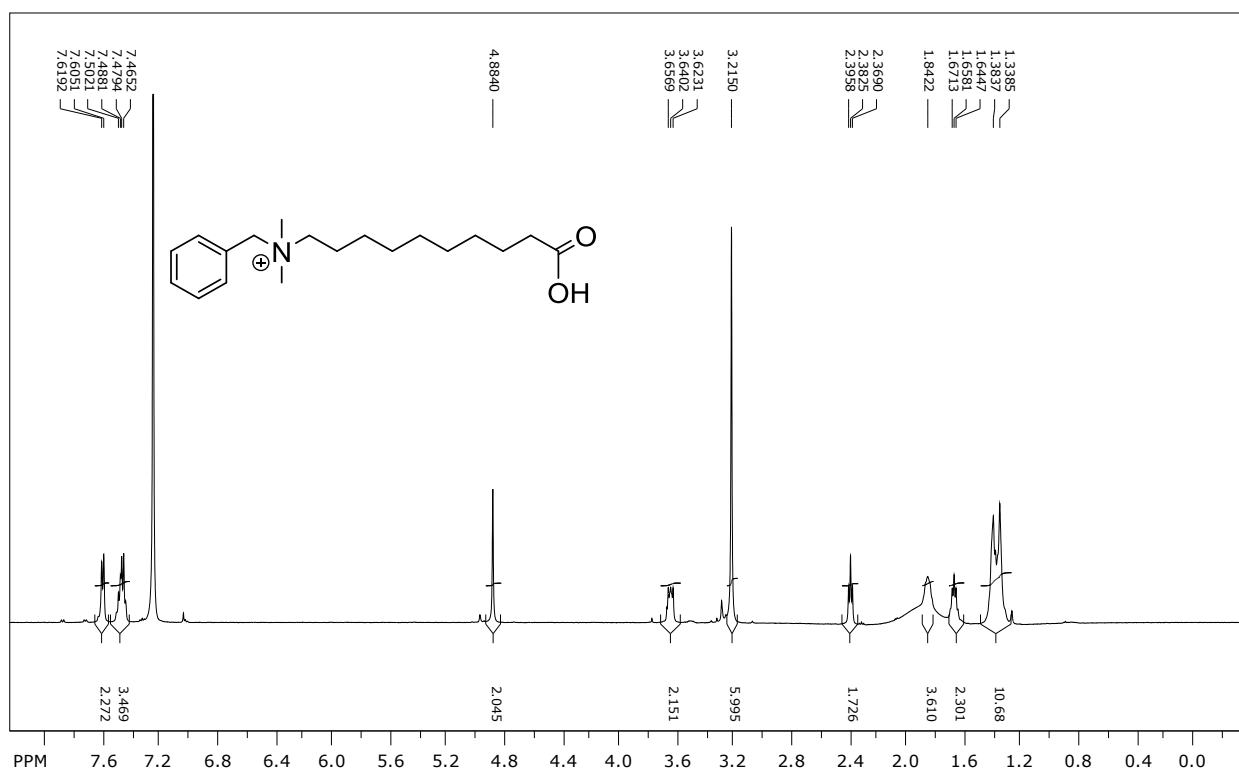


Figure S24. $^1\text{H-NMR}$ spectrum of ω -carboxylic acid- C_{10} -BAC in d_6 -DMSO.

