Supporting Information

Potential Metabolic Activation of Representative Alkylated Polycyclic Aromatic Hydrocarbons 1-Methylphenanthrene and 9-Ethylphenanthrene Associated with the Deepwater Horizon Oil Spill in Human Hepatoma (HepG2) Cells

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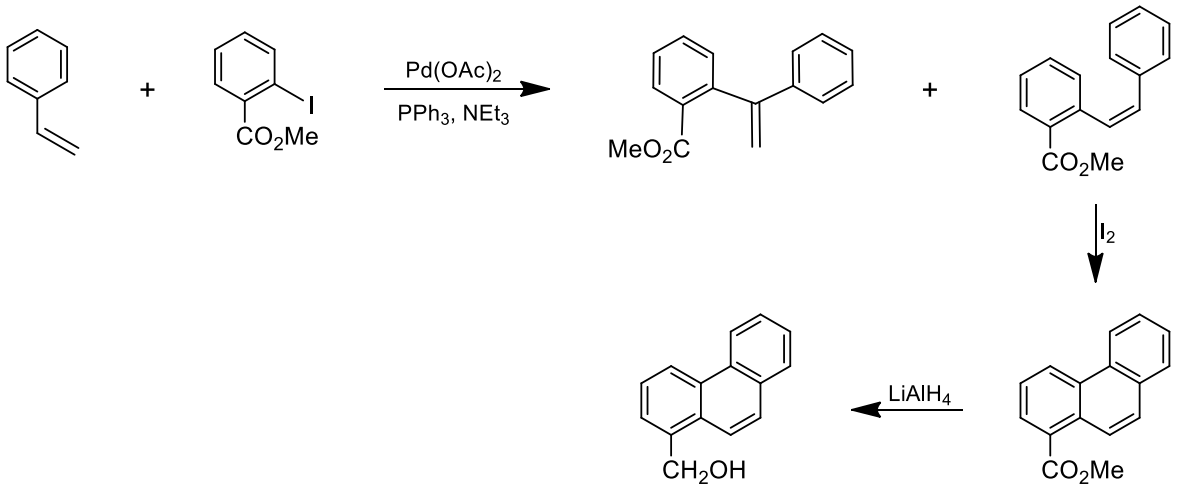
Table S1. Mass Transitions for 9-EP Metabolites in HepG2 Cells

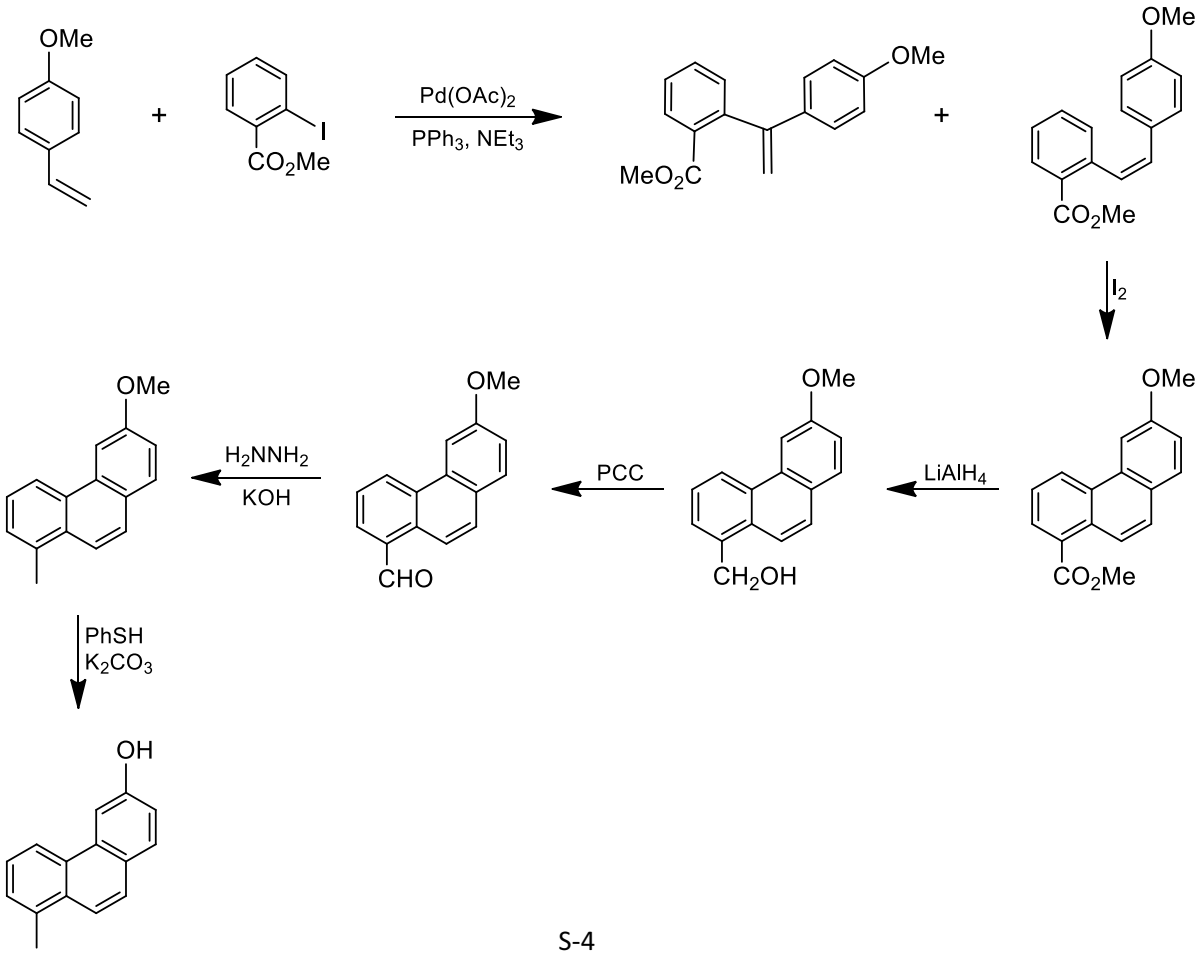
Metabolite No.	9-EP metabolites	Retention time (min)	Mode	*m/z*
—	Dehydrated O-sulfonated dihydrodiol	20.06	negative	301 $[M-H]^-$, 221 $[M-H-SO_3]^-$, 206 $[M-H-SO_3-CH_3]^-$, 188 $[M-H-SO_3-CH_3-H_2O]^-$
2	Bisdehydrated tetraol	16.70	positive	239 $[M+H]^+$, 220 $[M+H-H_2O-H]^+$, 192 $[M+H-H_2O-H-CO]^+$
3	O-sulfonated catechol	22.10	negative	317 $[M-H]^-$, 237 $[M-H-SO_3]^-$, 222 $[M-H-SO_3-CH_3]^-$

Figure S1: Synthetic Routes and details of synthesis of 1-hydroxy-1-MP and 6-hydroxy-1-MP

1-Hydroxy-1-methylphenanthrene:



6-Hydroxy-1-methylphenanthrene:



Materials and Methods

Methyl-2-iodobenzoate and palladium acetate were purchased from Alfa Aesar. Silica gel and all other chemicals and solvents were purchased from Fisher Scientific or Aldrich Chemicals.

Analyses

All NMR data was obtained on a Bruker Ultrashield 300, Avance II, 300 MHz. Negative mode mass spectroscopy data was obtained on a Thermo Fusion Orbitrap ESI-MS. Positive mode mass spectroscopy data was obtained on a Sciex 5800 Maldi TOF/TOF.

1-Hydroxy-1-methylphenanthrene[1]

(E)-Methyl 2-styrylbenzoate: Methyl 2-iodobenzoate (0.561 mL, 3.82 mmol) was dissolved in 20 mL anhydrous acetonitrile under an argon atmosphere in a 50 mL RB flask fitted with a reflux condenser and a magnetic stir bar. Styrene (0.526 mL, 4.58 mmol), palladium acetate (0.069 g, 0.305 mmol), triphenylphosphine (0.150 g, 0.572 mmol) and triethylamine (0.638 mL, 4.58 mmol) were added. The mixture was heated to 80°C for 21 hrs while stirring, cooled to room temperature and filtered through celite. The filtrate was washed with 25 mL water, dried over sodium sulfate, filtered and evaporated. The crude product was purified by silica gel chromatography using 30% methylene chloride / hexane as eluant to give (E)-methyl 2-styrylbenzoate (0.685 g, 2.87 mmol, 75 % yield). ^{1}H NMR ($CDCl_3$, 300 MHz): 3.91 (s, 3H); 7.0 (d, J=16.3Hz, 1H); 7.21-7.38 (m, 5H); 7.5 (-7.56 (m, 2H); 7.71 (d, J=7.8Hz, 1H); 7.92 (dd, J=6.4, 1.4Hz, 1H); 8.02 (d, J=16.2Hz, 1H).

Methyl phenanthrene-1-carboxylate: (E)-Methyl 2-styrylbenzoate (0.6 g, 2.52 mmol) was dissolved in 200 mL ether and 10 mL dichloromethane in a 250 mL photochemical reactor and air was bubbled through the solution for 10 min. Iodine (0.058 g, 0.227 mmol) was added and the mixture was photolyzed with a 450 W medium pressure Hg lamp for 7 hr. The solution was poured into a 500 mL separatory funnel and washed with 175 mL saturated sodium thiosulfate. The organic layer was dried over sodium sulfate, filtered and evaporated. The crude product was purified by silica gel chromatography using 30% ethyl acetate / hexane as eluant to give methyl phenanthrene-1-carboxylate (225 mg, 0.952 mmol, 37.8 % yield). ^{1}H NMR ($CDCl_3$, 300 MHz): 4.06 (s, 3H); 7.6-7.8 (m, 3H); 7.89 (d, J = 9.3Hz, 1H); 7.94 (dd, J = 1.9, 6.5 Hz, 1H); 8.23 (dd, J = 1.2, 7.4 Hz, 1H); 8.74 (d, J = 7.6Hz, 1H); 8.79 (dd, J = 0.6, 9.4Hz, 1H); 8.94 (d, J = 8.4Hz, 1H).

1-hydroxy-1-methylphenanthrene: Methyl phenanthrene-1-carboxylate (0.225 g, 0.952 mmol) was dissolved in anhydrous 10 mL THF under an argon atmosphere in a 25 mL RB flask with a magnetic stir bar. The mixture was cooled to 0°C while stirring and lithium aluminum hydride (0.145 g, 3.81 mmol) was added. The mixture was warmed to room temperature and stirring was continued for 4 hrs. The mixture was cooled to 0°C and 5 mL water was added to destroy excess lithium aluminum hydride and the precipitate was removed by filtration. The filtrate was extracted with 10 mL ethyl acetate and the organic layer was dried over sodium sulfate, filtered

and evaporated to give crude product which was dissolved in 5 mL methylene chloride leaving a white solid that was removed by filtration. The organic layer containing the crude product was purified by silica gel chromatography using 30% ethyl acetate / hexane as eluant to give 1-hydroxy-1-methylphenanthrene (26.7 mg, 0.128 mmol, 13.5 % yield). ^{1}H NMR ($CDCl_3$, 300 MHz): 5.21 (s, 2H); 7.64 (m, 4H); 7.83 (d, J=9.2 Hz, 1H); 7.91 (dd, J=1.3, 6.3 Hz, 1H); 8.08 (dd, J=0.6, 9.2 Hz, 1H); 8.71 (m, 2H). MS- = 206; MW = 208.2 g/moL.

6-Hydroxy-1-methylphenanthrene

(Z)-Methyl 2-(4-methoxystyryl)benzoate: This compound was made using the Heck coupling method.[1] Methyl 2-iodobenzoate (2.242 mL, 15.26 mmol) was dissolved in 60 mL anhydrous acetonitrile under an argon atmosphere in a 200 mL RB flask fitted with a reflux condenser and a magnetic stir bar. 1-Methoxy-styrene (2.465 mL, 18.32 mmol), palladium acetate (0.274 g, 1.221 mmol), triphenylphosphine (0.601 g, 2.290 mmol) and triethylamine (2.55 mL, 18.32 mmol) were added while stirring. The mixture was heated to 80°C for 21 hrs and filtered through celite. The filtrate was washed with 30 mL brine, dried over sodium sulfate, filtered and evaporated. The crude product was purified by silica gel chromatography using 30 % methylene chloride / hexane to give (Z)-methyl 2-(4-methoxystyryl)benzoate (3.02 g, 11.26 mmol, 73.7 % yield). ^{1}H NMR ($CDCl_3$, 300 MHz): 3.83 (s, 3H), 3.92 (s, 3H), 6.9 (m, 2H), 6.97 (d, J = 16.2 Hz, 1H), 7.29 (td, J = 1.2, 6.4 Hz, 1H), 7.49 (m, 3H), 7.7 (d, J = 6.8 Hz, 1H), 7.86 (d, J = 16.2 Hz, 1H), 7.9 (dd, J = 1.1, 6.4 Hz, 1H).

Methyl 6-methoxyphenanthrene-1-carboxylate: This compound was made using the cyclization method of Carme Pampin *et al.*[1] (Z)-Methyl 2-(4-methoxystyryl)benzoate (2.5 g, 9.32 mmol) was dissolved in 200 mL benzene in a 250 mL photochemical reactor and air was bubbled through the solution for 10 min. Iodine (0.213 g, 0.839 mmol) was added and the mixture was photolyzed with a 450 W medium pressure Hg lamp for 5 hrs. The solution was transferred to a 500 mL separatory funnel and washed with 150 mL saturated sodium thiosulfate. The organic layer was dried over sodium sulfate, filtered and evaporated. The crude product was purified by silica gel chromatograpy using 30 % ethyl acetate / hexane as eluant to give methyl 6-methoxyphenanthrene-1-carboxylate (0.596 g, 2.238 mmol, 24.02 % yield). ^{1}H NMR (300 MHz, $CDCl_3$): 3.73 (s, 3H); 3.87 (s, 3H); 6.57 (d, J = 12.12 Hz, 1H); 6.67 (d, J = 8.25 Hz, 2H); 6.99 (m, 2H); 7.28 (m, 2H); 7.89 (m, 1H).

(6-Methoxyphenanthren-1-yl)methanol: This compound was made using the method of Amin, S. *et al.*[2] Lithium aluminum hydride (0.144 g, 3.80 mmol) was suspended in 70 mL anhydrous THF in a 250 mL RB flask with a magnetic stir bar. Methyl 6-methoxyphenanthrene-1-carboxylate (0.596 g, 2.238 mmol) was dissolved in 35 mL anhydrous THF and added dropwise to the stirring solution. The mixture was stirred for 21 hrs and poured into 20 mL water then extracted with ether (3 x 15 mL). The combined organic layers were washed with 20 mL water, dried over sodium sulfate, filtered and evaporated. The crude product was purified by silica gel chromatography using 100 % hexane to 50 % ethyl acetate / hexane as eluant gradient to give (6-methoxyphenanthren-1-yl)methanol (0.423 g, 1.775 mmol, 79 % yield). ^{1}H NMR (300 MHz,

$CDCl_3$): 3.84 (s, 3H); 4.83 (s, 2H), 6.9 (d, J = 8.01 Hz, 2H); 7.01 (d, J = 16.44 Hz, 1H); 7.36 (m, 2H); 7.48 (d, J = 8.25 Hz, 2H); 7.64 (d, J = 7.86 Hz, 1H).

6-Methoxyphenanthrene-1-carbaldehyde: This compound was made using the method of Amin, S. *et al.*[2] Pyridinium chlorochromate (0.731 g, 3.39 mmol) was dissolved in 64 mL dichloromethane in a 200 mL RB flask with a magnetic stir bar. (6-Methoxyphenanthren-1-yl)methanol (0.425 g, 1.784 mmol) was dissolved in 26 mL dichloromethane and added dropwise over 10 min. The mixture was stirred for 3hrs and then diluted with 35 mL ether. The mixture was filtered through silica gel and evaporated to give crude product which was recrystallized from methylene chloride / hexane to give 6-methoxyphenanthrene-1-carbaldehyde (0.41 g, 1.735 mmol, 97 % yield). ^{1}H NMR (300 MHz, $CDCl_3$): 3.84 (s, 3H); 6.9 (m, 3H); 7.34 (m, 2H); 7.45 (m, 2H); 7.9 (m, 1H); 10.33 (s, 1H).

6-Methoxy-1-methylphenanthrene: This compound was made using the method of Amin, S. *et al.*[2] 6-Methoxyphenanthrene-1-carbaldehyde (0.4 g, 1.693 mmol) was dissolved in 50 mL diethylene glycol in a 100 mL RB flask with a magnetic stir bar. Hydrazine hydrate (3.38 ml, 69.4 mmol) and potassium hydroxide (0.380 g, 6.77 mmol) were added and the mixture was heated to reflux for 2hrs. The mixture was cooled to room temperature and stirring was continued while 15 mL conc. hydrochloric acid was added. The mixture was immediately extracted with methylene chloride (3 x 75 mL) and the organic layer was washed with 50 mL water, dried over sodium sulfate, filtered and evaporated to dryness. The crude product was purified by silica gel chromatography using 100 % hexane to 30 % ethyl acetate / hexane as eluant gradient to give 6-methoxy-1-methylphenanthrene (0.23 g, 1.035 mmol, 61.1 % yield). ^{1}H NMR (300 MHz, $CDCl_3$): 2.42 (s, 3H); 3.83 (s, 3H); 6.92 (m, 3H); 7.18 (m, 2H); 7.45 (d, J = 8.22 Hz, 2H); 7.56 (d, J = 6.99 Hz, 1H).

6-Hydroxy-1-methylphenanthrene: This compound was made using the method of Chakraborti, A. K. *et al.*[3] 6-Methoxy-1-methylphenanthrene (0.22 g, 0.990 mmol) was dissolved in 5 mL 1-methyl-2-pyrrolidinone under a nitrogen atmosphere in a 25 mL RB flask fitted with a reflux condenser and a magnetic stir bar. Thiophenol (0.102 mL, 0.990 mmol) and potassium carbonate (6.84 mg, 0.049 mmol) were added and the mixture was heated to reflux for 4 hrs. The solution was cooled to room temperature and 23 mL 5% sodium hydroxide was added slowly while stirring to make the solution basic. The aqueous layer was extracted with ether (3 x 15 mL). The aqueous layer was acidified to pH 3 with 6 N hydrochloric acid and extracted with ether (3 x 15 mL). The combined ether layers were washed with 5 mL brine, dried over sodium sulfate, filtered and evaporated. The crude product was purified by silica gel chromatography using 100 % hexane to 60 % ethyl acetate / hexane as eluant gradient to give 6-Hydroxy-1-methylphenanthrene (0.039 g, 0.187 mmol, 18.9 % yield). ^{1}H NMR (300 MHz, $CDCl_3$): 2.40 (s, 3H); 6.9 (m, 2H); 7.15 (m, 2H); 7.39 (d, J = 8.22 Hz, 2H); 7.56 (d, J = 7.05 Hz, 1H). MS+ = 210.03; MW = 208.3 g/moL.

References:

1. Carme Pampin, M.; Estevez, J.C.; Estevez, R. J.; Maestro, M. and Castedo, L. (2003) Heck-mediated synthesis and photochemically induced cyclization of [2-(2-styrylphenyl)ethylcarbamic acid ethyl esters and 2-styrylbenzoic acid methyl esters-total synthesis of naphtha[2,1f]-isoquinolines (2-azachrysenses). *Tetrahedron 59*, 7231-7243.
2. Amin, S.; Balanikas, G.; Huie, K. and Hecht, S. S. (1988) Synthesis and tumor-initiating activities of dimethylchrysenes. *Chem Res Toxicol 1*, 349-355.
3. Chakraborti, A. K.; Sharma, L. and Nayak, M. K. (2002) Demand-based thiolate anion generation under virtually neutral conditions: influence of steric and electronic factors on chemo and regioselective cleavage of aryl alkyl ethers. *J. Org. Chem. 67*, 6406-6414.

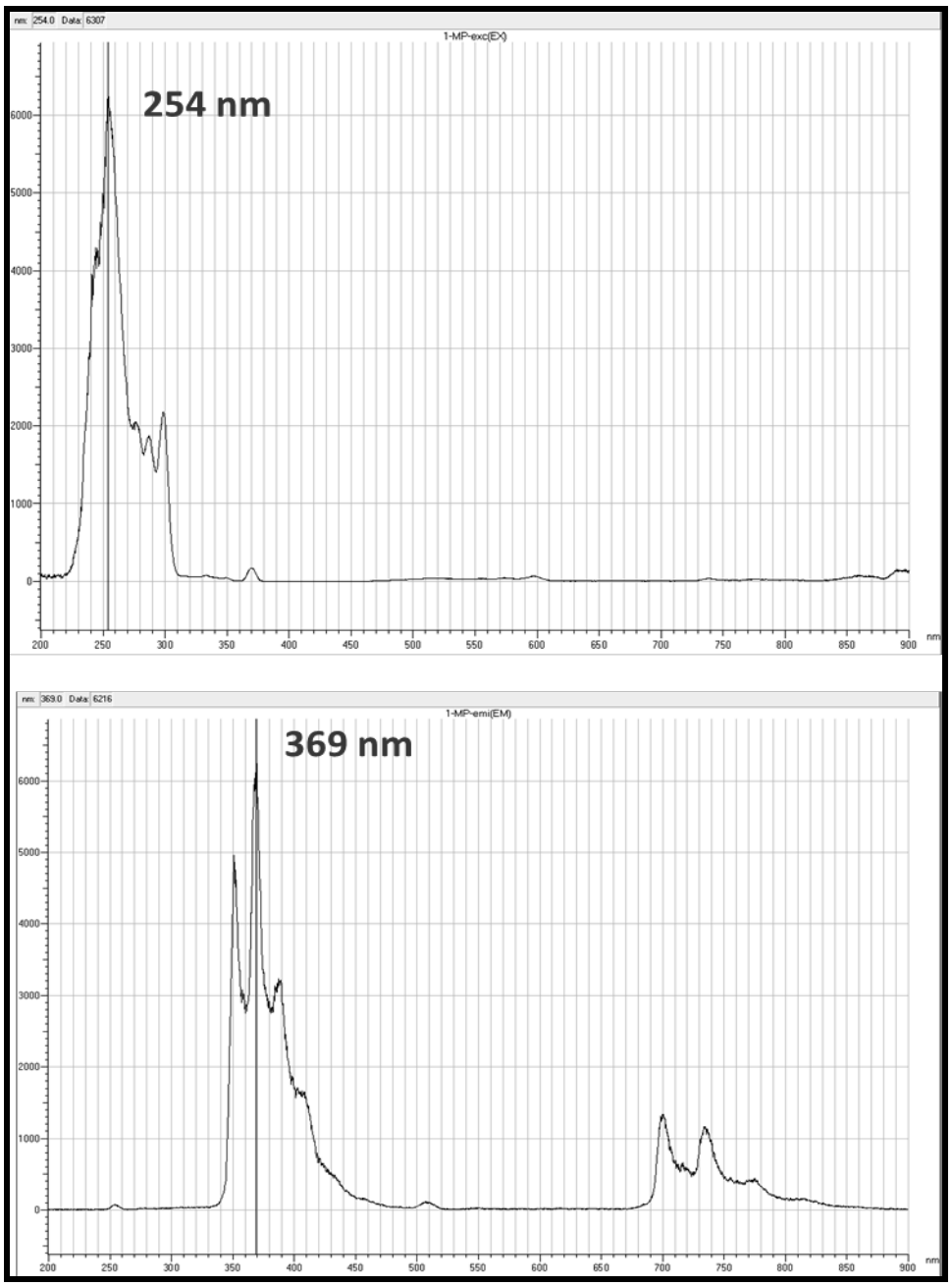


Figure S2. Excitation wavelength and emission wavelength spectra of 1-MP.

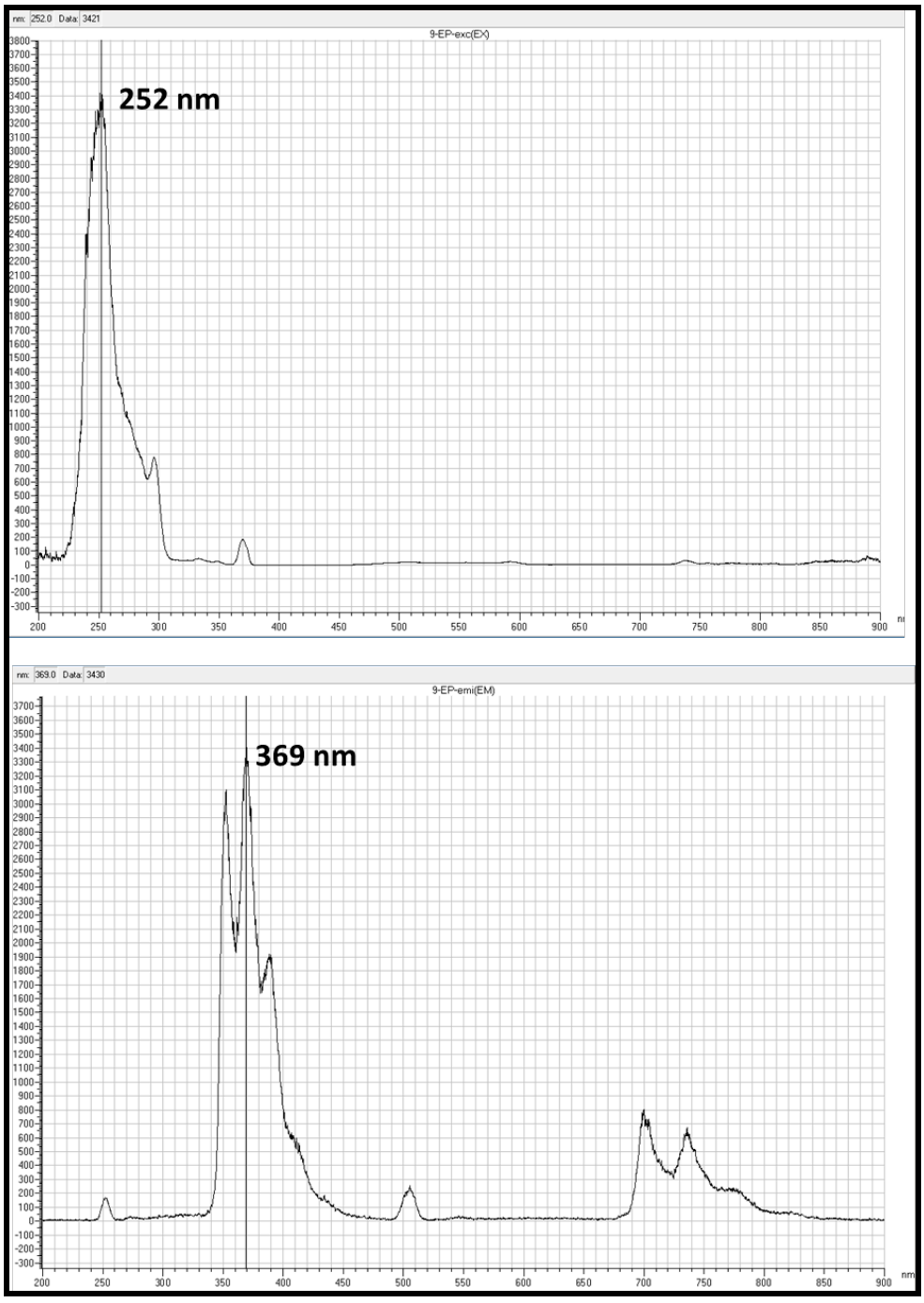


Figure S3. Excitation wavelength and emission wavelength spectra of 9-EP.

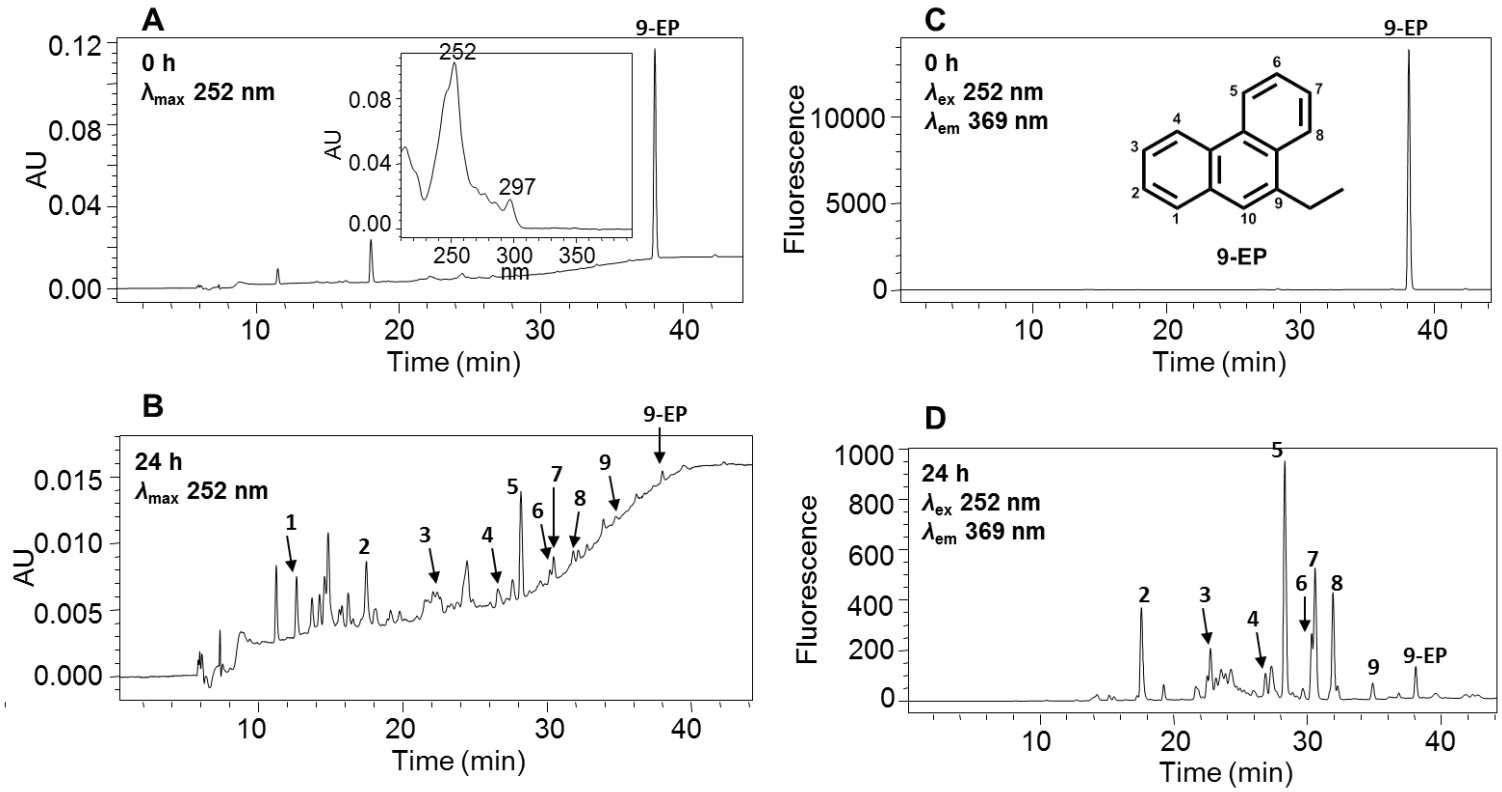


Figure S4. HPLC detection of 9-EP metabolites in human HepG2 cells. (A) UV chromatogram at λ_{max} 252 nm at 0 h. (B) UV chromatogram at λ_{max} 252 nm at 24 h. (C) FLR chromatogram at λ_{ex} 252 nm and λ_{em} 369 nm at 0 h. (D) FLR chromatogram at λ_{ex} 252 nm and λ_{em} 369 nm at 24 h.

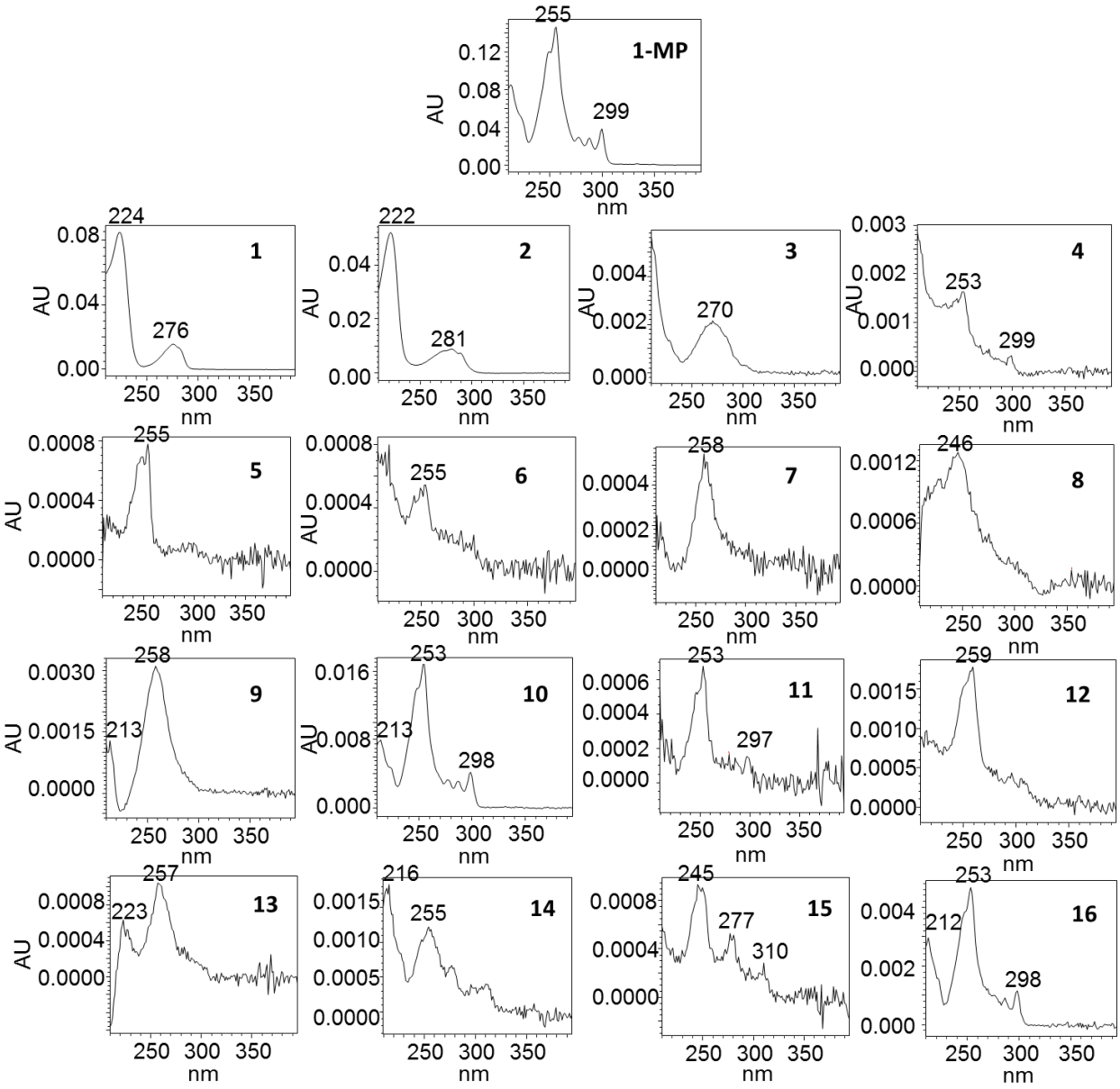


Figure S5. UV spectra of 1-MP and its metabolites in HepG2 cells.

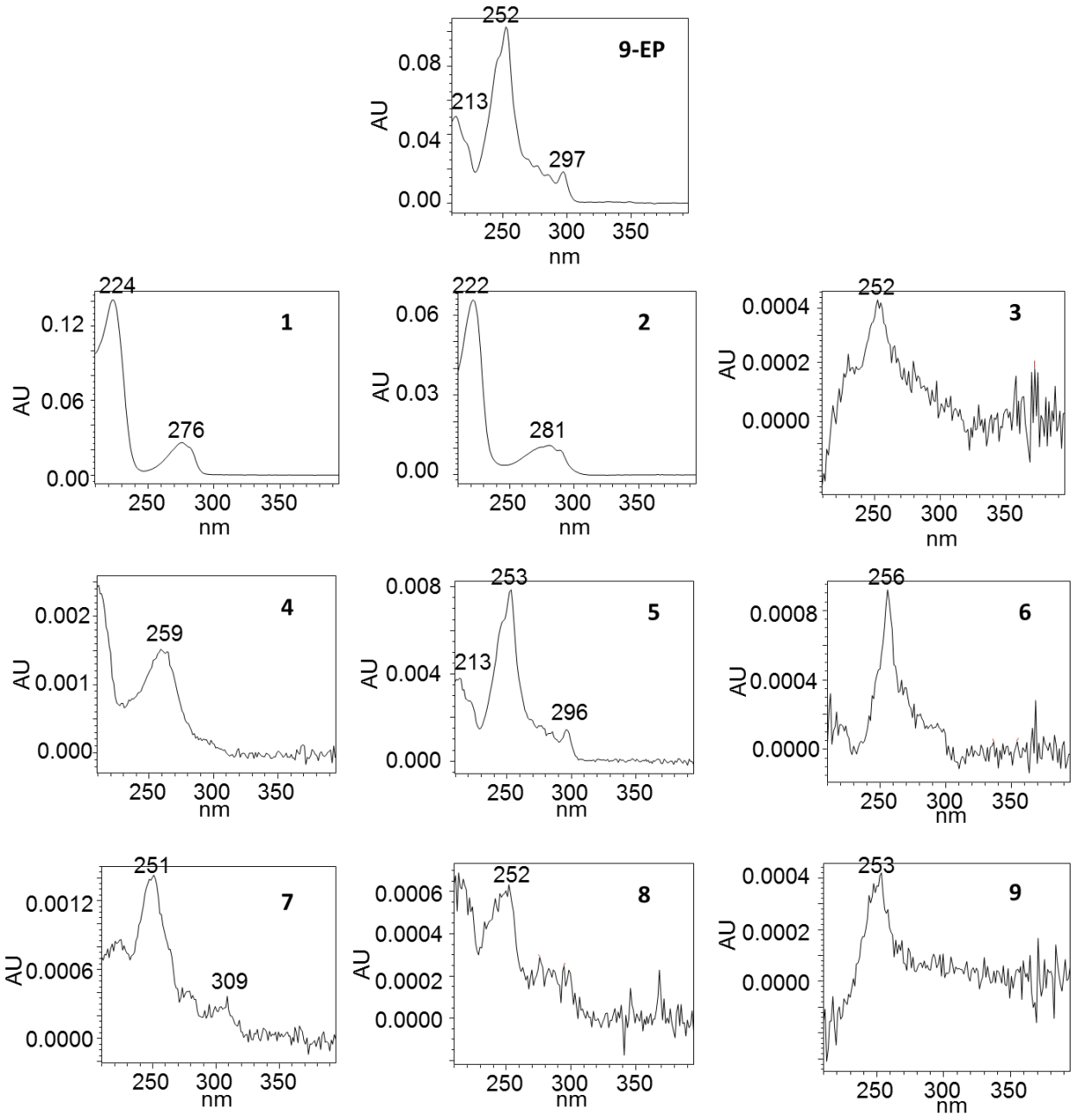


Figure S6. UV spectra of 9-EP and its metabolites in HepG2 cells.

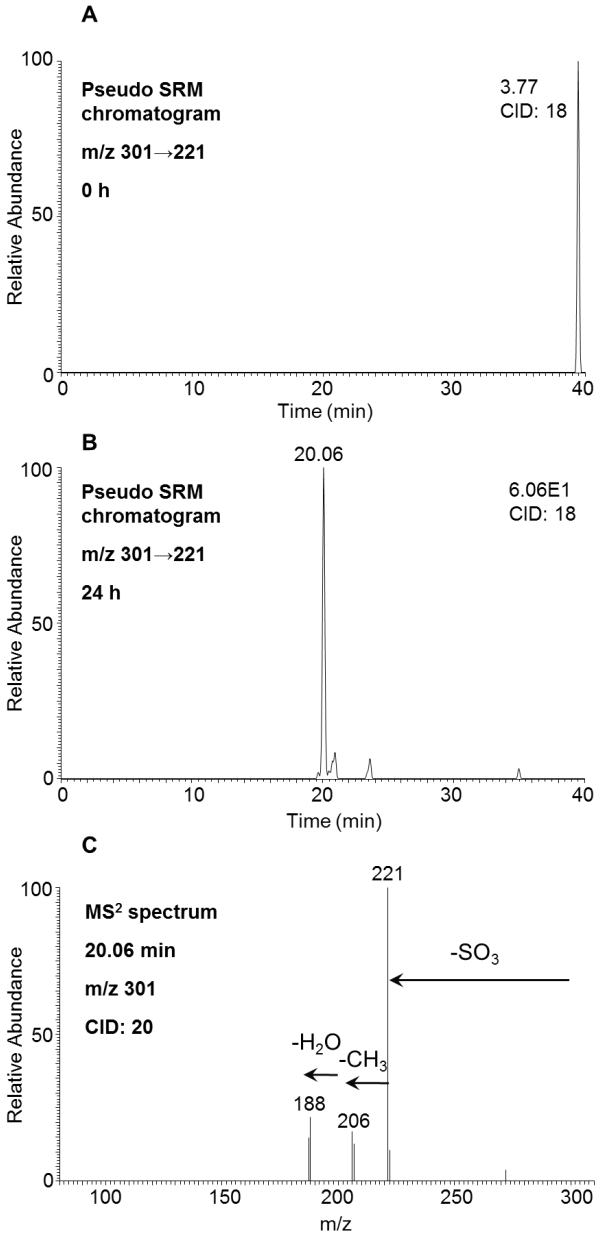


Figure S7. Detection of monodehydrated O-monosulfonated-9-EP-dihydrodiol in human HepG2 cells. (A) Extracted ion chromatogram of pseudo SRM transition at 0 h. (B) Extracted ion chromatogram of pseudo SRM transition at 24 h. (C) MS^2 spectrum of the peak at 20.06 min.

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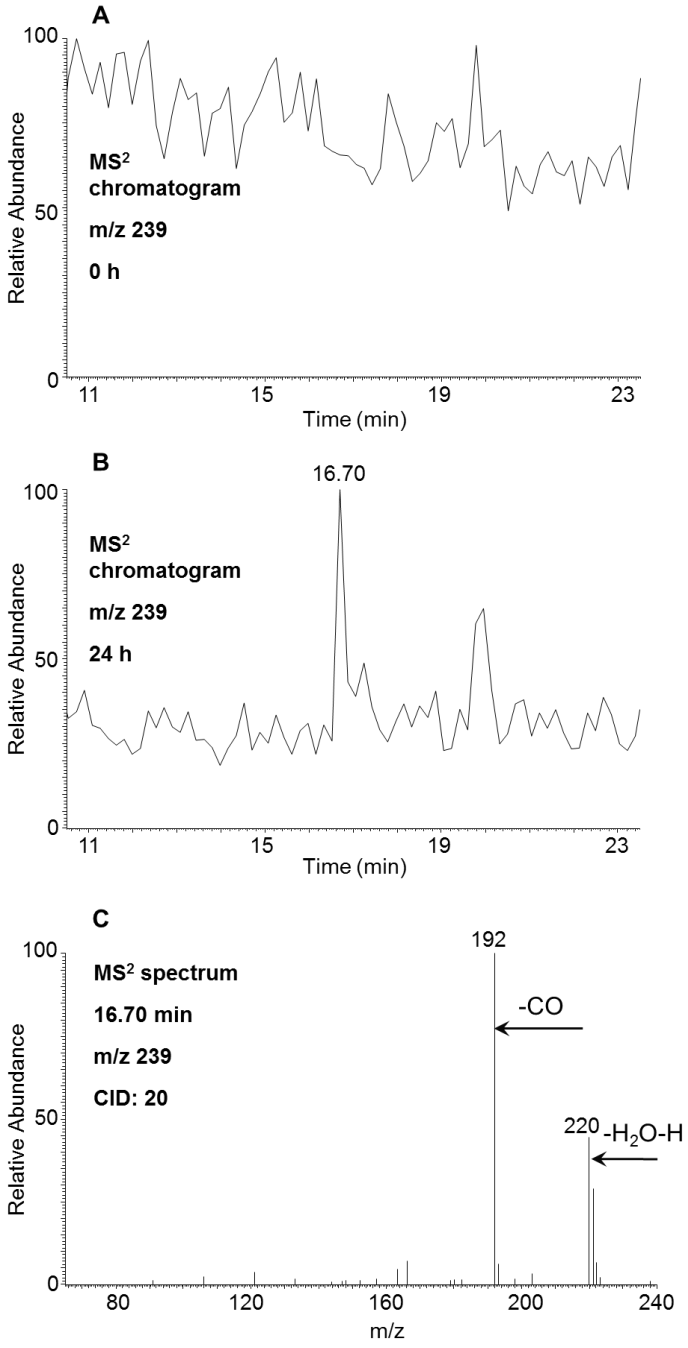

Figure S8. Detection of bisdehydrated 9-EP-tetraol in human HepG2 cells. (A) MS^2 chromatogram at 0 h. (B) MS^2 chromatogram at 24 h. (C) MS^2 spectrum of the peak at 16.70 min.

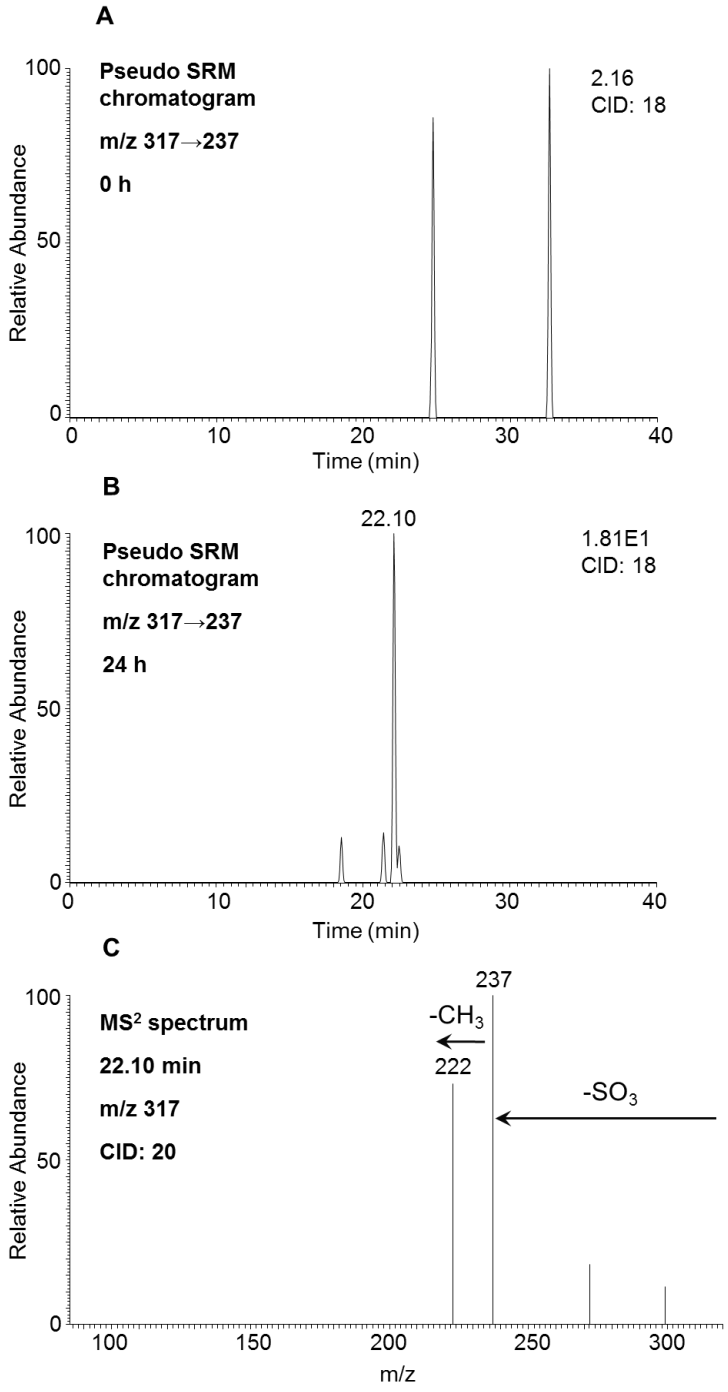

Figure S9. Detection of O-monosulfonated-9-EP-catechol in human HepG2 cells. (A) Extracted ion chromatogram of pseudo SRM transition at 0 h. (B)) Extracted ion chromatogram of pseudo SRM transition at 24 h. (C) MS^2 spectrum of the peak at 22.10 min.

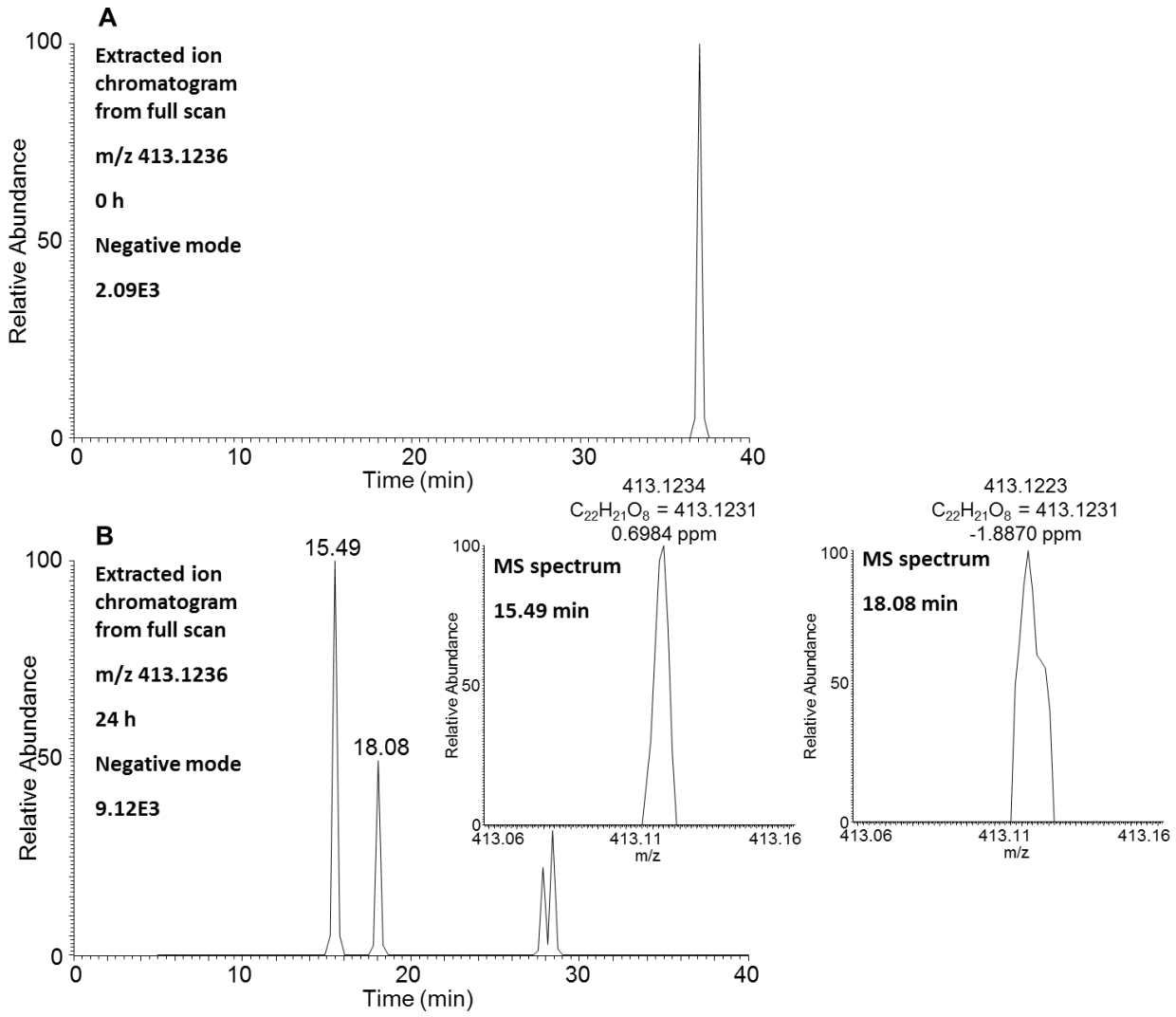

Figure S10. Detection of either O-monoglucuronosyl-9-EP-catechols or O-monoglucuronosyl-9-EP-bis-phenols in human HepG2 cells. (A) Extracted ion chromatogram of Orbitrap full scan at 0 h. (B) Extracted ion chromatogram of Orbitrap full scan at 24 h and MS spectra of the peaks at 15.49 min and 18.08 min.

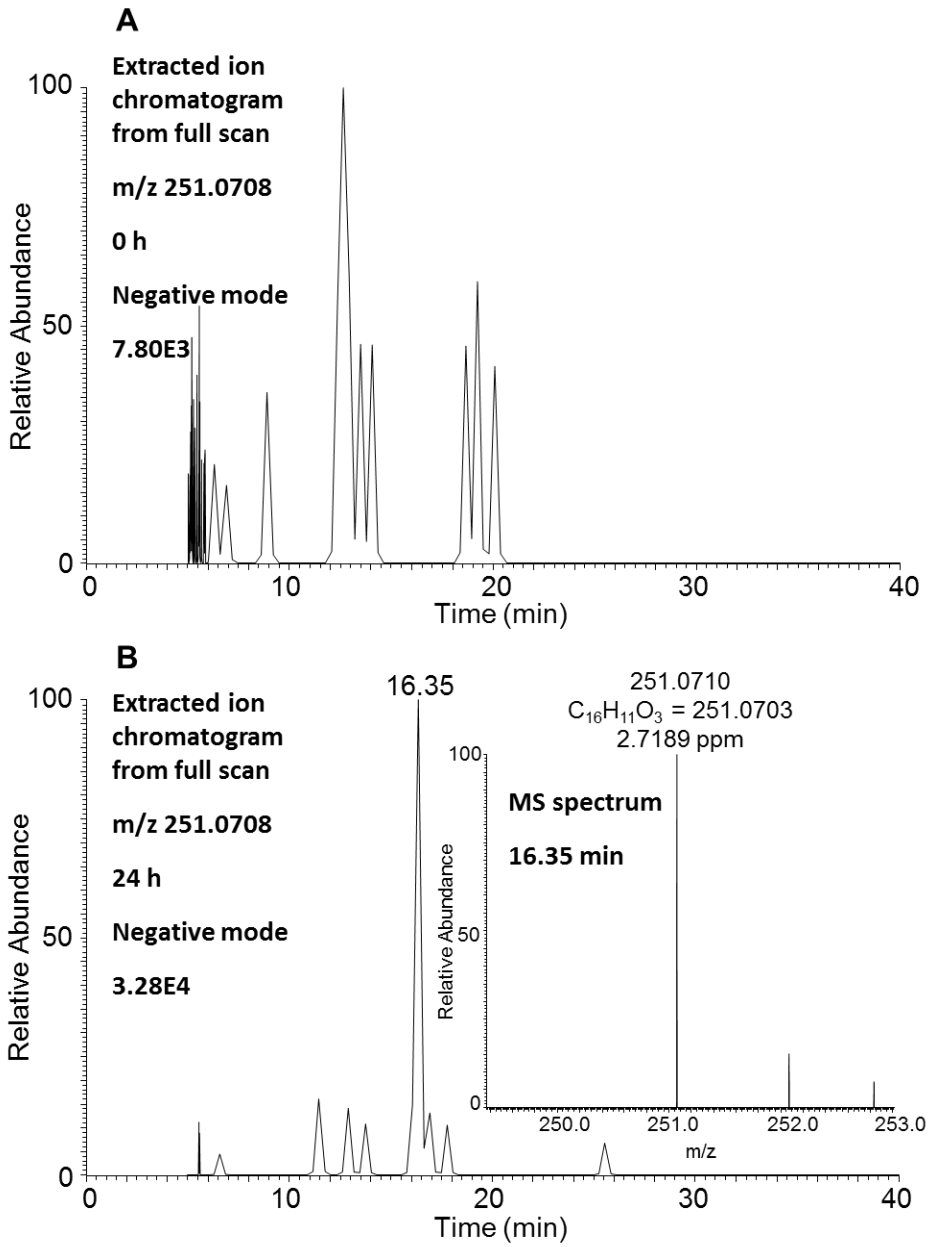

Figure S11. Detection of monohydroxy-9-EP-dione in human HepG2 cells. (A) Extracted ion chromatogram of Orbitrap full scan at 0 h. (B) Extracted ion chromatogram of Orbitrap full scan at 24 h and MS spectrum of the peak at 16.35 min.