# SUPPORTING INFORMATION

# Microsomal Metabolism of Prochiral Polychlorinated Biphenyls Results in the Enantioselective Formation of Chiral Metabolites

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**Abbreviations**

| | |
|---|---|
| BDM: | Chiral-Dex B-DM column |
| BNF: | β-Naphthoflavone |
| CD: | Chirasil-Dex column |
| CFA: | Clofibric acid |
| CYP: | Cytochrome P450 |
| DEX: | Dexamethasone |
| DMSO: | Dimethyl sulfoxide |
| EF: | Enantiomeric fraction |
| GC-MS: | Gas chromatograph-mass spectrometer |
| GC-μECD: | Gas chromatograph-micro electron capture detector |
| INH: | Isoniazid |
| MTBE: | Methyl *tert*-butyl ether |
| NADPH: | Nicotine adenine dinucleotide phosphate (reduced) |
| nd: | Not determined |
| nr: | Not resolved |
| OH-PCB: | Hydroxylated polychlorinated biphenyl metabolite |
| PB: | Phenobarbital |
| PCB: | Polychlorinated biphenyl |
| PCB 102: | 2,’2’,4,5,6’-Pentachlorobiphenyl |
| PCB 51: | 2,2’,4,6’-Tetrachlorobiphenyl |
| RT: | Retention time |
| RRT: | Relative retention time |
| SIM: | selected ion monitoring |

**Table S1.** Sources of liver microsomes prepared from male Sprague Dawley rats pretreated with different inducers of specific cytochrome P450 isoforms.*

| **Species** | **Sex** | **Inducer** | **Catalog number** | **Lot number** |
|---|---|---|---|---|
| Rat[$] | male | Corn oil | - | - |
| Rat[#] | male | β-Naphthoflavone (BNF, CYP1A inducer) | R1083 | 1110154 |
| Rat[#] | male | Clofibric Acid (CFA, CYP4A inducer) | R1063 | 0610080 |
| Rat[#] | male | Dexamethasone (DEX, CYP3A inducer) | R1093 | 1110156 |
| Rat[#] | male | Isoniazid (INH, CYP2E inducer) | R1088 | 1110155 |
| Rat[#] | male | Phenobarbital (PB, CYP2B inducer) | R1078 | 1010045 |

* The same microsomal preparations were used in our earlier study investigating the species-dependent metabolism of chiral PCB 136.[1]
[$] Microsomes prepared from corn oil treated male rats as reported previously.[2]
[#] Liver microsomes were purchased from Xenotech (Lenexa, KS, USA).
nd: Not determined

**Table S2.** Sources of liver microsomes prepared from different species.*

| **Species** | **Sex** | **Catalog number** | **Lot number** |
|---|---|---|---|
| Rat[$] | Male | - | - |
| Dog[#] | Female | D1500 | 0410049 |
| Guinea pig[#] | Male | G1000 | 0710094 |
| Hamster[#] | Male | S1000 | 0710396 |
| Monkey[#] | Male | P2000 | 1010321 |
| Rabbit[#] | Male | L1000 | 0810371 |

* The same microsomal preparations were used in our earlier study investigating the species-dependent metabolism of chiral PCB 136.[1]
[$] Microsomes prepared from corn oil treated male rats as reported previously.[2]
[#] Liver microsomes were purchased from Xenotech (Lenexa, KS, USA).
nd: Not determined

**Table S3A.** The resolution of the major OH-PCB 51 atropisomers (analyzed as methylated derivative) improved with decreasing temperature on a gas chromatograph-electron capture detector (GC-μECD) equipped with a Chirasil-Dex (CD) column.[a]

| Temperature (°C)[#] | Retention time (min) | Resolution[3] | Enantiomeric Fraction |
|---|---|---|---|
| 140 | 176.6 | 1.28 | 0.48 |
| 145 | 130.7 | 1.34 | 0.49 |
| 150 | 100.9 | 1.32 | 0.52 |
| 160 | 62.2 | 0.85 | 0.51 |
| 170 | 41.9 | 0.74 | 0.50 |
| 180 | 31.0 | 0.46 | 0.49 |
| 200 | 21.9 | nr | nd |

[#] Carrier gas flow was at 3 mL/min to determine how temperature affects the enantioselective separation of the OH-PCB 51 metabolite. nr: not resolved, nd: not determined.

**Table S3B.** The resolution of the major OH-PCB 102 atropisomers (analyzed as methylated derivative) improved with decreasing temperature on a GC-μECD equipped with a CD column.[a]

| Temperature (°C)[#] | Retention time (min) | Resolution[3] | Enantiomeric Fraction |
|---|---|---|---|
| 140 | 337.2 | 0.83 | 0.50 |
| 150 | 182.3 | 0.77 | 0.50 |
| 160 | 105.5 | 0.71 | 0.50 |
| 170 | 65.8 | 0.65 | 0.50 |
| 180 | 44.7 | 0.60 | 0.50 |
| 200 | 26.9 | 0.44 | 0.50 |

[#] Carrier gas flow was at 3 mL/min to determine how temperature affects the enantioselective separation of the OH-PCB 102 metabolite.

[a] Large scale incubations were performed with rat liver microsomes prepared from dexamethasone-pretreated rats and contained phosphate buffer (0.1 M, pH 7.4), liver microsomes (0.1 mg/mL) and NADPH (0.5 mM). The respective PCB congener in DMSO (0.5% of the incubation volume) was added to give a final concentration of 50 μM in a final volume of 16 mL as described in the Experimental Section. After extraction with hexane-MTBE (1:1, *v/v*) and derivatization with diazomethane, the extracts were dried and racemized by heating of the residue at 300 °C for 2 hours. The resulting racemized sample was subject to enantioselective analyses using GC-μECD equipped with a CD capillary column (25 m length, 250 μm inner diameter, 0.25 μm film thickness; Agilent, Santa Clara, CA, USA).

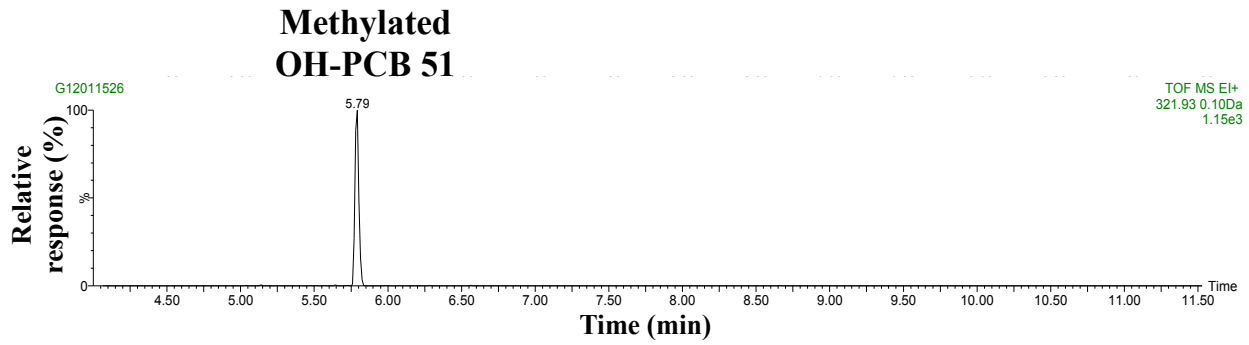


**Figure S1A. Representative gas chromatogram showing the formation of OH-PCB 51 (RT 5.79 min; relative retention time (RRT) 0.665; *m/z* 321.9) from prochiral PCB 51 in incubations with rat liver microsomes prepared from male corn oil pretreated rats (analyzed as the corresponding methylated derivative).** The metabolite was tentatively identified as OH-PCB 51 based on its RRT and mass spectrum (Figure S1B). diOH-PCB 51, a metabolite observed in microsomal incubations with other rat liver microsomes or liver microsomes prepared from other mammalian species, was not detected. The incubation was performed in a shaking water bath at 37 °C for 2 hours and, after extraction with hexane-MTBE (1:1, *v/v*) and derivatization with diazomethane, the organic extract was analyzed on a Waters GCT Premier gas chromatograph-mass spectrometer as described in the Experimental Section.

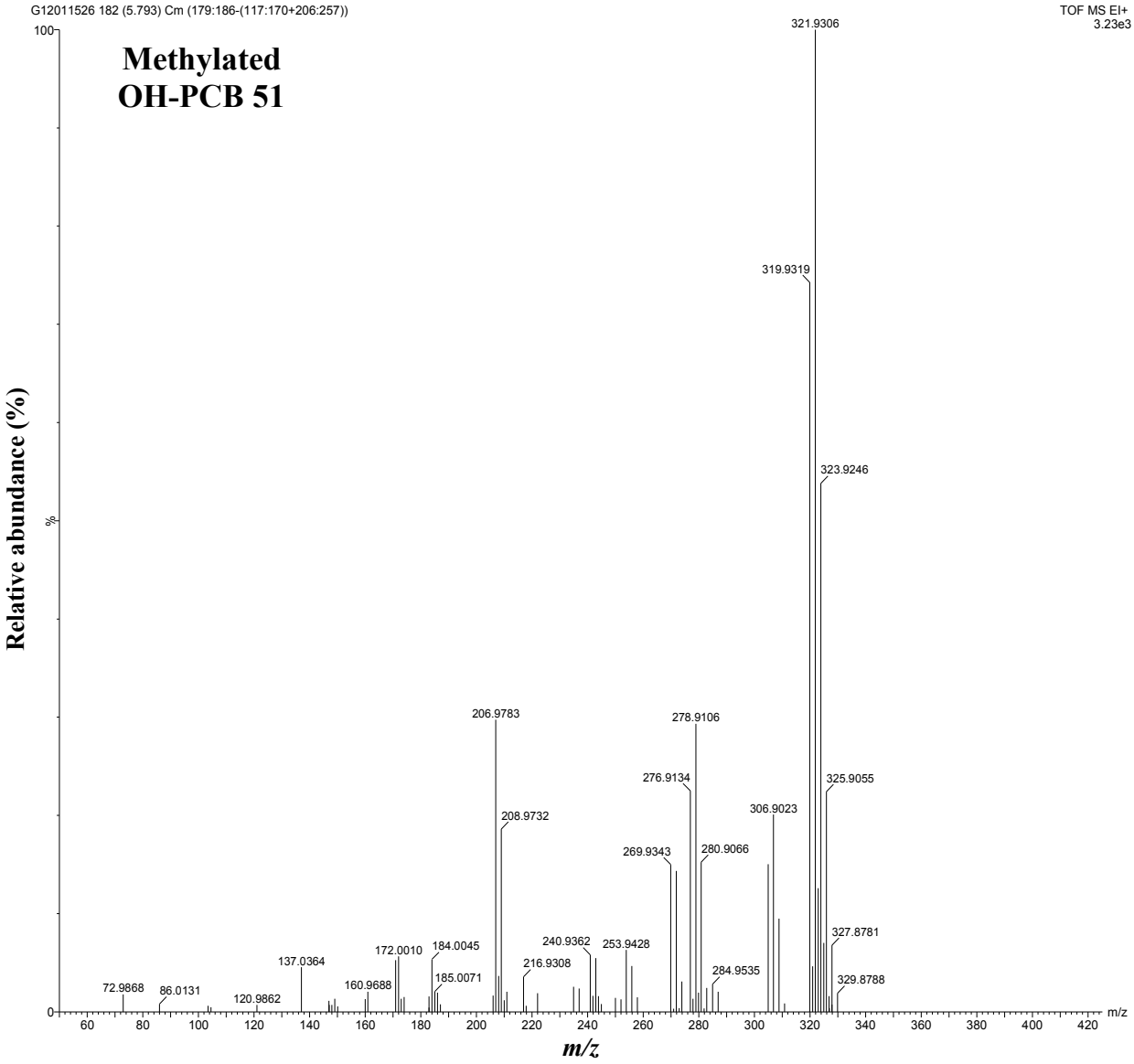


**Figure S1B. Mass spectrum of methylated OH-PCB 51 (RT 5.79 min; RRT 0.665) obtained from incubations of PCB 51 with rat liver microsomes prepared from male corn oil pretreated rats after derivatization.** The accurate mass determination of the monoisotopic $[M]^+$ (*m/z* 319.9319) versus the *m/z* calculated for $C_{13}H_8O^{35}Cl_4$ (*m/z* 319.9329); the isotope pattern of the molecular ion (1:1.3:0.7) compared to the theoretical ratio (1:1.3:0.6); and the fragmentation pattern were consistent with a monohydroxylated tetrachlorobiphenyl (as the corresponding methylated derivative). See the manuscript for a discussion of the chemical structure of this metabolite.

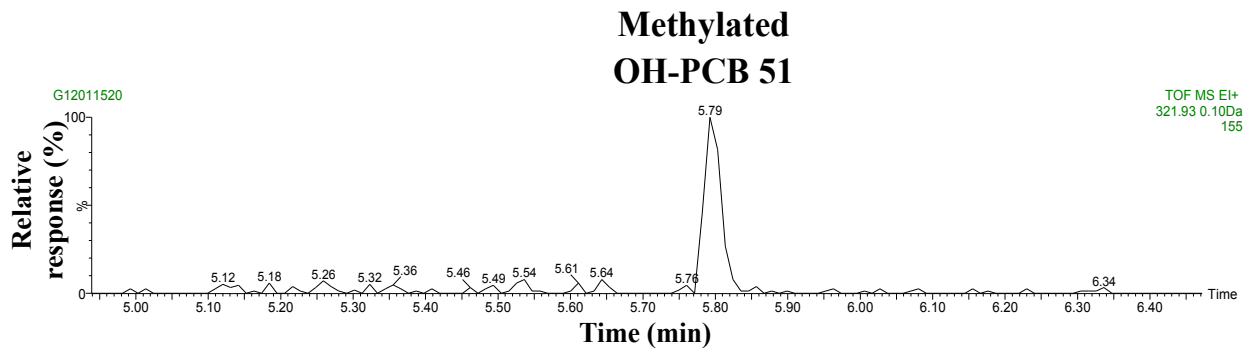


**Figure S2. Representative gas chromatogram showing the formation of OH-PCB 51 (RT 5.79 min; *m/z* 321.9) from prochiral PCB 51 in incubations with rat liver microsomes prepared from male β-naphthoflavone pretreated rats (analyzed as the corresponding methylated derivative).** The metabolite was tentatively identified based on the retention time. diOH-PCB 51, a metabolite observed in microsomal incubations with other rat liver microsomes or liver microsomes prepared from other mammalian species, was not detected. The incubation was performed in a shaking water bath at 37 °C for 2 hours and, after extraction with hexane-MTBE (1:1, *v/v*) and derivatization with diazomethane, the organic extract was analyzed on a Waters GCT Premier gas chromatograph-mass spectrometer as described in the Experimental Section.

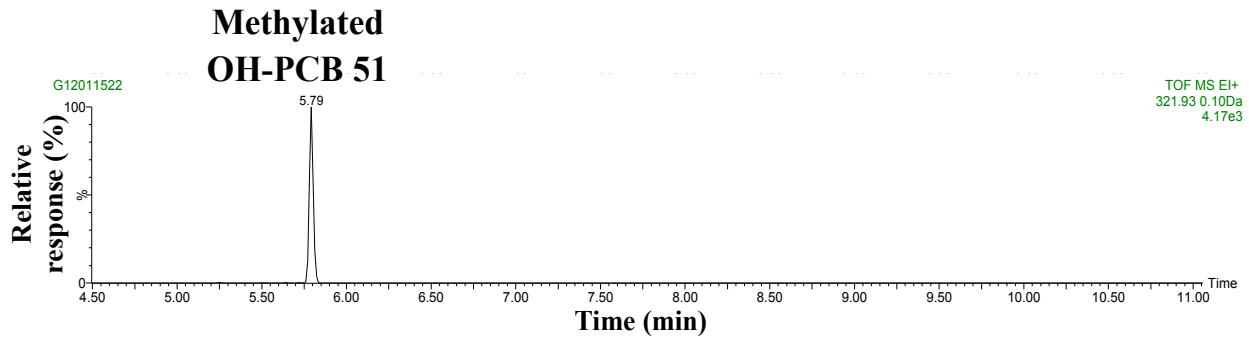


**Figure S3A. Representative gas chromatogram showing the formation of OH-PCB 51 (RT 5.79 min; RRT 0.665; *m/z* 321.9) from prochiral PCB 51 in incubations with rat liver microsomes prepared from male clofibric acid pretreated rats (analyzed as the corresponding methylated derivative).** The metabolite was tentatively identified based on its RRT and mass spectrum (Figure S3B). In addition, diOH-PCB 51 (RT 6.68 min; RRT 0.767) was tentatively identified based on its RRT (data not shown). The incubation was performed in a shaking water bath at 37 °C for 2 hours and, after extraction with hexane-MTBE (1:1, *v/v*) and derivatization with diazomethane, the organic extract was analyzed on a Waters GCT Premier gas chromatograph-mass spectrometer as described in the Experimental Section.

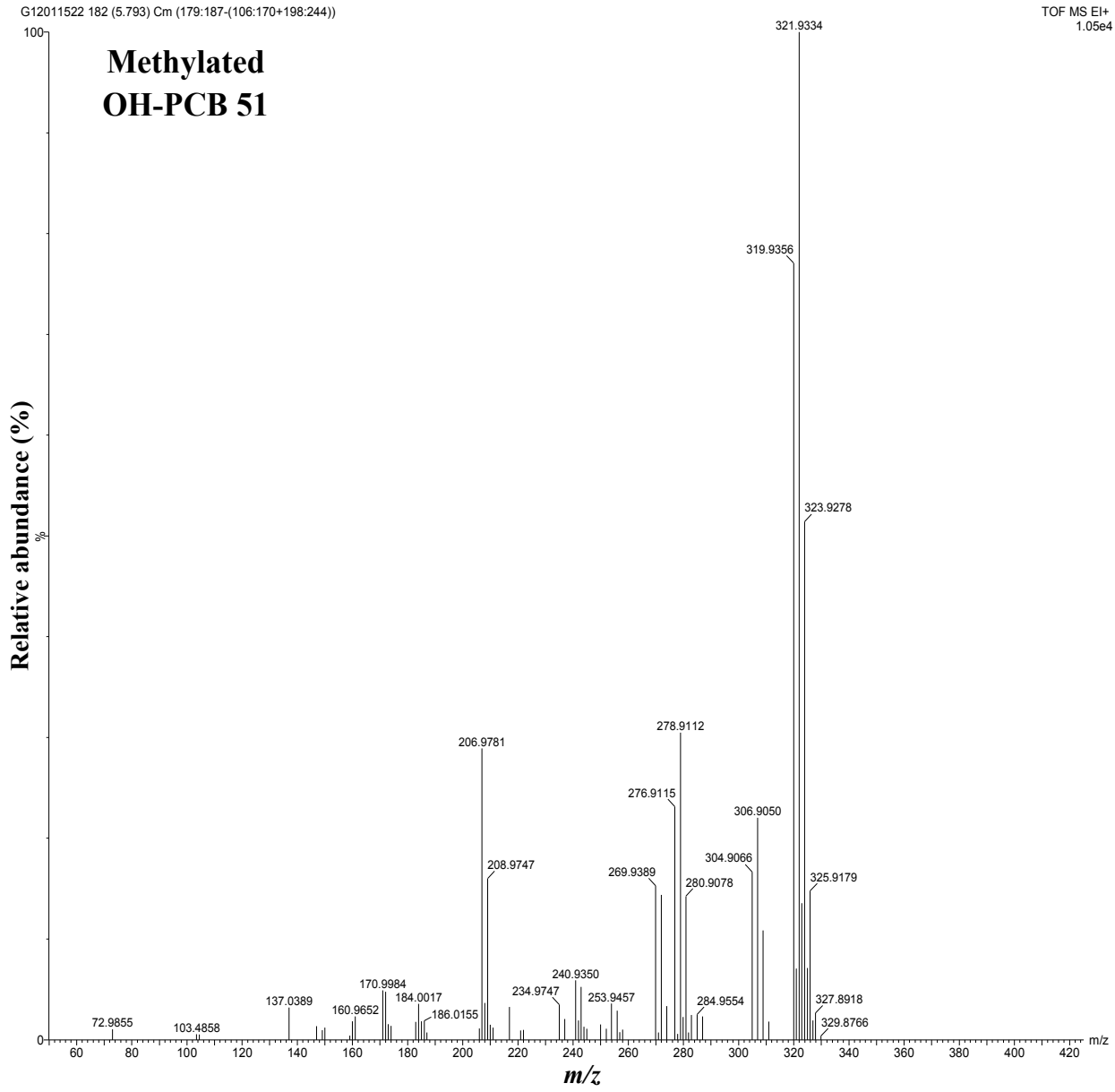


**Figure S3B. Mass spectrum of methylated OH-PCB 51 (RT 5.79 min; RRT 0.665) obtained from incubations of PCB 51 with rat liver microsomes prepared from male clofibric acid pretreated rats after derivatization.** The accurate mass determination of the monoisotopic $[M]^+$ (*m/z* 319.9356) versus the *m/z* calculated for $C_{13}H_8O^{35}Cl_4$ (*m/z* 319.9329); the isotope pattern of the molecular ion (1:1.3:0.7) compared to the theoretical ratio (1:1.3:0.6); and the fragmentation pattern were consistent with a monohydroxylated tetrachlorobiphenyl (as the corresponding methylated derivative). See the manuscript for a discussion of the chemical structure of this metabolite.

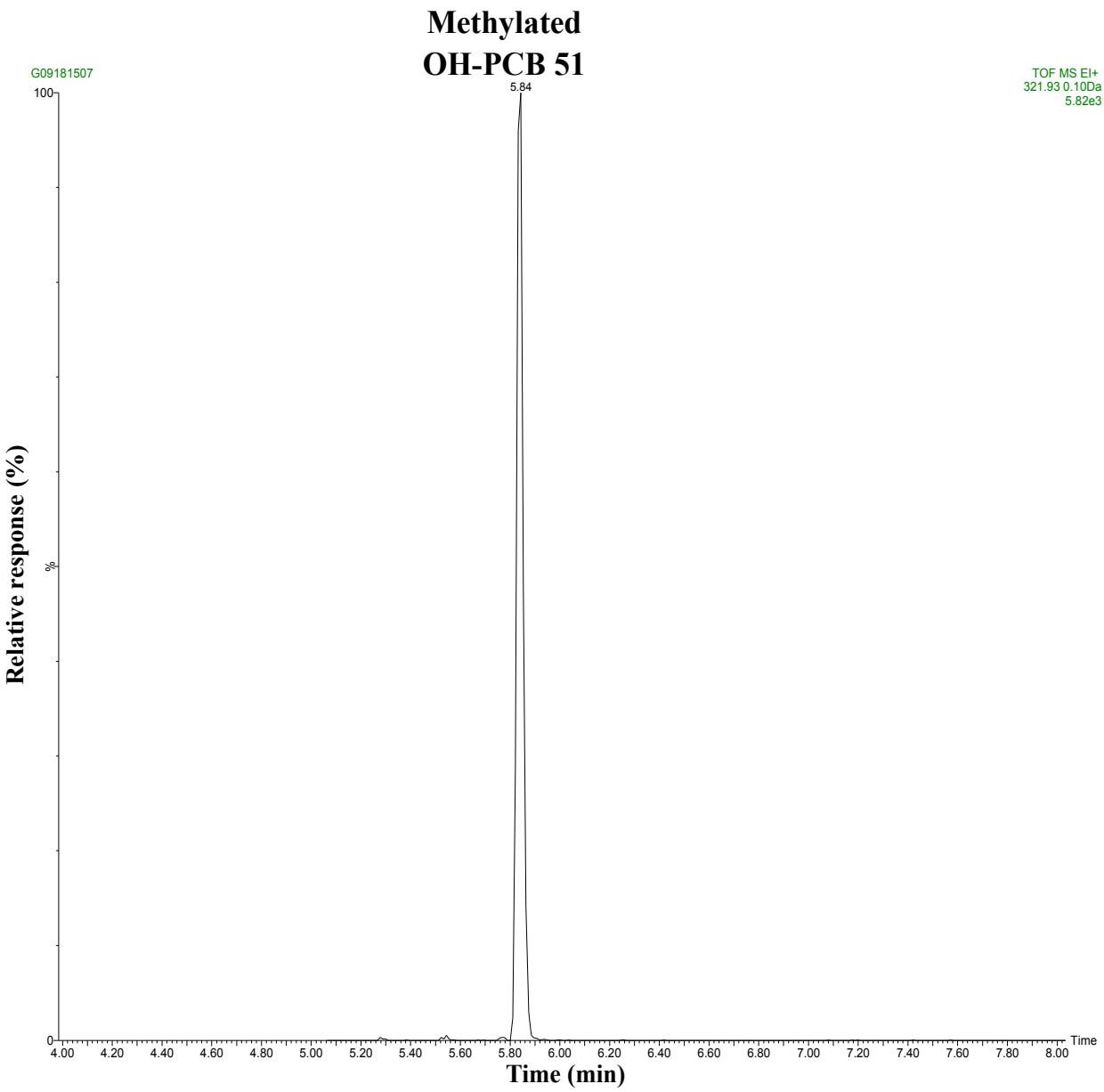


**Figure S4A. Representative gas chromatogram showing the formation of OH-PCB 51 (RT 5.84 min; RRT 0.663; *m/z* 321.9) from prochiral PCB 51 in incubations with rat liver microsomes prepared from male dexamethasone pretreated rats (analyzed as the corresponding methylated derivative).** The metabolite was tentatively identified based on its RRT and mass spectrum (Figure S4B). diOH-PCB 51, a metabolite observed in microsomal incubations with other rat liver microsomes or liver microsomes prepared from other mammalian species, was not detected. The incubation was performed in a shaking water bath at 37 °C for 2 hours and, after extraction with hexane-MTBE (1:1, *v/v*) and derivatization with diazomethane, the organic extract was analyzed on a Waters GCT Premier gas chromatograph-mass spectrometer as described in the Experimental Section.

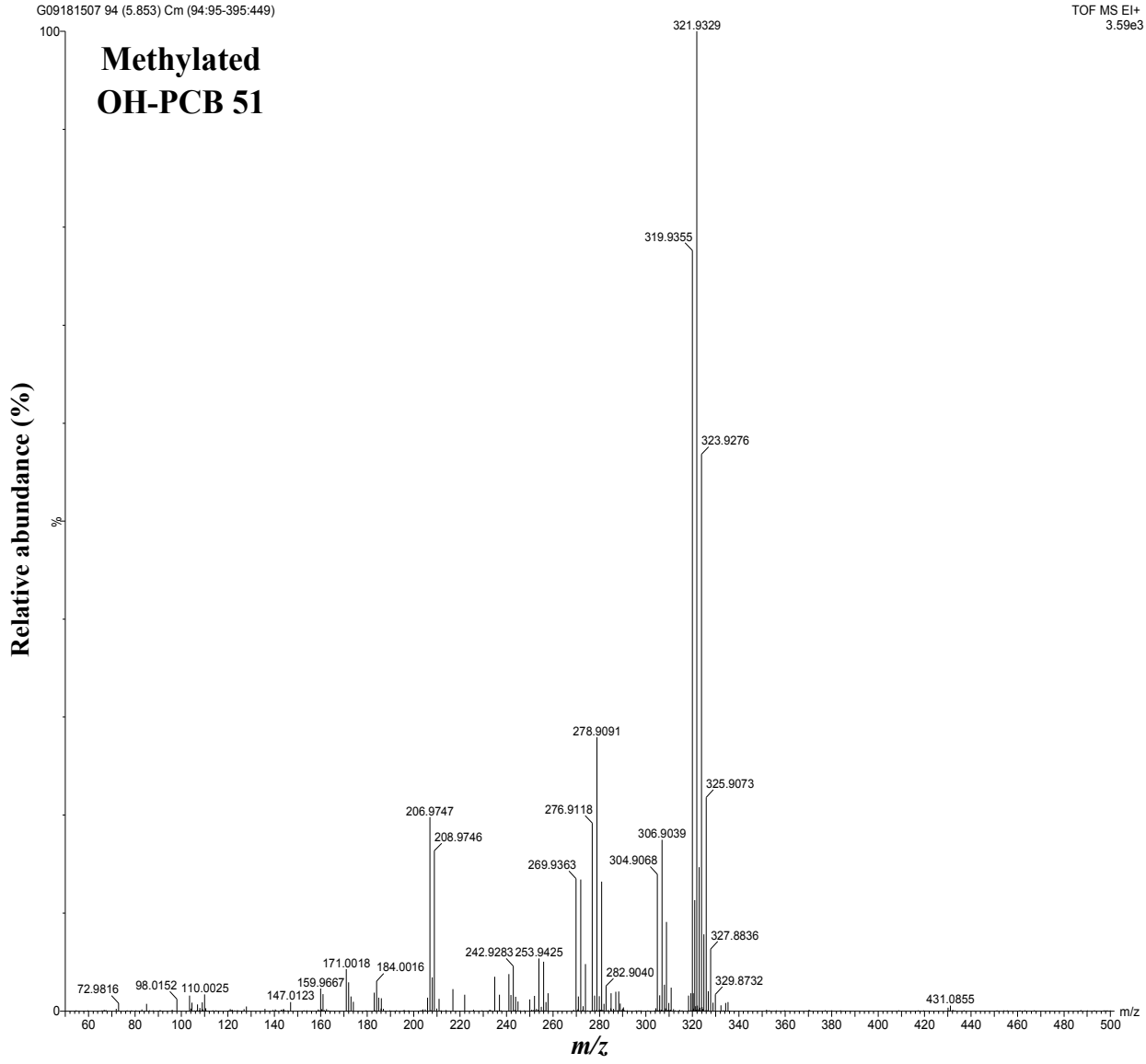


**Figure S4B. Mass spectrum of methylated OH-PCB 51 (RT 5.84; RRT 0.663) obtained from incubations of PCB 51 with rat liver microsomes prepared from male dexamethasone pretreated rats after derivatization.** The accurate mass determination of the monoisotopic $[M]^+$ (*m/z* 319.9355) versus the *m/z* calculated for $C_{13}H_8O^{35}Cl_4$ (*m/z* 319.9329); the isotope pattern of the molecular ion (1:1.3:0.7) compared to the theoretical ratio (1:1.3:0.6); and the fragmentation pattern were consistent with a monohydroxylated tetrachlorobiphenyl (as the corresponding methylated derivative). See the manuscript for a discussion of the chemical structure of this metabolite.

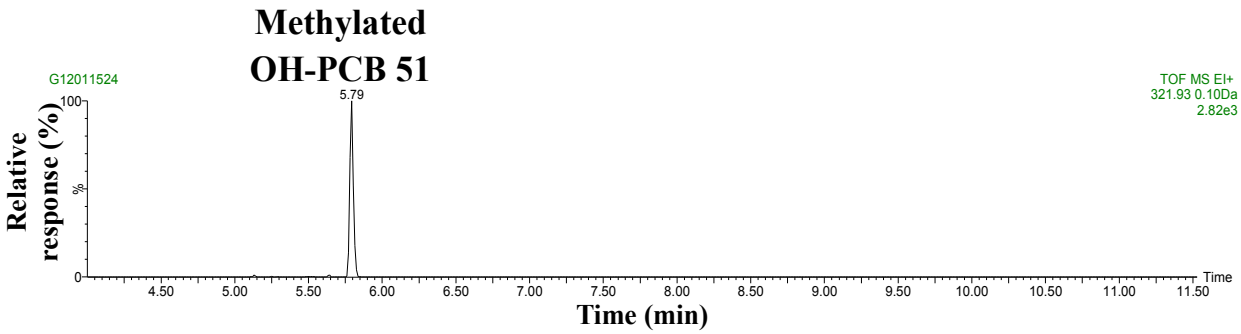


**Figure S5A. Representative gas chromatogram showing the formation of OH-PCB 51 (RT 5.79 min; RRT 0.665; *m/z* 321.9) from prochiral PCB 51 in incubations with rat liver microsomes prepared from male isoniazid pretreated rats (analyzed as the corresponding methylated derivative).** The metabolite was tentatively identified based on its RRT and mass spectrum (Figure S5B). In addition, diOH-PCB 51 (RT 6.65 min; RRT 0.763) was tentatively identified based on its RRT (data not shown). The incubation was performed in a shaking water bath at 37 °C for 2 hours and, after extraction with hexane-MTBE (1:1, *v/v*) and derivatization with diazomethane, the organic extract was analyzed on a Waters GCT Premier gas chromatograph-mass spectrometer as described in the Experimental Section.

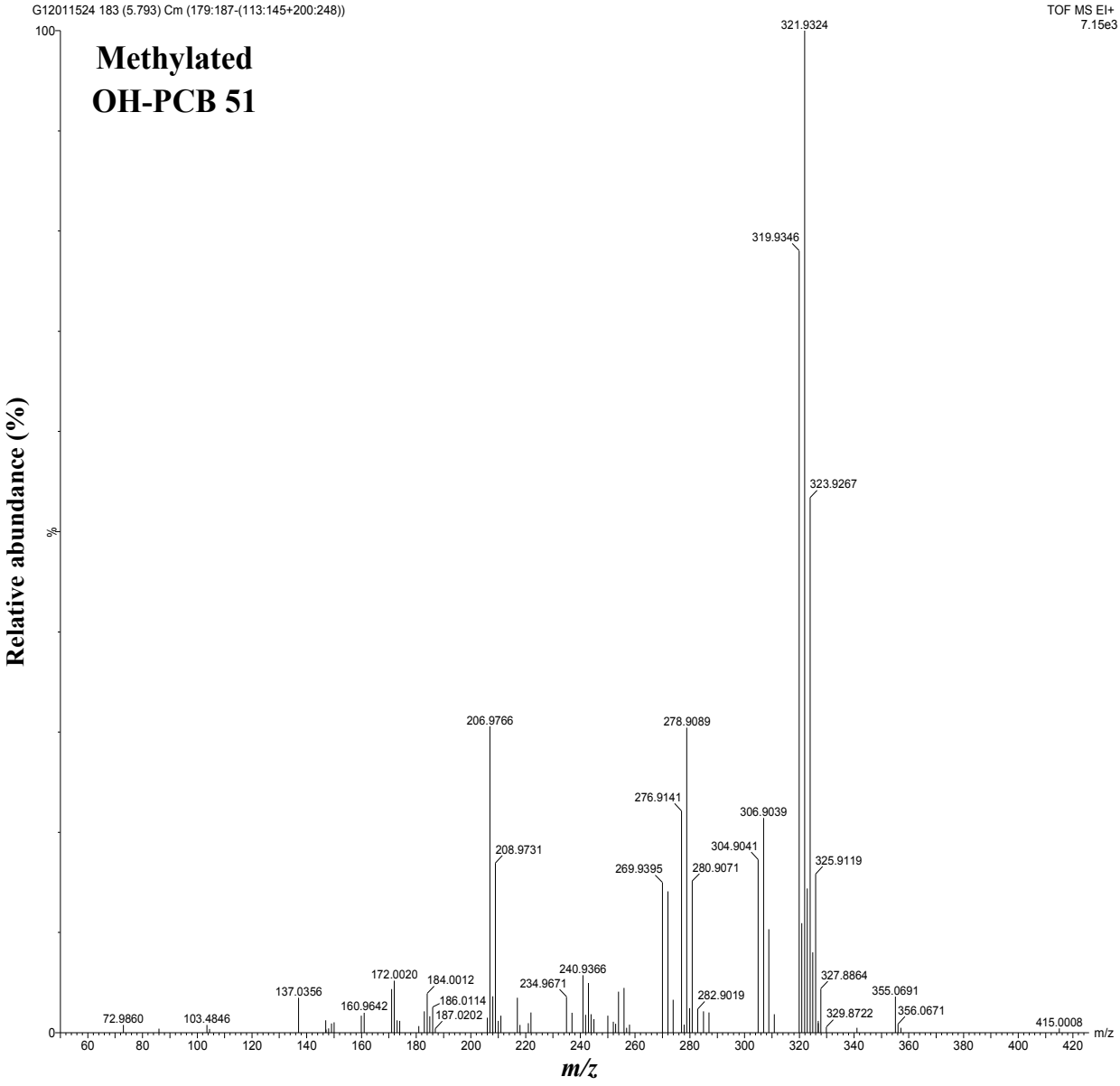


**Figure S5B. Mass spectrum of methylated OH-PCB 51 (RT 5.79 min; RRT 0.665) obtained from incubations of PCB 51 with rat liver microsomes prepared from male isoniazid pretreated rats after derivatization.** The accurate mass determination of the monoisotopic $[M]^+$ (*m/z* 319.9346) versus the *m/z* calculated for $C_{13}H_8O^{35}Cl_4$ (*m/z* 319.9329); the isotope pattern of the molecular ion (1:1.3:0.7) compared to the theoretical ratio (1:1.3:0.6); and the fragmentation pattern were consistent with a monohydroxylated tetrachlorobiphenyl (as the corresponding methylated derivative). See the manuscript for a discussion of the chemical structure of this metabolite.

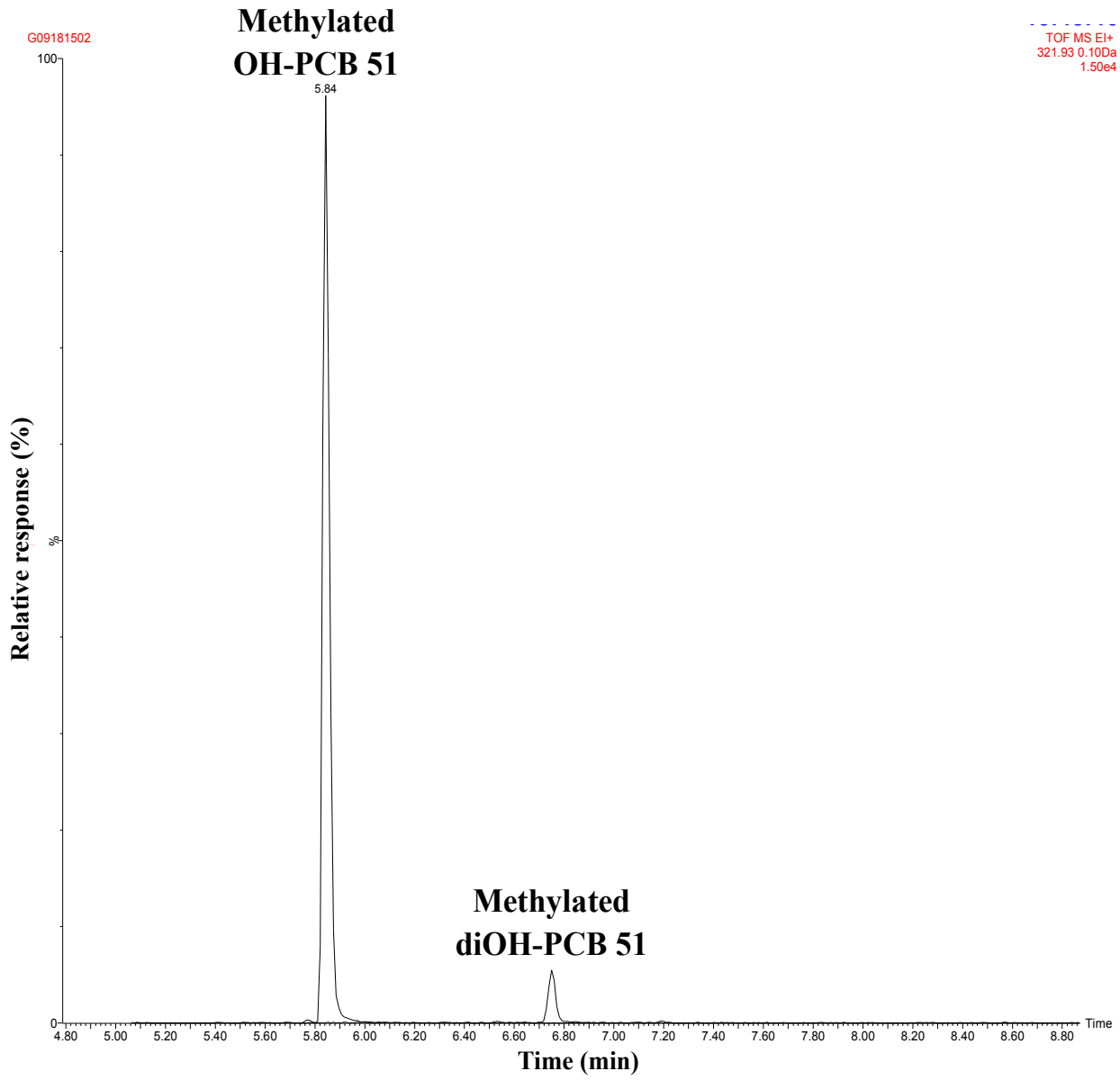


**Figure S6A. Representative gas chromatogram showing the formation of two metabolites, OH-PCB 51 (RT 5.84 min; RRT 0.662; *m/z* 321.9) and diOH-PCB 51 (RT 6.75 min; RRT 0.765; *m/z* 351.9), from prochiral PCB 51 in incubations with rat liver microsomes prepared from phenobarbital pretreated male rats (analyzed as the corresponding methylated derivative).** The metabolites were tentatively identified based on their RRTs and mass spectra (Figures S6B and S6C, respectively). The incubation was performed in a shaking water bath at 37 °C for 2 hours and, after extraction with hexane-MTBE (1:1, *v/v*) and derivatization with diazomethane, the organic extract was analyzed on a Waters GCT Premier gas chromatograph-mass spectrometer as described in the Experimental Section.

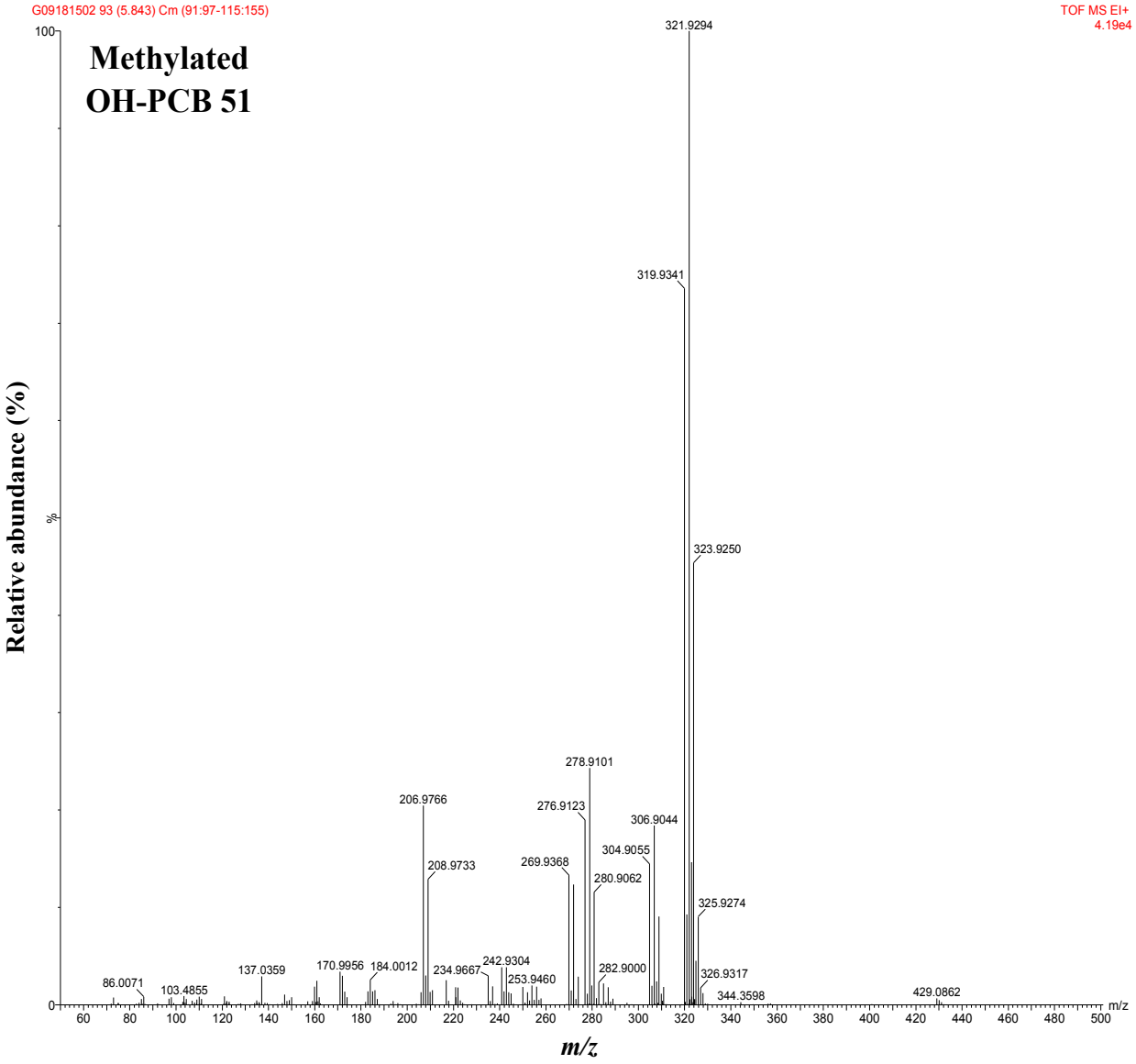


**Figure S6B. Mass spectrum of methylated OH-PCB 51 (RT 5.84 min; RRT 0.662) obtained from incubations of PCB 51 with rat liver microsomes prepared from phenobarbital pretreated male rats after derivatization.** The accurate mass determination of the monoisotopic $[M]^+$ (*m/z* 319.9346) versus the *m/z* calculated for $C_{13}H_8O^{35}Cl_4$ (*m/z* 319.9329); the isotope pattern of the molecular ion (1:1.4:0.6) compared to the theoretical ratio (1:1.3:0.6); and the fragmentation pattern were consistent with a monohydroxylated tetrachlorobiphenyl (as the corresponding methylated derivative). See the manuscript for a discussion of the chemical structure of this metabolite.

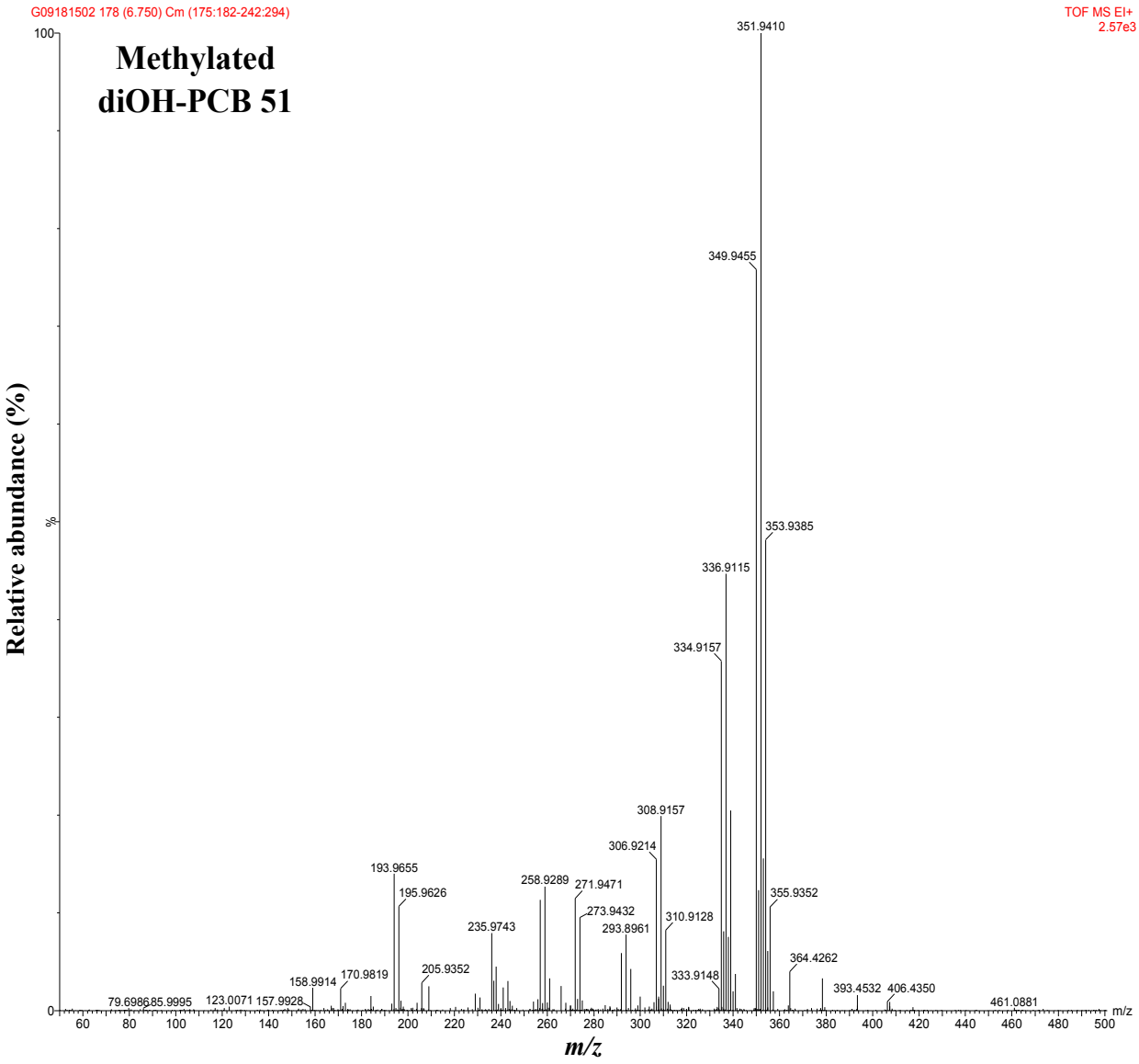


**Figure S6C. Mass spectrum of diOH-PCB 51 (RT 6.75 min; RRT 0.765) obtained from incubations of PCB 51 with rat liver microsomes prepared from phenobarbital pretreated male rats after derivatization.** The accurate mass determination of the monoisotopic $[M]^+$ (*m/z* 349.9455) versus the *m/z* calculated for $C_{14}H_{10}O_2{}^{35}Cl_4$ (*m/z* 349.9435); the isotope pattern of the molecular ion (1:1.3:0.6) compared to the theoretical ratio (1:1.3:0.6); and the fragmentation pattern were consistent with a dihydroxylated tetrachlorobiphenyl (as the corresponding methylated derivative). See the manuscript for a discussion of the chemical structure of this metabolite.

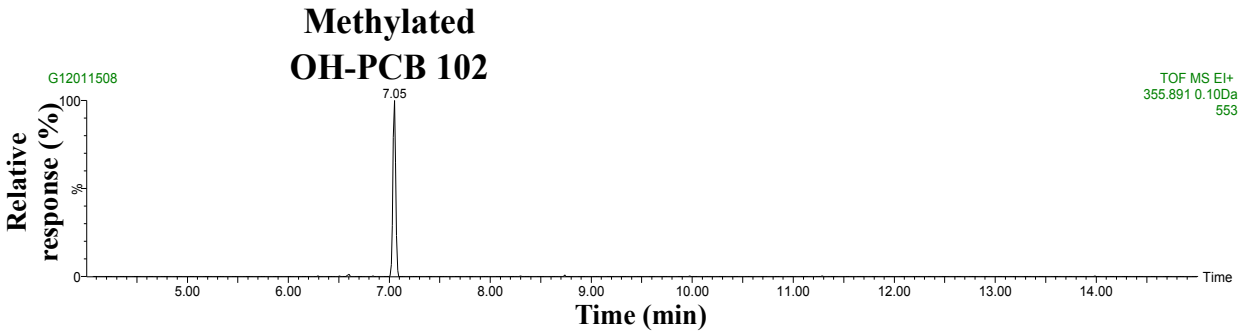


**Figure S7A. Representative gas chromatogram showing the formation of OH-PCB 102 (RT 7.05 min; RRT 0.810; *m/z* 355.9) from prochiral PCB 102 in incubations with rat liver microsomes prepared from corn oil pretreated male rats (analyzed as the corresponding methylated derivative).** The metabolite was tentatively identified based on its RRT and mass spectrum (Figure S7B). In addition, methylated diOH-PCB 102 (RT 8.36 min; RRT 0.961) was tentatively identified based on its RRT (data not shown). The incubation was performed in a shaking water bath at 37 °C for 2 hours and, after extraction with hexane-MTBE (1:1, *v/v*) and derivatization with diazomethane, the organic extract was analyzed on a Waters GCT Premier gas chromatograph-mass spectrometer as described in the Experimental Section.

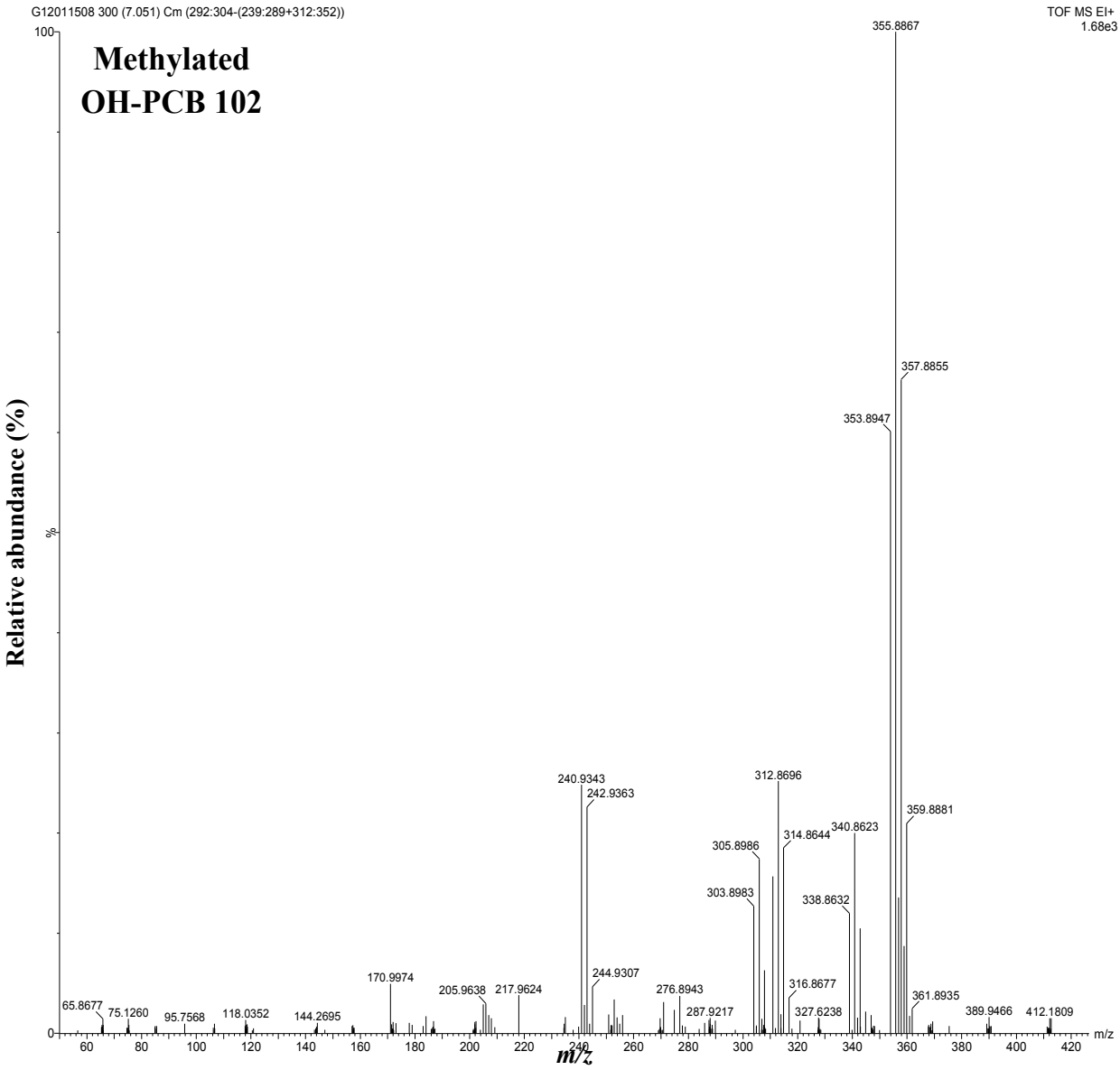


**Figure S7B. Mass spectrum of methylated OH-PCB 102 (RT 7.05; RRT 0.810) obtained from incubations of PCB 102 with rat liver microsomes prepared from male corn oil pretreated rats after derivatization.** The accurate mass determination of the monoisotopic $[M]^+$ (*m/z* 353.8947) versus the *m/z* calculated for $C_{13}H_7O_1{}^{35}Cl_5$ (*m/z* 353.8940 ); the isotope pattern of the molecular ion (1:1.7:1.1:0.3) compared to the theoretical ratio (1:1.6:1:0.3); and the fragmentation pattern were consistent with a monohydroxylated pentachlorobiphenyl (as the corresponding methylated derivative). See the manuscript for a discussion of the chemical structure of this metabolite.

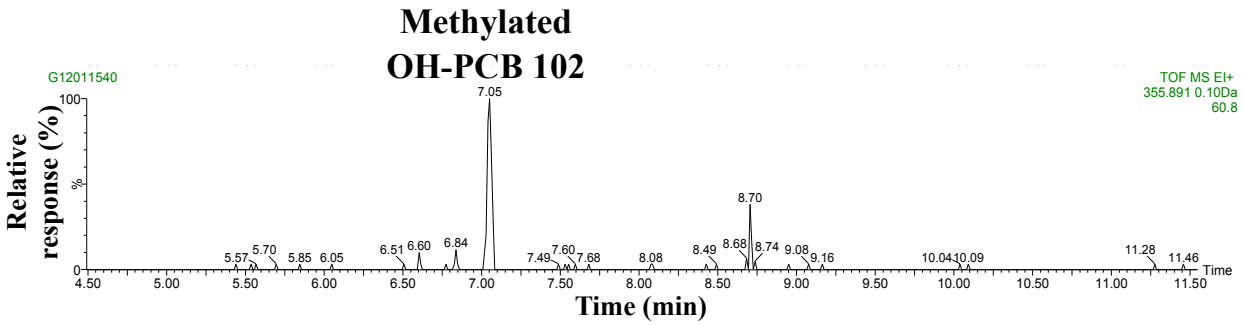


**Figure S8. Representative gas chromatogram showing the formation of OH-PCB 102 (RT 7.05 min; RRT 0.810; *m/z* 355.9) from prochiral PCB 102 in incubations with rat liver microsomes prepared from male β-naphthoflavone pretreated rats (analyzed as the corresponding methylated derivative).** The metabolite was tentatively identified based on its RRT. diOH-PCB 102, a metabolite observed in microsomal incubations with other rat liver microsomes or liver microsomes prepared from other mammalian species, was not detected. The incubation was performed in a shaking water bath at 37 °C for 2 hours and, after extraction with hexane-MTBE (1:1, *v/v*) and derivatization with diazomethane, the organic extract was analyzed on a Waters GCT Premier gas chromatograph-mass spectrometer as described in the Experimental Section.

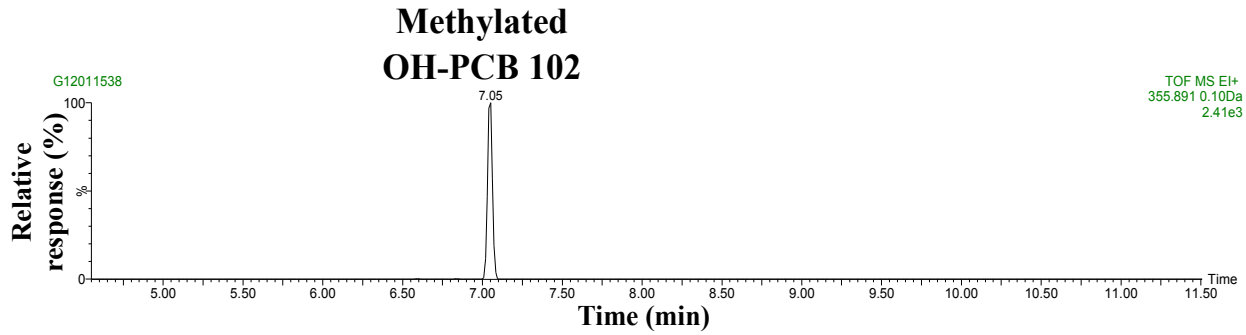


**Figure S9A. Representative gas chromatogram showing the formation of OH-PCB 102 (RT 7.05 min; RRT 0.810; *m/z* 355.9) from prochiral PCB 102 in incubations with rat liver microsomes prepared from male clofibric acid pretreated rats (analyzed as the corresponding methylated derivative).** The metabolite was tentatively identified based on its RRT and mass spectrum (Figure S9B). In addition, diOH-PCB 102 (RT 8.36 min; RRT 0.960), as dimethylated derivative, was tentatively identified based on its RRT (data not shown). The incubation was performed in a shaking water bath at 37 °C for 2 hours and, after extraction with hexane-MTBE (1:1, *v/v*) and derivatization with diazomethane, the organic extract was analyzed on a Waters GCT Premier gas chromatograph-mass spectrometer as described in the Experimental Section.

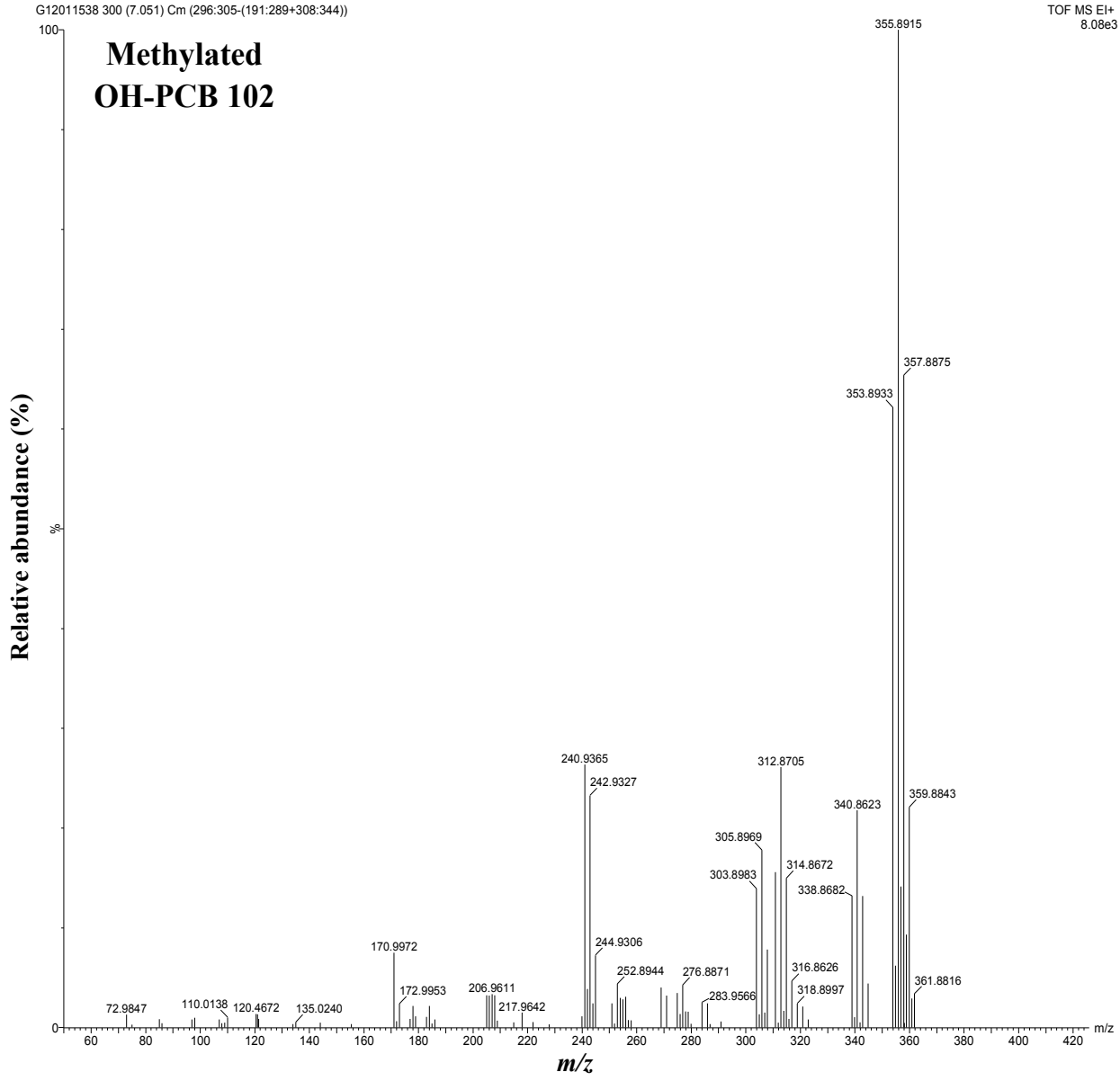


**Figure S9B. Mass spectrum of methylated OH-PCB 102 (RT 7.05 min; RRT 0.810) obtained from incubations of PCB 102 with rat liver microsomes prepared from male clofibric acid rats after derivatization.** The accurate mass determination of the monoisotopic $[M]^+$ (*m/z* 353.8933) versus the *m/z* calculated for $C_{13}H_7O_1{}^{35}Cl_5$ (*m/z* 353.8940); the isotope pattern of the molecular ion (1:1.6:1.1:0.4) compared to the theoretical ratio (1:1.6:1:0.3); and the fragmentation pattern were consistent with a monohydroxylated pentachlorobiphenyl (as the corresponding methylated derivative). See the manuscript for a discussion of the chemical structure of this metabolite.

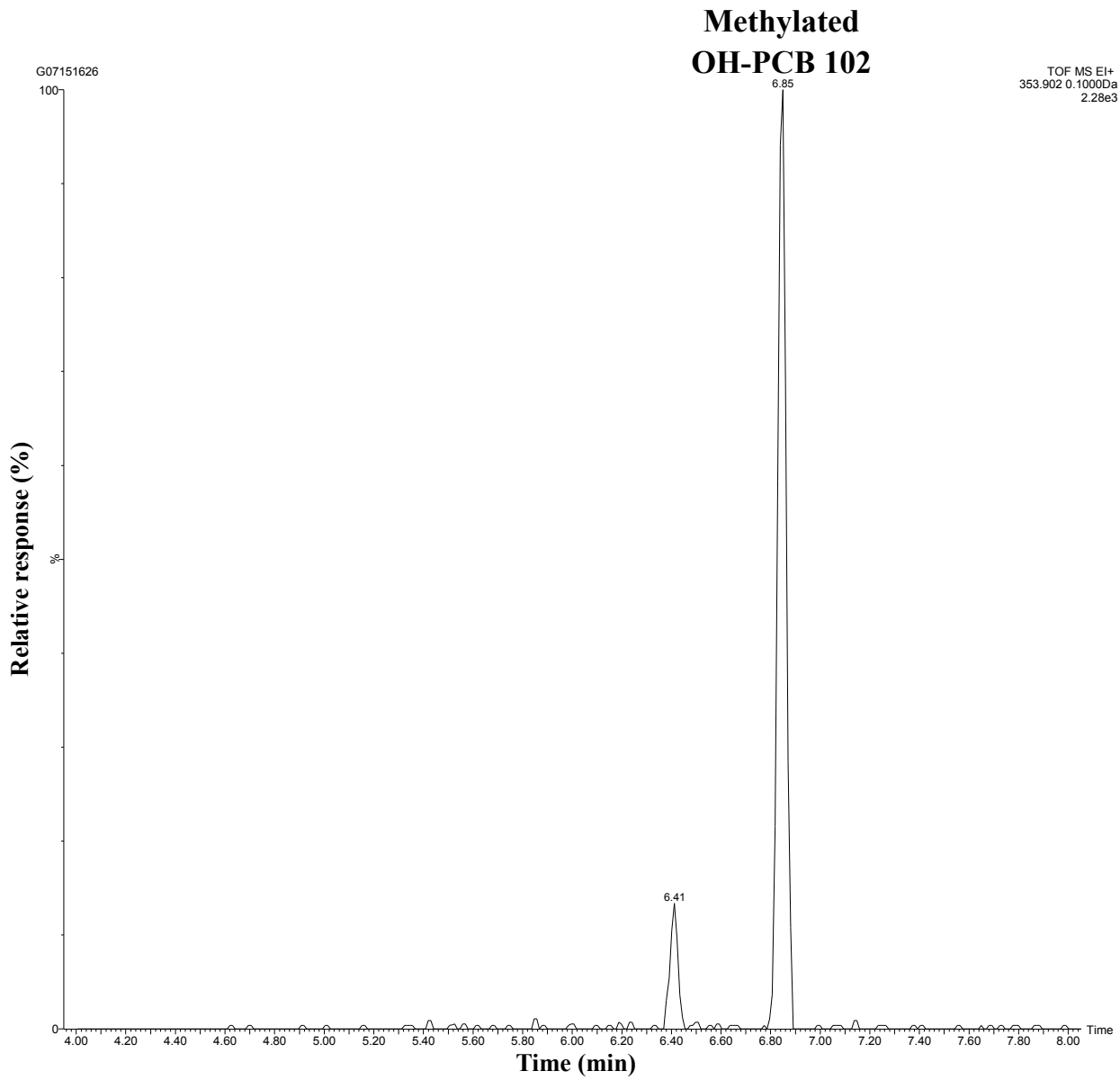


**Figure S10A. Representative gas chromatogram showing the formation of OH-PCB 102 (RT 6.85 min; RRT 0.815; *m/z* 355.9) from prochiral PCB 102 in incubations with rat liver microsomes prepared from male dexamethasone pretreated rats (analyzed as the corresponding methylated derivative).** The metabolite was tentatively identified based on its mass spectrum (Figure S10B); however, its RRT was slightly higher than the average RRT of OH-PCB 102, possibly because the sample was not analyzed in the same sequence as all other samples. diOH-PCB 102, a metabolite observed in microsomal incubations with other rat liver microsomes or liver microsomes prepared from other mammalian species, was not detected. The incubation was performed in a shaking water bath at 37 °C for 2 hours and, after extraction with hexane-MTBE (1:1, *v/v*) and derivatization with diazomethane, the organic extract was analyzed on a Waters GCT Premier gas chromatograph-mass spectrometer as described in the Experimental Section.

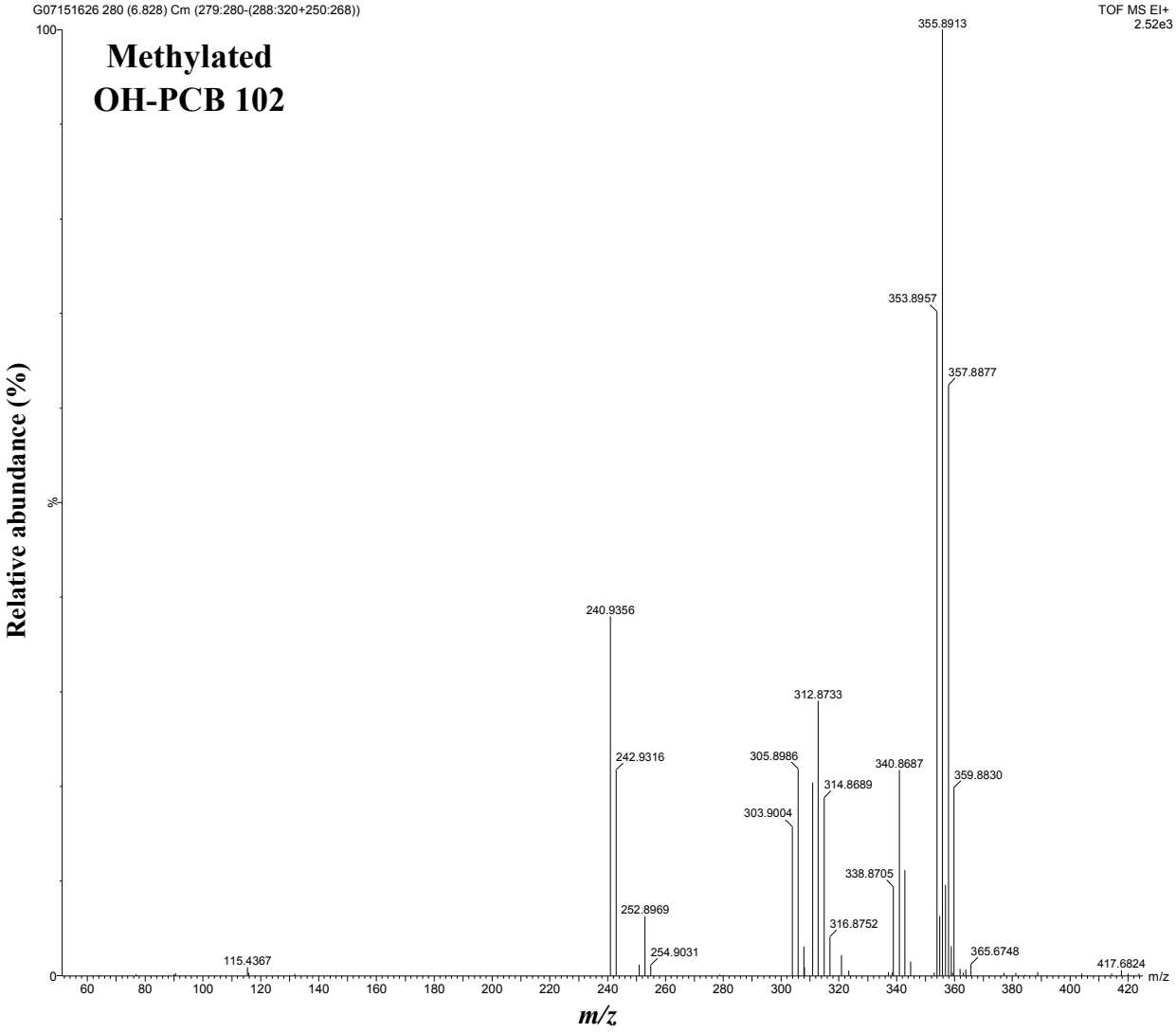


**Figure S10B. Mass spectrum of methylated OH-PCB 102 (RT 6.85 min; RRT 0.815) obtained from incubations of PCB 102 with rat liver microsomes prepared from male dexamethasone pretreated rats after derivatization.** The accurate mass determination of the monoisotopic $[M]^+$ (*m/z* 353.8957) versus the *m/z* calculated for $C_{13}H_7O_1{}^{35}Cl_5$ (*m/z* 353.8940); the isotope pattern of the molecular ion (1:1.4:0.9:0.3) compared to the theoretical ratio (1:1.6:1:0.3); and the fragmentation pattern were consistent with a monohydroxylated pentachlorobiphenyl (as the corresponding methylated derivative). See the manuscript for a discussion of the chemical structure of this metabolite.

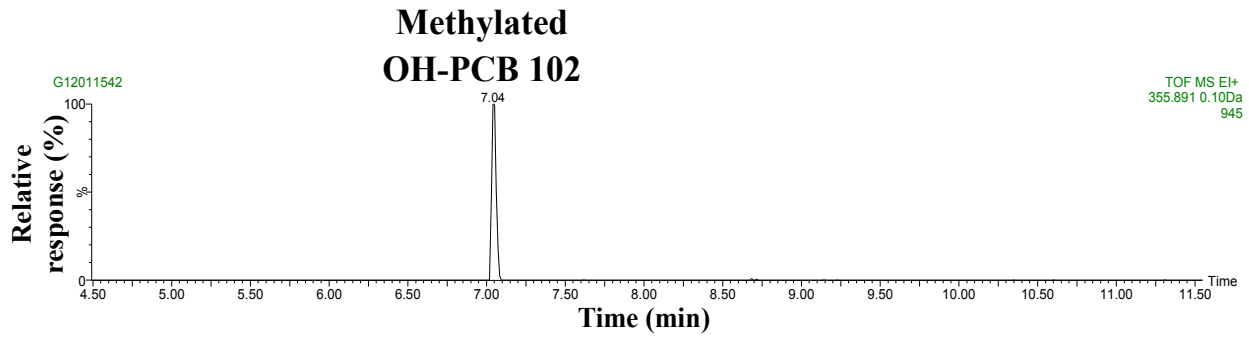


**Figure S11A. Representative gas chromatogram showing the formation of OH-PCB 102 (RT 7.04 min; RRT 0.808; *m/z* 355.9) from prochiral PCB 102 in incubations with rat liver microsomes prepared from male isoniazid pretreated rats (analyzed as the corresponding methylated derivative).** The metabolite was tentatively identified based on its RRT and mass spectrum (Figure S11B). In addition, diOH-PCB 102 (RT 8.35 min; RRT 0.96), as dimethylated derivative, was tentatively identified based on its RRT (data not shown). The incubation was performed in a shaking water bath at 37 °C for 2 hours and, after extraction with hexane-MTBE (1:1, *v/v*) and derivatization with diazomethane, the organic extract was analyzed on a Waters GCT Premier gas chromatograph-mass spectrometer as described in the Experimental Section.

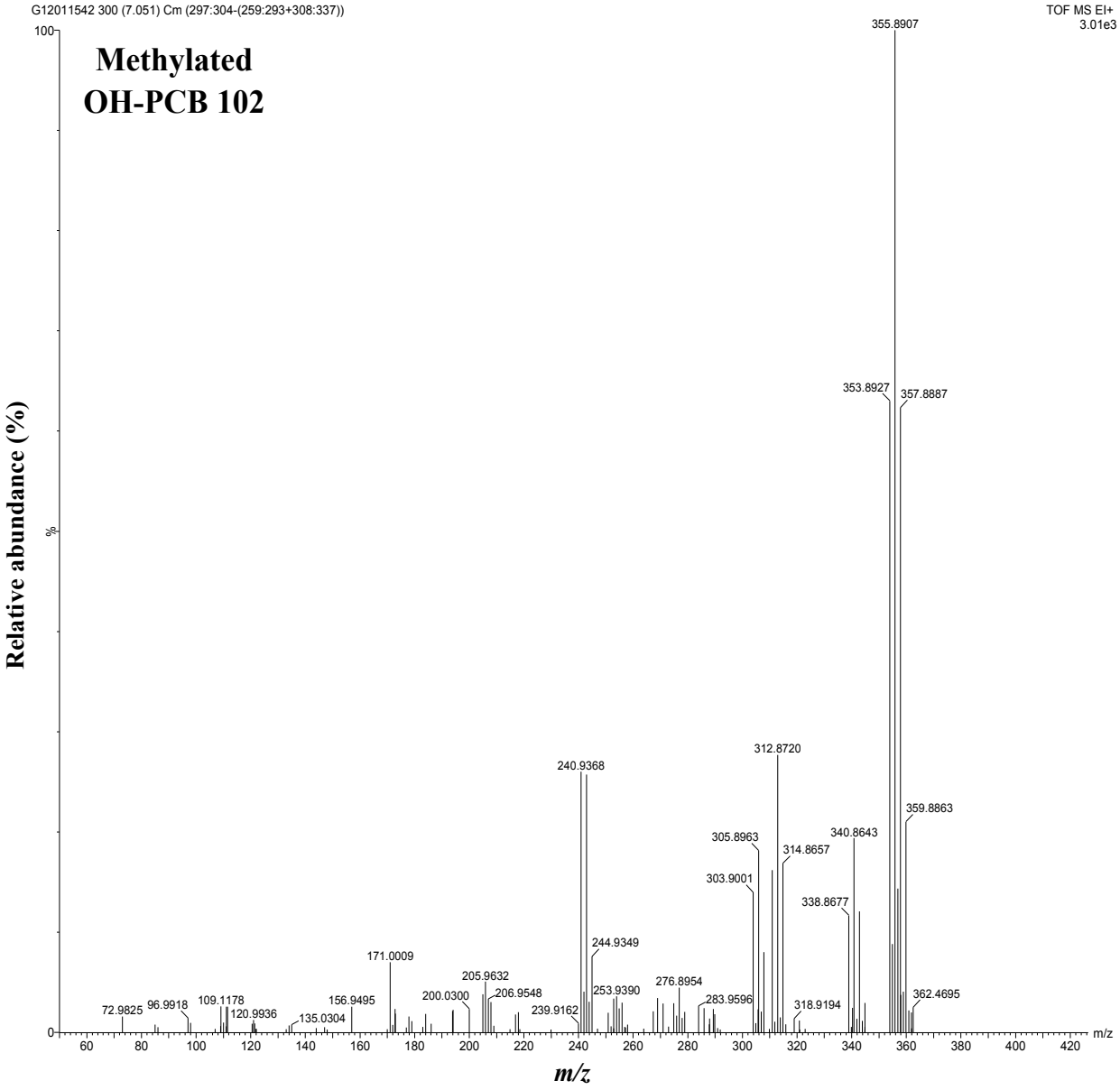


**Figure S11B. Mass spectrum of methylated OH-PCB 102 (RT 7.045 min; RRT 0.808) obtained from incubations of PCB 102 with rat liver microsomes prepared from male isoniazid pretreated rats after derivatization.** The accurate mass determination of the monoisotopic $[M]^+$ (*m/z* 353.8960 versus the *m/z* calculated for $C_{13}H_7O_1{}^{35}Cl_5$ (*m/z* 353.8940); the isotope pattern of the molecular ion (1:1.6:1:0.3) compared to the theoretical ratio (1:1.6:1:0.3); and the fragmentation pattern were consistent with a monohydroxylated pentachlorobiphenyl (as the corresponding methylated derivative). See the manuscript for a discussion of the chemical structure of this metabolite.

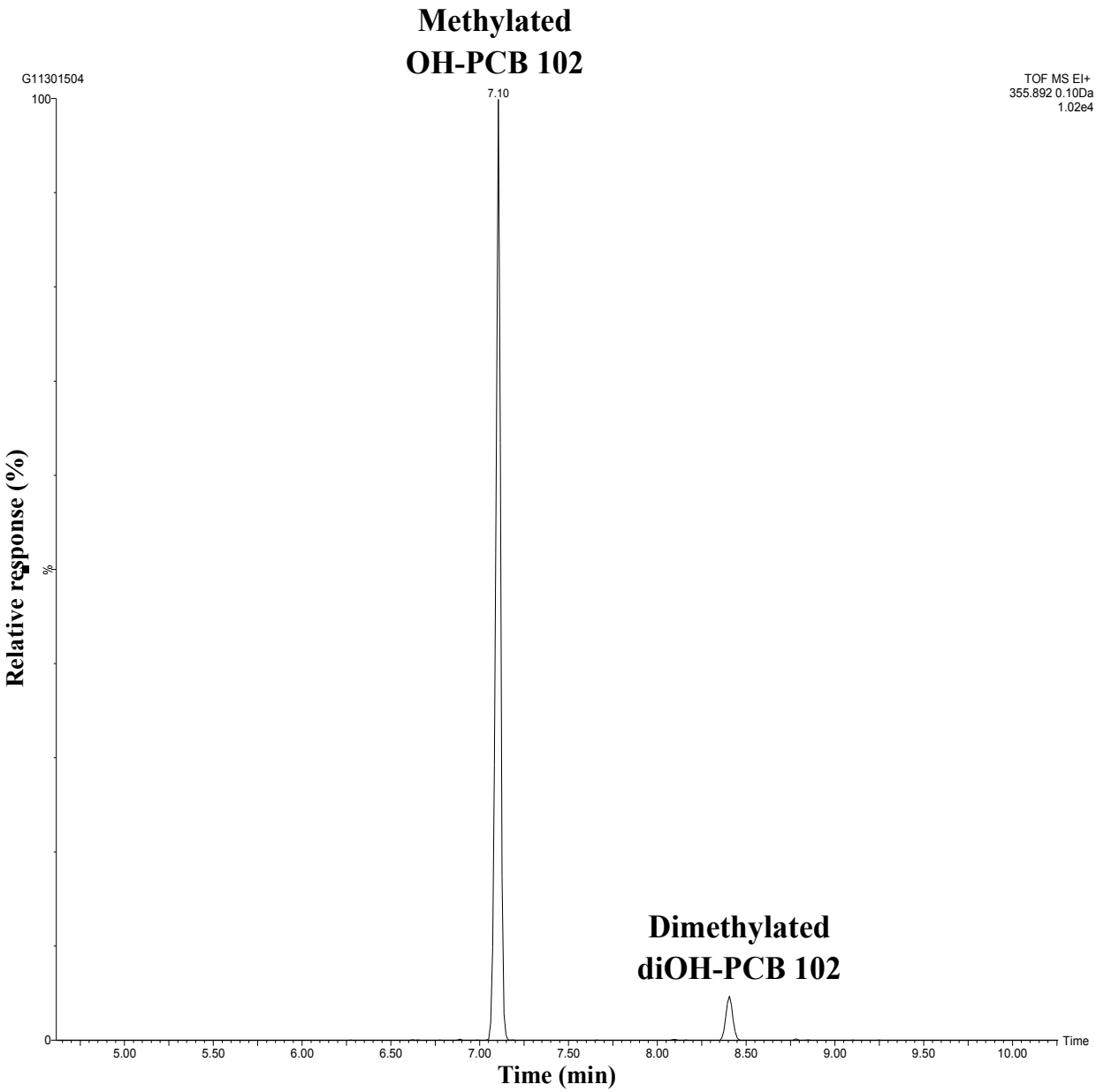


**Figure S12A. Representative gas chromatogram showing the formation of two metabolites, OH-PCB 102 (RT 7.10 min; RRT 0.808; *m/z* 355.9) and diOH-PCB 102 (RT 8.40 min; RRT 0.956; *m/z* 385.9), from prochiral PCB 102 in incubations with rat liver microsomes prepared from male phenobarbital pretreated rats (analyzed as the corresponding methylated derivative).** The metabolites were tentatively identified based on their RRTs and mass spectra (Figures S12B and S12C, respectively). The incubation was performed in a shaking water bath at 37 °C for 2 hours and, after extraction with hexane-MTBE (1:1, *v/v*) and derivatization with diazomethane, the organic extract was analyzed on a Waters GCT Premier gas chromatograph-mass spectrometer as described in the Experimental Section.

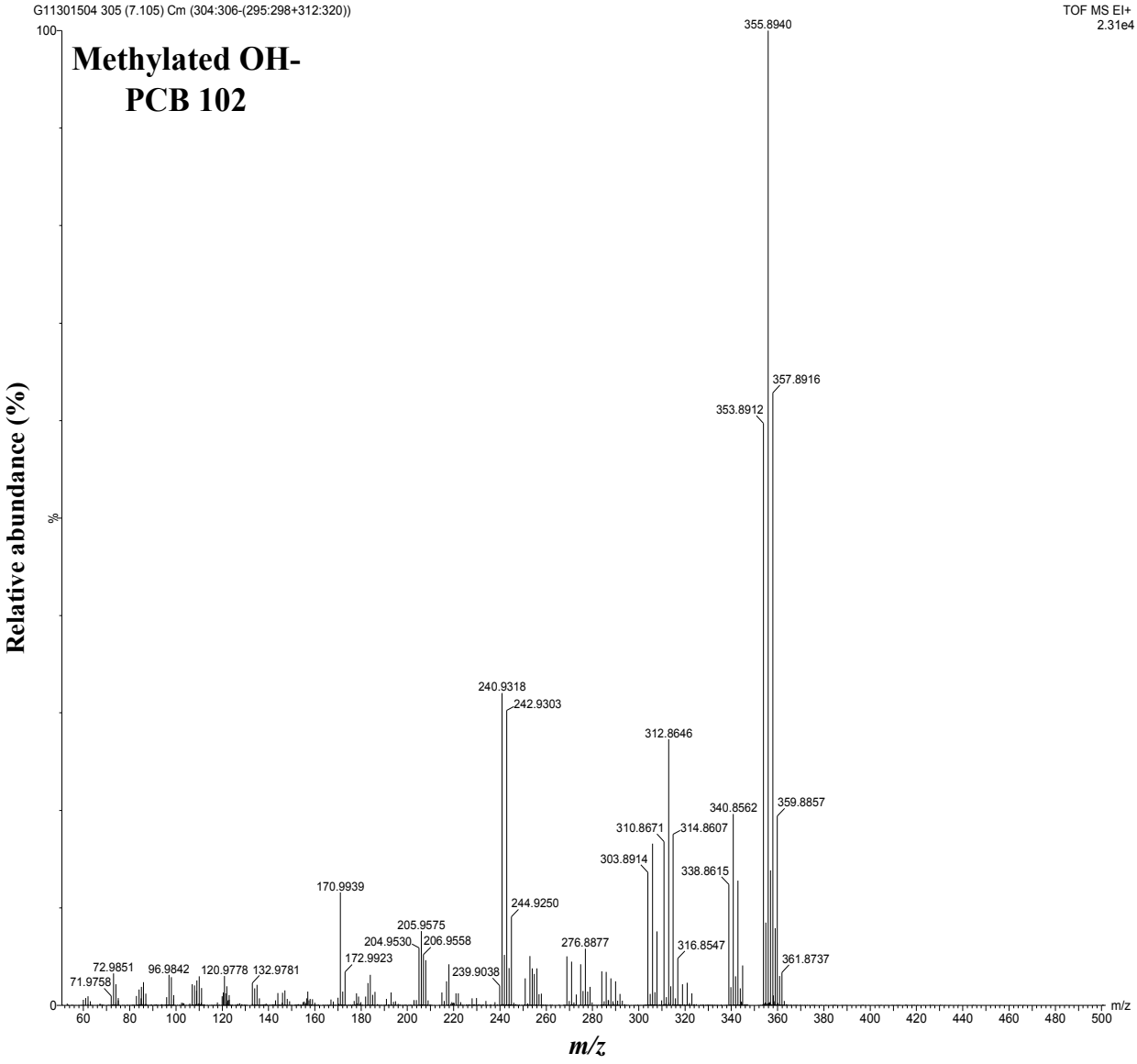


**Figure S12B. Mass spectrum of methylated OH-PCB 102 (RT 7.10 min; RRT 0.808) obtained from incubations of PCB 102 with rat liver microsomes prepared from male phenobarbital pretreated rats after derivatization.** The accurate mass determination of the monoisotopic $[M]^+$ (*m/z* 353.8912) versus the *m/z* calculated for $C_{13}H_7O_1{}^{35}Cl_5$ (*m/z* 353.8940); the isotope pattern of the molecular ion (1:1.4:1:0.3) compared to the theoretical ratio (1:1.6:1:0.3); and the fragmentation pattern were consistent with a monohydroxylated pentachlorobiphenyl (as the corresponding methylated derivative). See the manuscript for a discussion of the chemical structure of this metabolite.

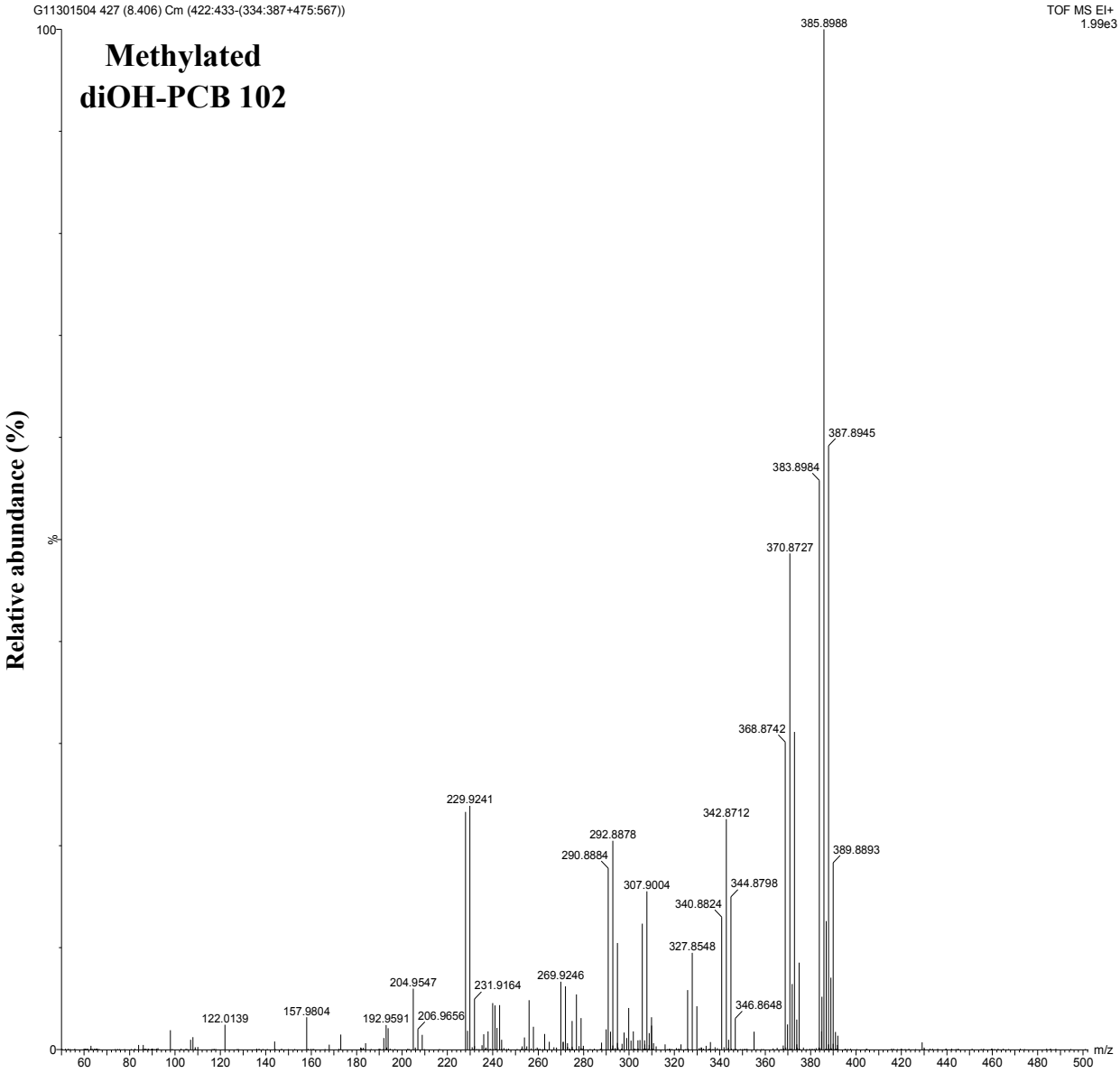


**Figure S12C. Mass spectrum of methylated diOH-PCB 102 (RT 8.40 min; RRT 0.9656) obtained from incubations of PCB 102 with rat liver microsomes prepared from male phenobarbital pretreated rats after derivatization.** The accurate mass determination of the monoisotopic $[M]^+$ (*m/z* 383.8984) did not match the *m/z* calculated for $C_{14}H_9O_2{}^{35}Cl_5$ (*m/z* 383.9045) due to a co-eluting impurity; however, the isotope pattern of the molecular ion (1:1.7:1.1:0.4) compared to the theoretical ratio (1:1.6:1:0.3) and the fragmentation pattern were consistent with a dihydroxylated pentachlorobiphenyl (as the corresponding methylated derivative). See the manuscript for a discussion of the chemical structure of this metabolite.

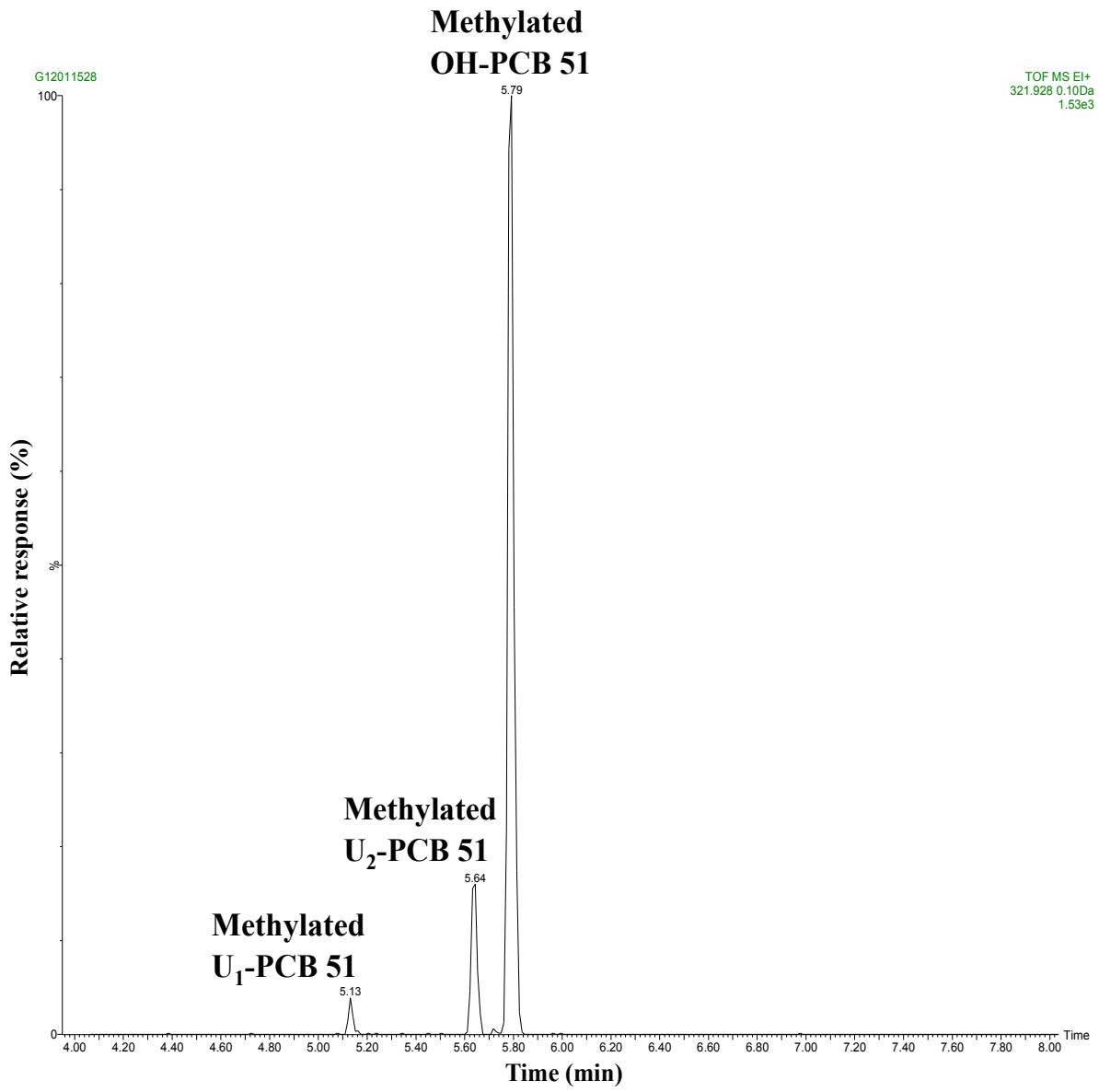


**Figure S13A. Representative gas chromatogram showing the formation of OH-PCB 51 (RT 5.79 min; RRT 0.664; *m/z* 321.9) and two unknown metabolites, $U_1$-PCB 51 (RT 5.13 min; RRT 0.558; *m/z* 321.9) and $U_2$-PCB 51 (RT 5.64 min; RRT 0.646; *m/z* 321.9), from prochiral PCB 51 in incubations with female dog liver microsomes (analyzed as the corresponding methylated derivative).** The methylated derivative of OH-PCB 51 was tentatively identified based on its RRT and mass spectrum (Figure S13B). The mass spectra of $U_1$-PCB 51 and methylated $U_2$-PCB 51, both as methylated derivatives, are shown in Figures S13C and S13D, respectively. diOH-PCB 51, a metabolite observed in microsomal incubations with other rat liver microsomes or liver microsomes prepared from other mammalian species, was not detected. The incubation was performed in a shaking water bath at 37 °C for 2 hours and, after extraction with hexane-MTBE (1:1, *v/v*) and derivatization with diazomethane, the organic extract was analyzed on a Waters GCT Premier gas chromatograph-mass spectrometer as described in the Experimental Section.

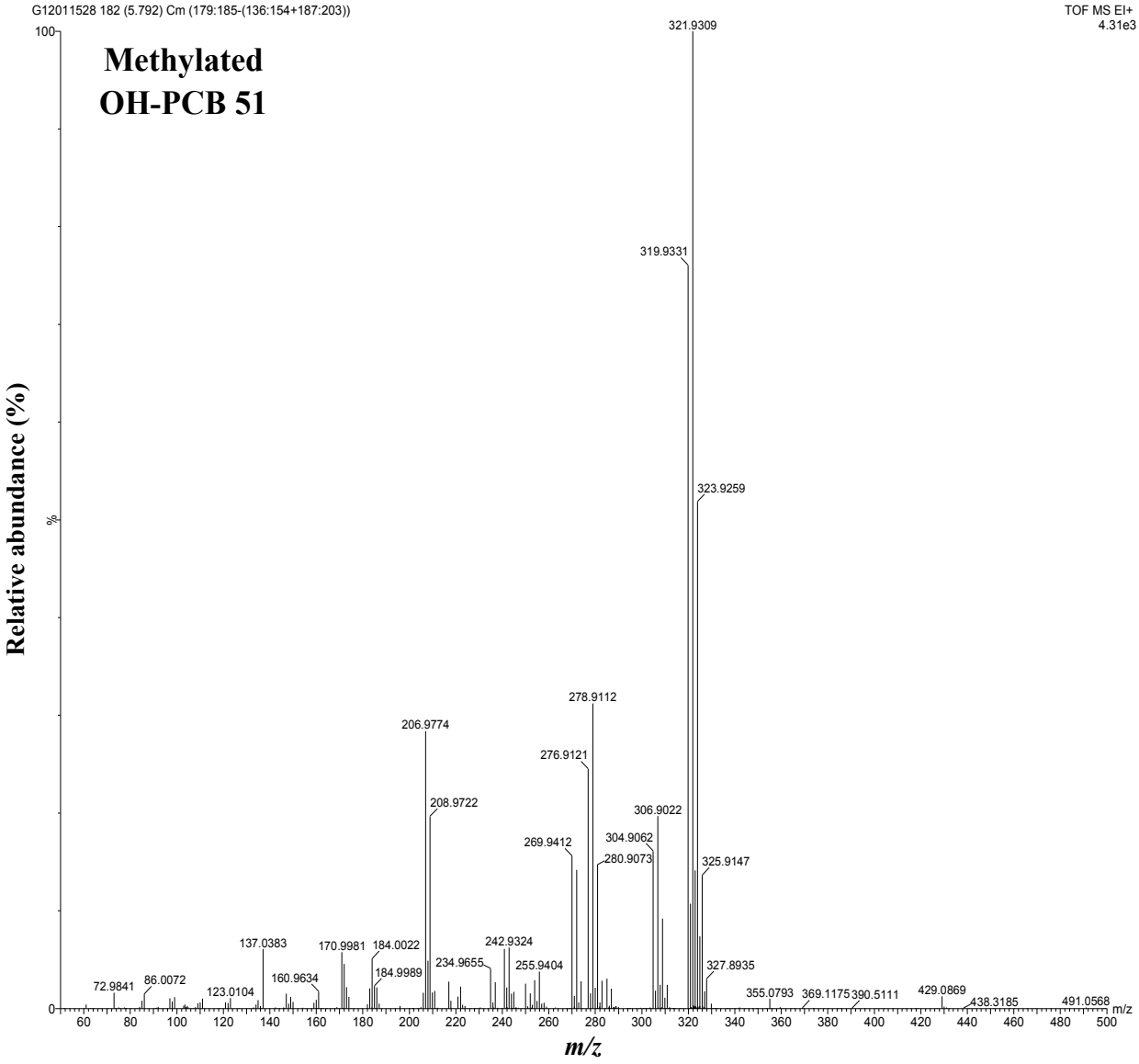


**Figure S13B. Mass spectrum of methylated OH-PCB 51 (RT 5.79 min; RRT 0.664) obtained from incubations of PCB 51 with female dog liver microsomes after derivatization.** The accurate mass determination of the monoisotopic $[M]^+$ (*m/z* 319.9331) versus the *m/z* alculated for $C_{13}H_8O^{35}Cl_4$ (*m/z* 319.9329); the isotope pattern of the molecular ion (1:1.3:0.7) compared to the theoretical ratio (1:1.3:0.6); and the fragmentation pattern were consistent with a monohydroxylated tetrachlorobiphenyl (as the corresponding methylated derivative). See the manuscript for a discussion of the chemical structure of this metabolite.

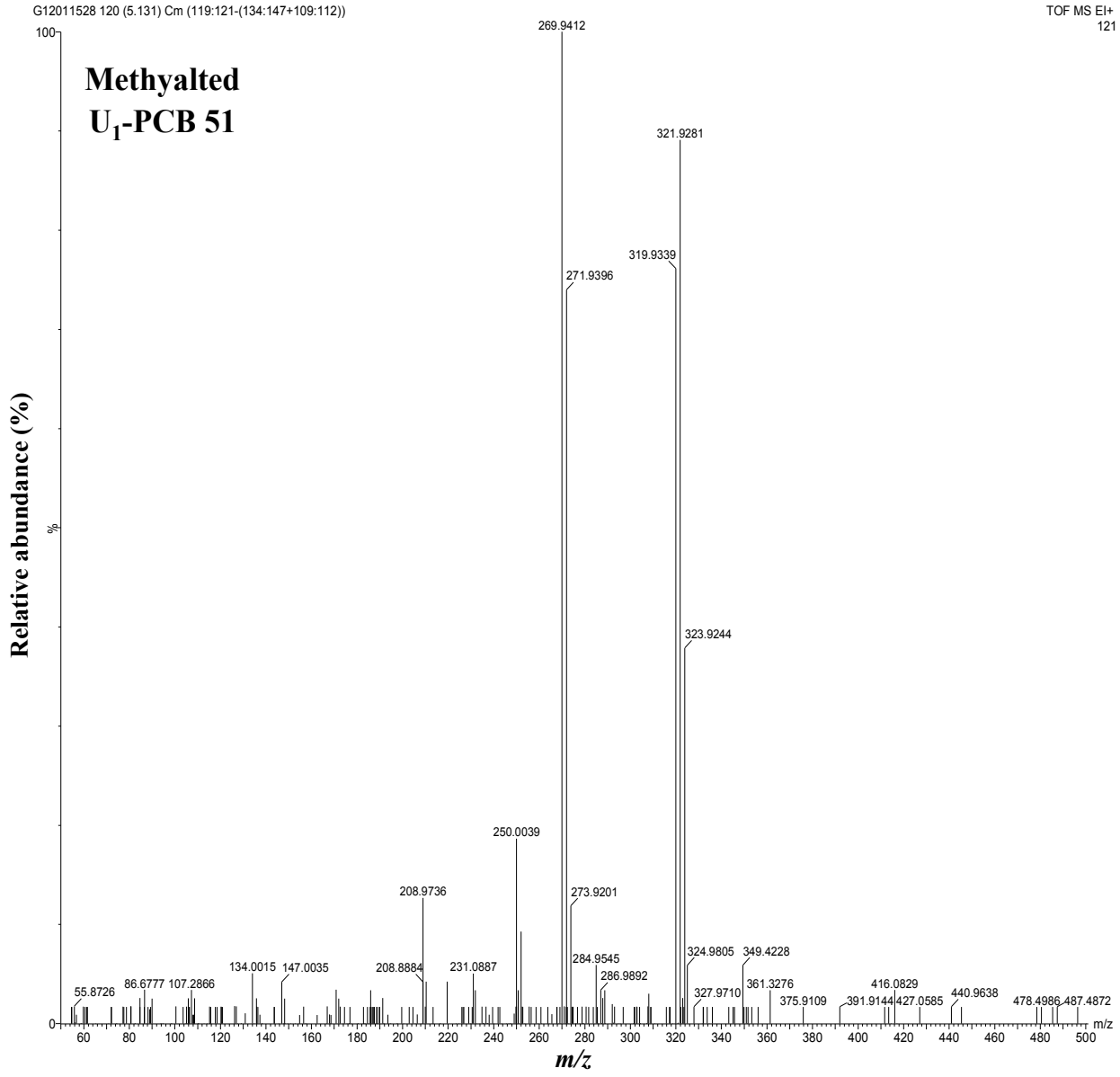


**Figure S13C. Mass spectrum of methylated $U_1$-PCB 51 (RT 5.13 min; RRT 0.588) obtained from incubations of PCB 51 with female dog liver microsomes after derivatization.** The accurate mass determination of the monoisotopic $[M]^+$ (*m/z* 319.9316) was consistent with *m/z* calculated for $C_{13}H_8O^{35}Cl_4$ (*m/z* 319.9329); however, the isotope pattern of the molecular ion (1:1:0.5) did not match to the theoretical ratio (1:1.3:0.6) because of the low levels of this metabolite. Interestingly, the mass spectrum of this minor metabolite shows a distinct $[M-50]^+$ fragment. This fragment is characteristic of a dibenzofuran daughter ion formed from the loss of $CH_3$ from an *ortho* methoxy group in one phenyl ring and an *ortho* chlorine in the other phenyl ring,[4-6] thus suggesting the formation of an *ortho* monohydroxylated metabolite of PCB 51. Similarly, earlier studies demonstrate the formation of *ortho* hydroxylated PCB metabolite in incubations of 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) with dog liver microsomes[7] or in incubations of 2,2',3,3',6,6'-hexachlorobiphenyl (PCB 136) with recombinant dog CYP2B11.[8] Additional studies are needed to confirm the formation and structure of this minor metabolite.

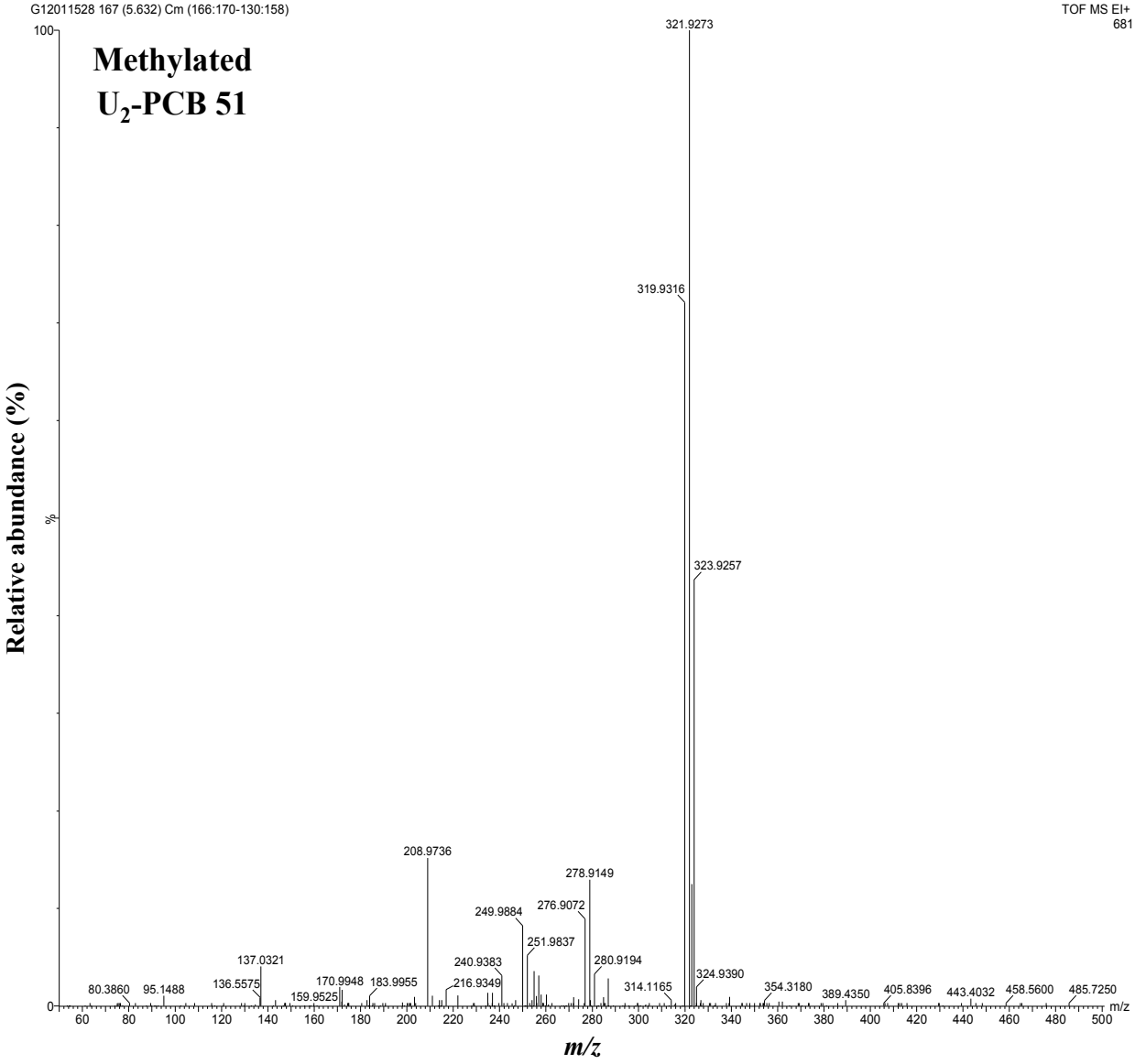


**Figure S13D. Mass spectrum of methylated $U_2$-PCB 51 (RT 5.64 min; RRT 0.646) obtained from incubations of PCB 51 with female dog liver microsomes after derivatization.** The accurate mass determination of the monoisotopic $[M]^+$ (*m/z* 319.9316) versus the *m/z* calculated for $C_{13}H_8O^{35}Cl_4$ (*m/z* 319.9329); the isotope pattern of the molecular ion (1:1.4:0.6) compared to the theoretical ratio (1:1.3:0.6); and the recognizable fragmentation pattern were consistent with a monohydroxylated tetrachlorobiphenyl (as the corresponding methylated derivative).

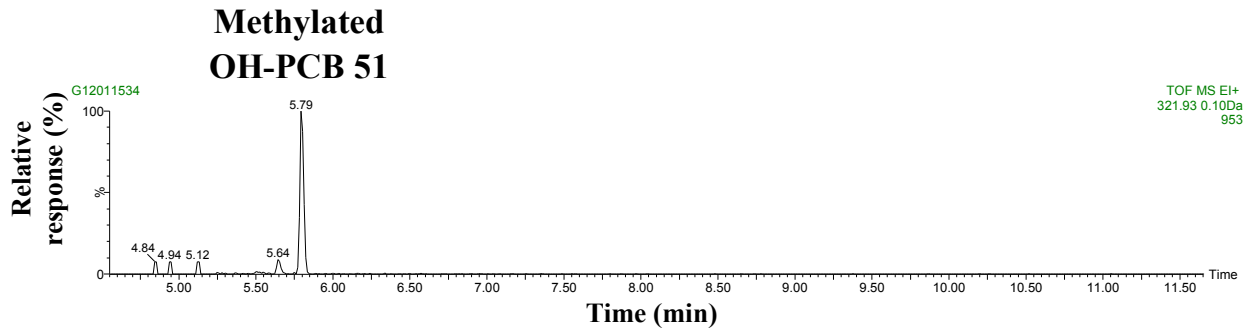


**Figure S14A. Representative gas chromatogram showing the formation of OH-PCB 51 (RT 5.79 min; RRT 0.664; *m/z* 321.9) from prochiral PCB 51 in incubations with male Guinea pig liver microsomes (analyzed as the corresponding methylated derivative).** The metabolite was tentatively identified based on its RRT and mass spectrum (Figure S14B). diOH-PCB 51, a metabolite observed in microsomal incubations with other rat liver microsomes or liver microsomes prepared from other mammalian species, was not detected. The incubation was performed in a shaking water bath at 37 °C for 2 hours and, after extraction with hexane-MTBE (1:1, *v/v*) and derivatization with diazomethane, the organic extract was analyzed on a Waters GCT Premier gas chromatograph-mass spectrometer as described in the Experimental Section.

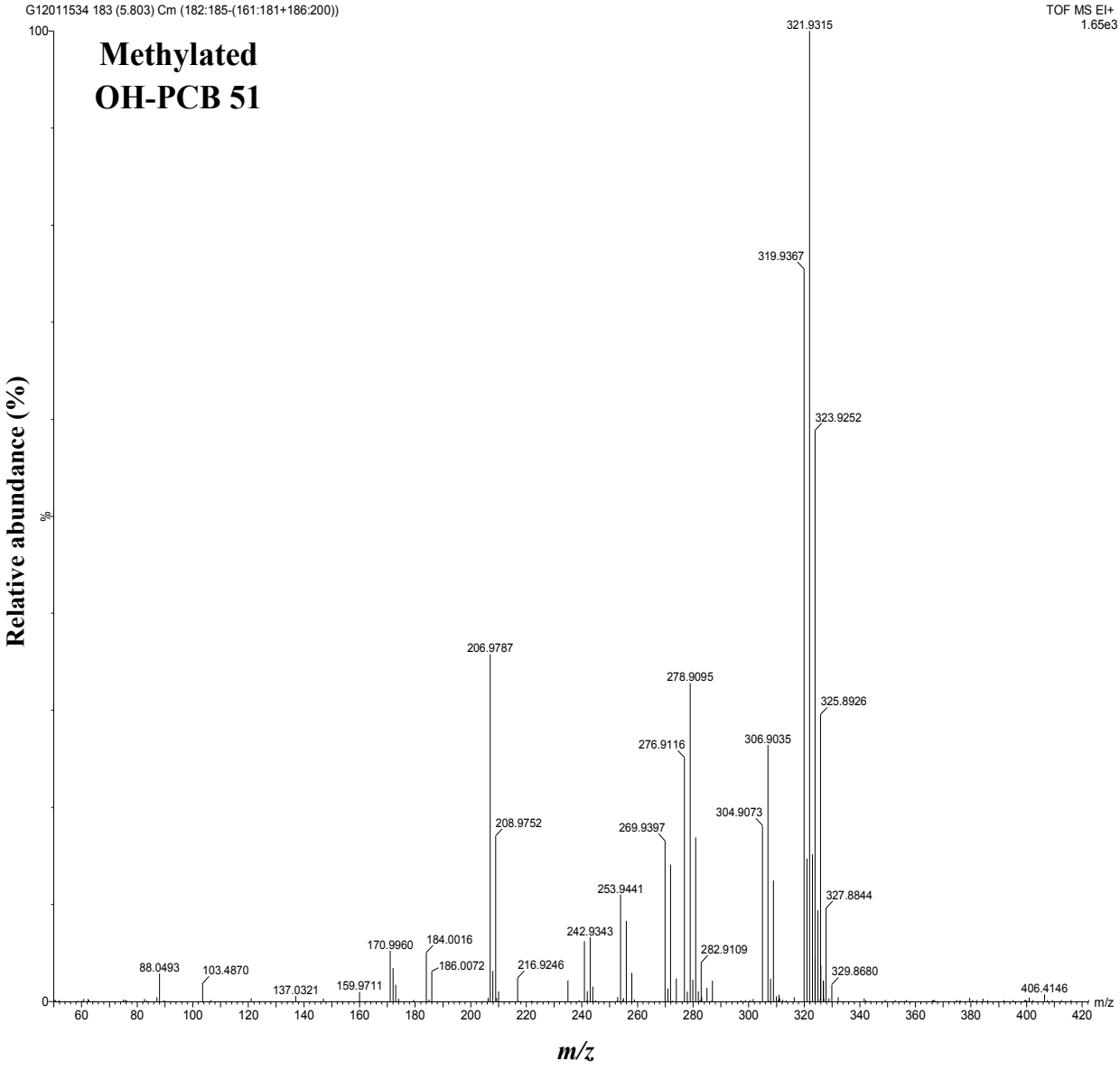


**Figure S14B. Mass spectrum of methylated OH-PCB 51 (RT 5.79 min; RRT 0.664) obtained from incubations of PCB 51 with male Guinea pig liver microsomes after derivatization.** The accurate mass determination of the monoisotopic $[M]^+$ (*m/z* 319.9367) did not match the *m/z* 319.9329 (calculated for $C_{13}H_8O^{35}Cl_4$) due to a co-eluting impurity; however, the isotope pattern of the molecular ion (1:1.3:0.7) compared to the theoretical ratio (1:1.3:0.6) and the fragmentation pattern were consistent with a monohydroxylated tetrachlorobiphenyl (as the corresponding methylated derivative). See the manuscript for a discussion of the chemical structure of this metabolite.

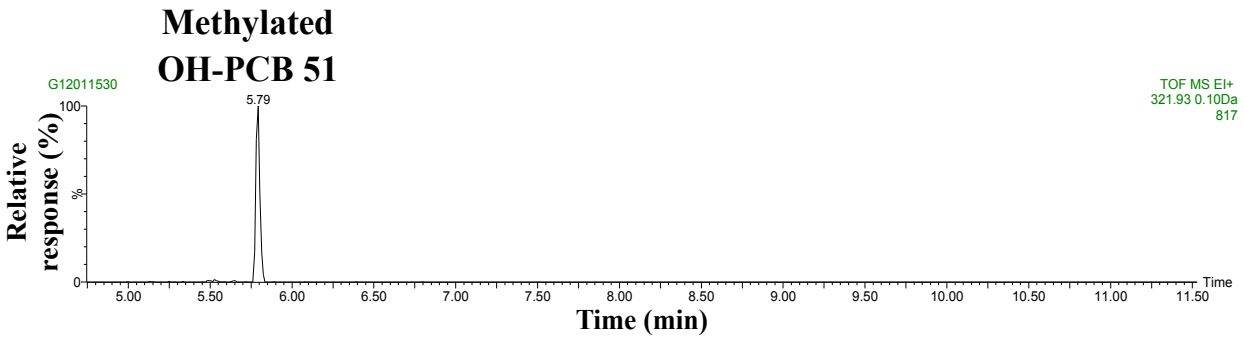


**Figure S15A. Representative gas chromatogram showing the formation of OH-PCB 51 (RT 5.79 min; RRT 0.66; *m/z* 321.9) from prochiral PCB 51 in incubations with male hamster liver microsomes (analyzed as the corresponding methylated derivative).** The metabolite was tentatively identified based on its RRT and mass spectrum (Figure S15B). diOH-PCB 51, a metabolite observed in microsomal incubations with other rat liver microsomes or liver microsomes prepared from other mammalian species, was not detected. The incubation was performed in a shaking water bath at 37 °C for 2 hours and, after extraction with hexane-MTBE (1:1, *v/v*) and derivatization with diazomethane, the organic extract was analyzed on a Waters GCT Premier gas chromatograph-mass spectrometer as described in the Experimental Section.

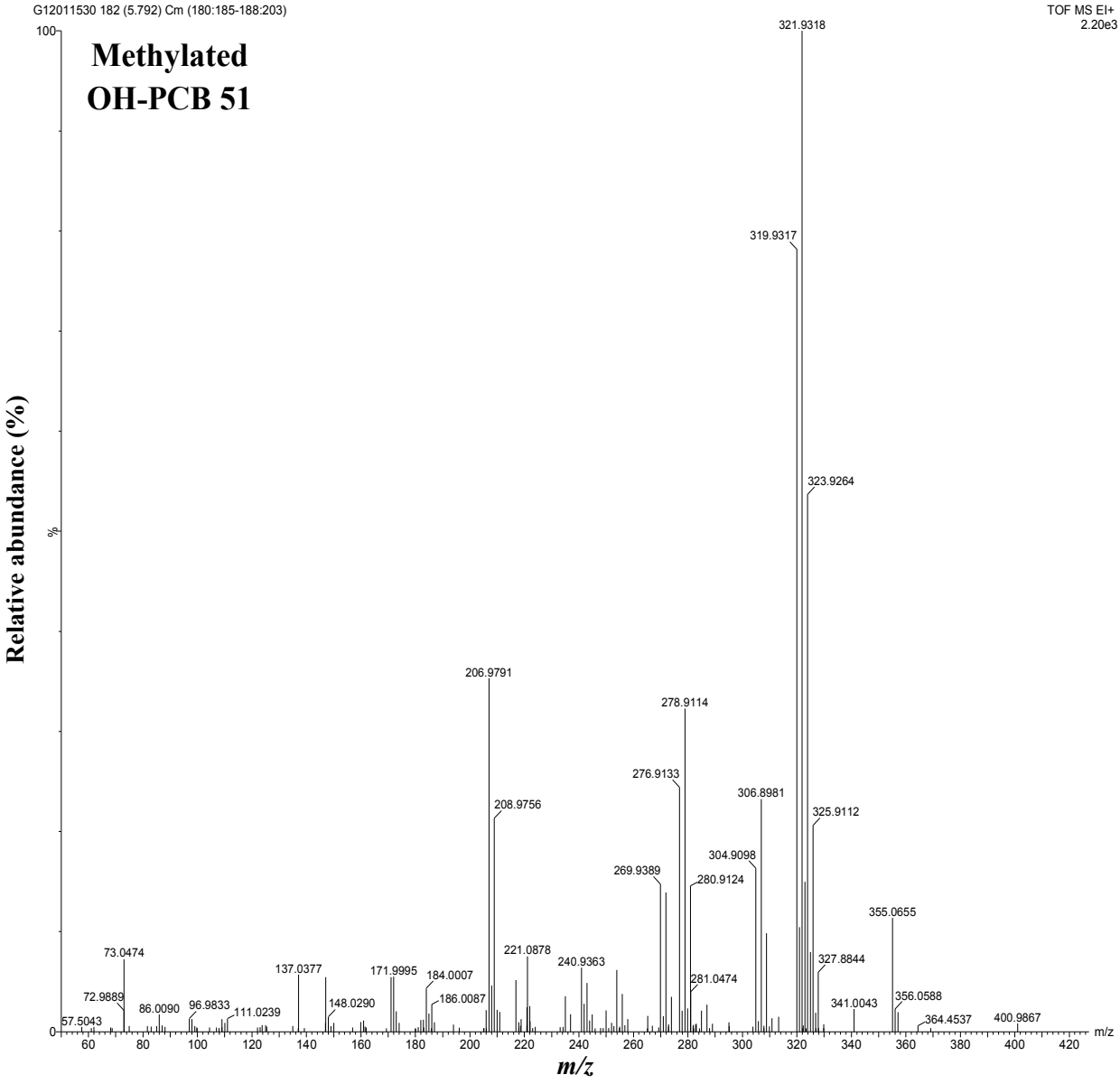


**Figure S15B. Mass spectrum of methylated OH-PCB 51 (RT 5.79 min; RRT 0.666) obtained from incubations of PCB 51 with male hamster liver microsomes after derivatization.** The accurate mass determination of the monoisotopic $[M]^+$ (*m/z* 319.9317) versus the *m/z* calculated for $C_{13}H_8O^{35}Cl_4$ (*m/z* 319.9329); the isotope pattern of the molecular ion (1:1.3:0.7) compared to the theoretical ratio (1:1.3:0.6); and the fragmentation pattern were consistent with a monohydroxylated tetrachlorobiphenyl (as the corresponding methylated derivative). See the manuscript for a discussion of the chemical structure of this metabolite.

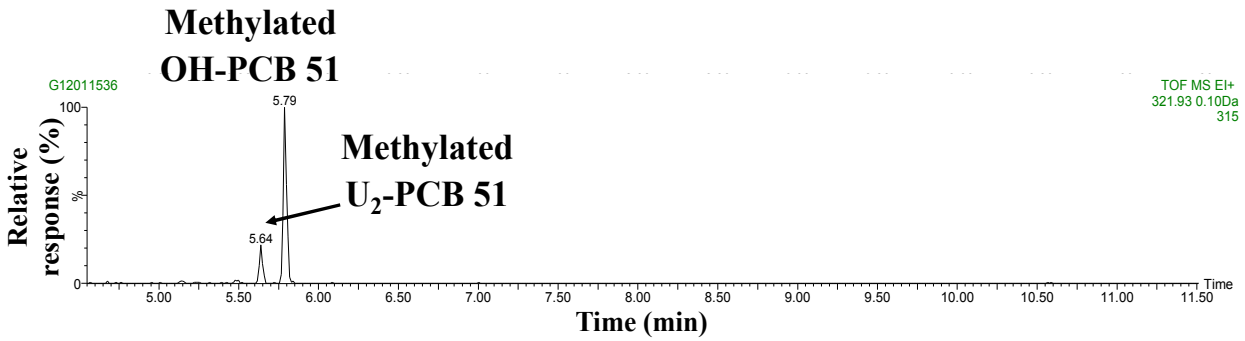


**Figure S16. Representative gas chromatogram showing the formation of OH-PCB 51 (RT 5.79 min; RRT 0.665; *m/z* 321.9) and an unknown metabolite, $U_2$-PCB 51 (RT 5.64 min; RRT 0.648; *m/z* 321.9), from prochiral PCB 51 in incubations with male monkey liver microsomes (analyzed as the corresponding methylated derivative).** Both metabolites were tentatively identified based on their RRT. diOH-PCB 51, a metabolite observed in microsomal incubations with other rat liver microsomes or liver microsomes prepared from other mammalian species, was not detected. The incubation was performed in a shaking water bath at 37 °C for 2 hours and, after extraction with hexane-MTBE (1:1, *v/v*) and derivatization with diazomethane, the organic extract was analyzed on a Waters GCT Premier gas chromatograph-mass spectrometer as described in the Experimental Section.

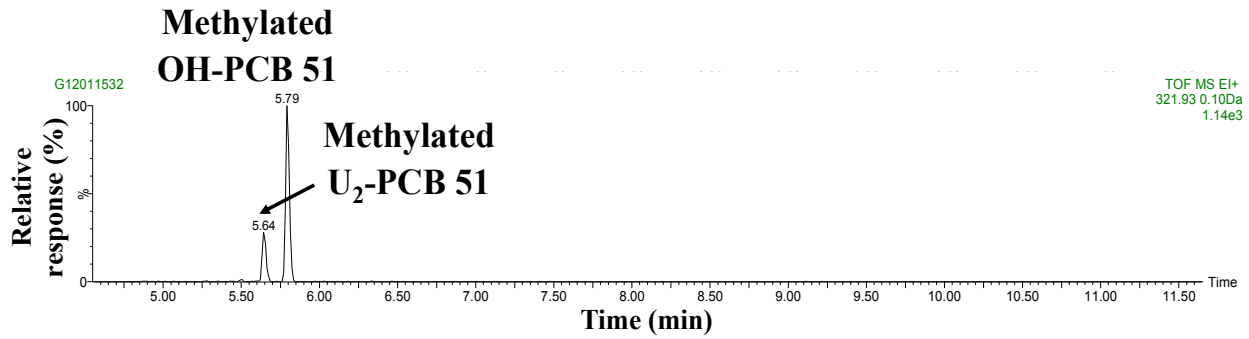


**Figure S17A. Representative gas chromatogram showing the formation of OH-PCB 51 (RT 5.79 min; RRT 0.664; *m/z* 321.9) and an unknown metabolite, $U_2$-PCB 51 (RT 5.64 min; RRT 0.647; *m/z* 321.9), from prochiral PCB 51 in incubations with male rabbit liver microsomes (analyzed as the corresponding methylated derivative).** OH-PCB 51, as methylated derivative, was tentatively identified based on its RRT and mass spectrum (Figure S17B). A peak with a RRT corresponding to $U_2$-PCB 51 was observed. diOH-PCB 51, a metabolite observed in microsomal incubations with other rat liver microsomes or liver microsomes prepared from other mammalian species, was not detected. The incubation was performed in a shaking water bath at 37 °C for 2 hours and, after extraction with hexane-MTBE (1:1, *v/v*) and derivatization with diazomethane, the organic extract was analyzed on a Waters GCT Premier gas chromatograph-mass spectrometer as described in the Experimental Section.

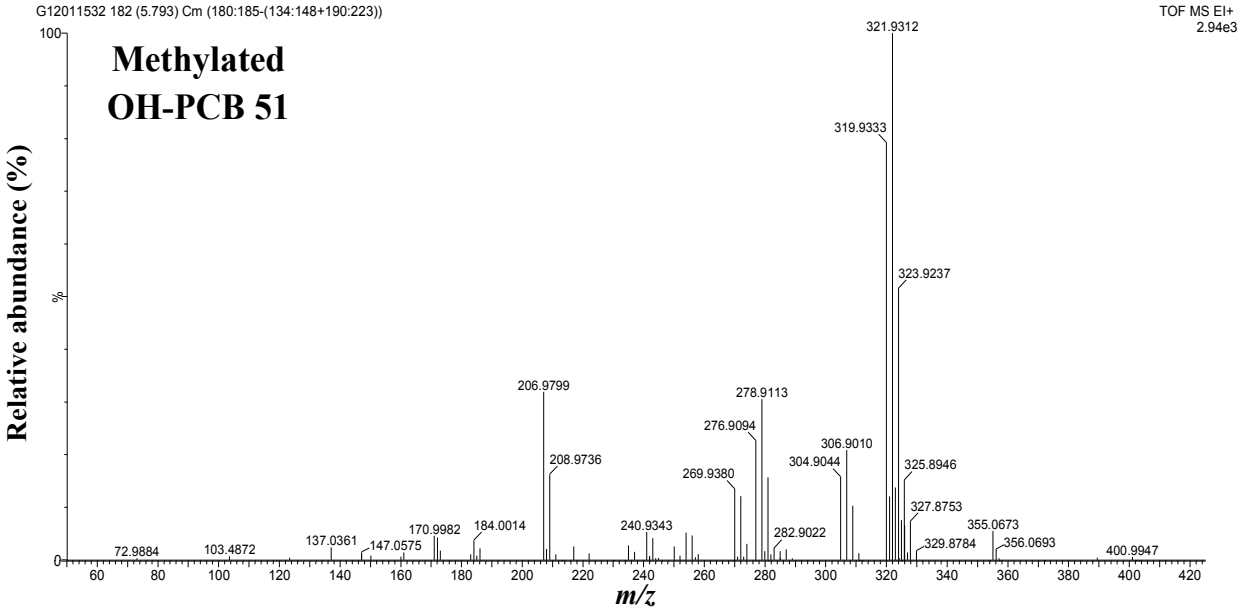


**Figure S17B. Mass spectrum of methylated OH-PCB 51 (RT 5.79 min; RRT 0.664) obtained from incubations of PCB 51 with male rabbit liver microsomes after derivatization.** The accurate mass determination of the monoisotopic $[M]^+$ (*m/z* 319.9333) versus the *m/z* calculated for $C_{13}H_8O^{35}Cl_4$ (*m/z* 319.9329); the isotope pattern of the molecular ion (1:1.3:0.6) compared to the theoretical ratio (1:1.3:0.6); and the fragmentation pattern were consistent with a monohydroxylated tetrachlorobiphenyl (as the corresponding methylated derivative). See the manuscript for a discussion of the chemical structure of this metabolite.

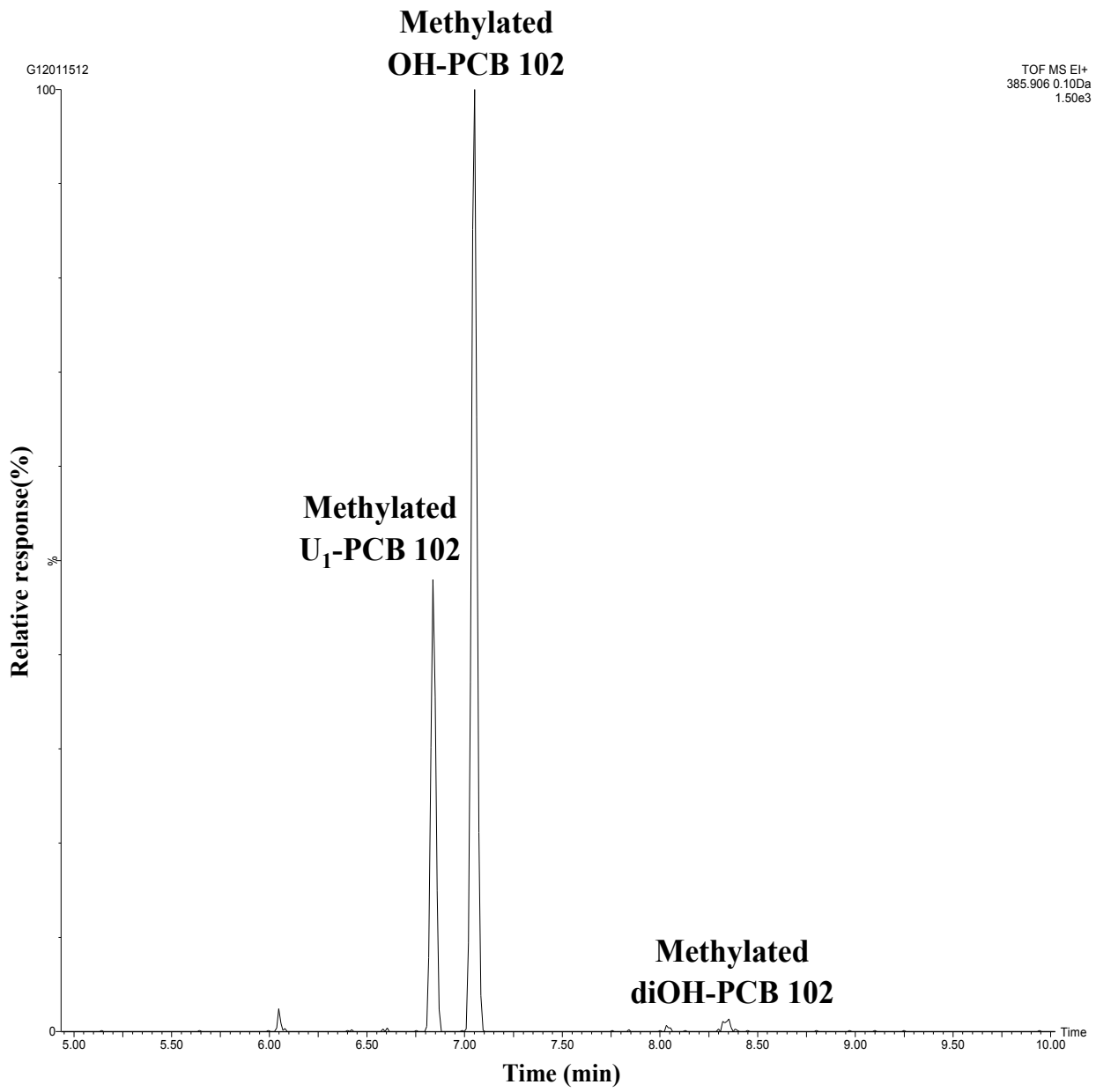


**Figure S18A. Representative gas chromatogram showing the formation of OH-PCB 102 (RT 7.05 min; RRT 0.810; *m/z* 355.9), diOH-PCB 102 (RT 8.34 min; RRT 0.959; *m/z* 385.9) and one unknown metabolite, $U_1$-PCB 102 (RT 6.84 min; RRT 0.786; *m/z* 355.9), formed from prochiral PCB 102 in incubations with female dog liver microsomes (analyzed as the corresponding methylated derivative).** The methylated derivative of OH-PCB 102 was tentatively identified based on its RRT and mass spectrum (Figures S18B). diOH-PCB 102, as dimethylated derivative, was tentatively identified based on its RRT. The mass spectrum of the unidentified metabolite $U_1$-PCB 102, a monohydroxylated pentachlorobiphenyl (as methylated derivative), is shown in Figure S18C. The incubation was performed in a shaking water bath at 37 °C for 2 hours and, after extraction with hexane-MTBE (1:1, *v/v*) and derivatization with diazomethane, the organic extract was analyzed on a Waters GCT Premier gas chromatograph-mass spectrometer as described in the Experimental Section.

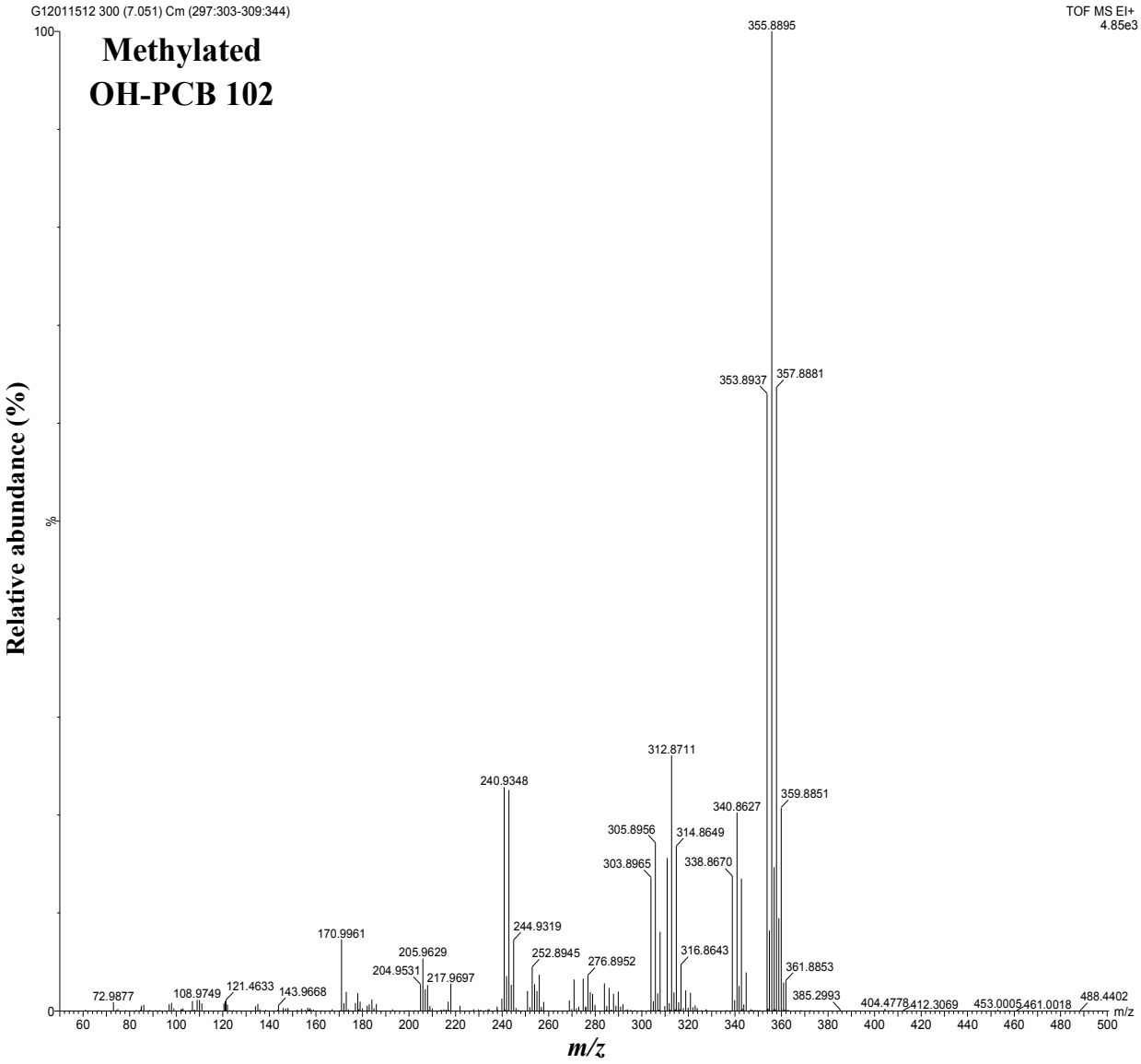


**Figure S18B. Mass spectrum of methylated OH-PCB 102 (RT 7.05 min; RRT 0.810) obtained from incubations of PCB 102 with female dog liver microsomes after derivatization.** The accurate mass determination of the monoisotopic $[M]^+$ (*m/z* 353.8937) versus the *m/z* calculated for $C_{13}H_7O_1{}^{35}Cl_5$ (*m/z* 353.8940); the isotope pattern of the molecular ion (1:1.6:1:0.3) compared to the theoretical ratio (1:1.6:1:0.3); and the fragmentation pattern were consistent with a monohydroxylated pentachlorobiphenyl (as the corresponding methylated derivative). See the manuscript for a discussion of the chemical structure of this metabolite.

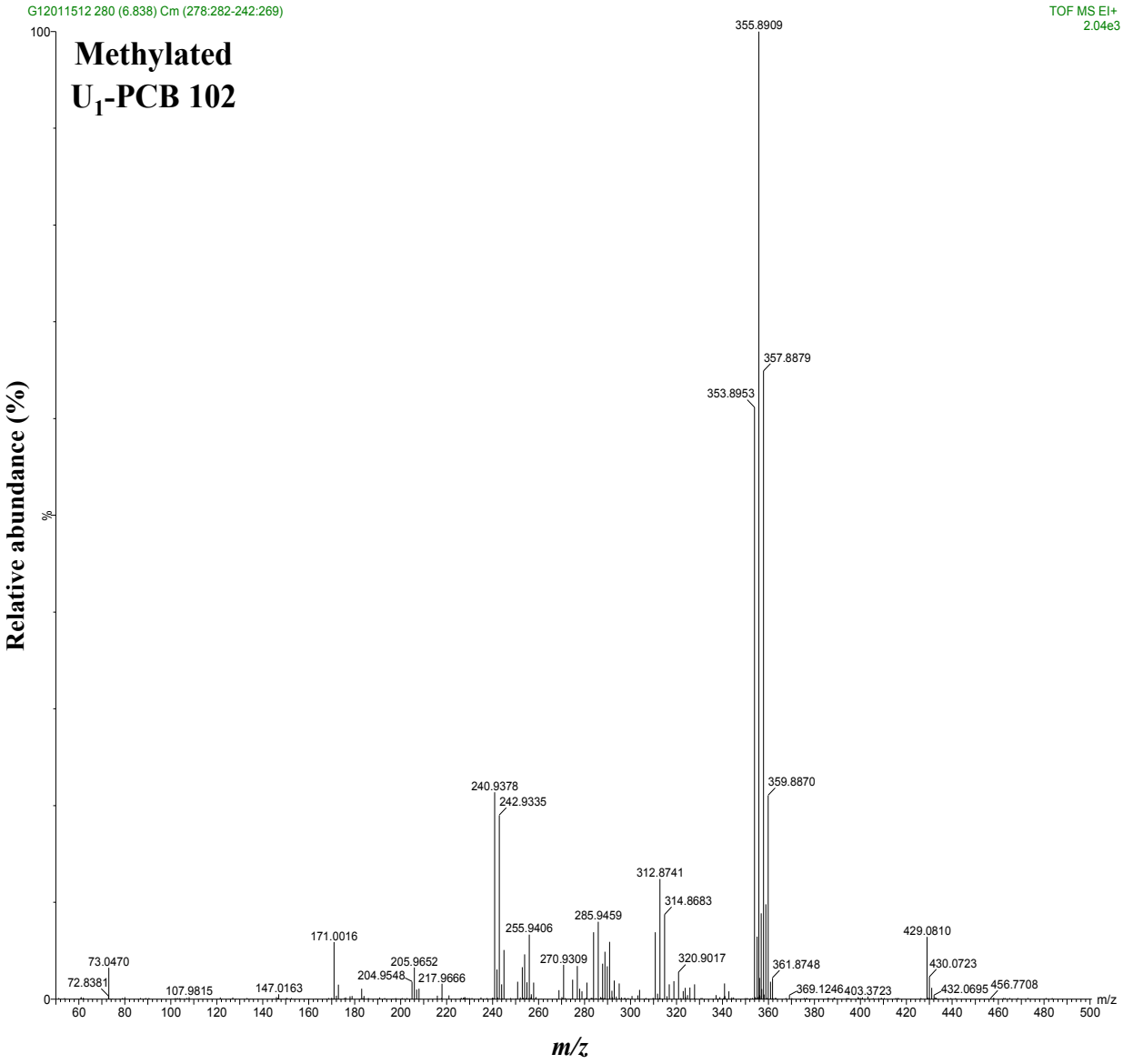


**Figure S18C. Mass spectrum of methylated $U_1$-PCB 102 (RT 6.84 min; RRT 0.786) obtained from incubations of PCB 102 with female dog liver microsomes after derivatization.** The accurate mass determination of the monoisotopic $[M]^+$ (*m/z* 353.8953) versus the *m/z* calculated for $C_{13}H_7O_1{}^{35}Cl_5$ (*m/z* 353.8940); the isotope pattern of the molecular ion (1:1.6:1.1:0.3) compared to the theoretical ratio (1:1.6:1:0.3); and the fragmentation pattern were consistent with a monohydroxylated pentachlorobiphenyl (as the corresponding methylated derivative). See the manuscript for a discussion of the chemical structure of this metabolite.

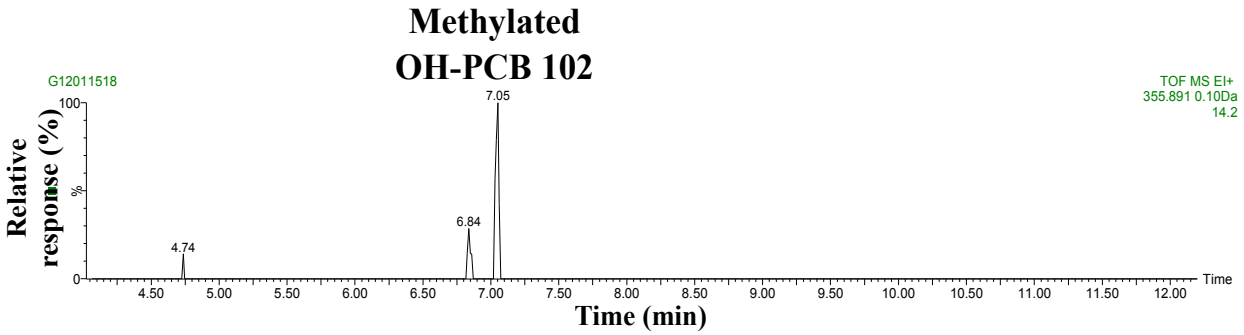


**Figure S19. Representative gas chromatogram showing the formation of OH-PCB 102 (RT 7.05 min; RRT 0.811; *m/z* 355.9) from prochiral PCB 102 in incubations with Guinea pig liver microsomes (analyzed as the corresponding methylated derivative).** The metabolite was tentatively identified based on its RRT. diOH-PCB 102, a metabolite observed in microsomal incubations with other rat liver microsomes or liver microsomes prepared from other mammalian species, was not detected. The incubation was performed in a shaking water bath at 37 °C for 2 hours and, after extraction with hexane-MTBE (1:1, *v/v*) and derivatization with diazomethane, the organic extract was analyzed on a Waters GCT Premier gas chromatograph-mass spectrometer as described in the Experimental Section.

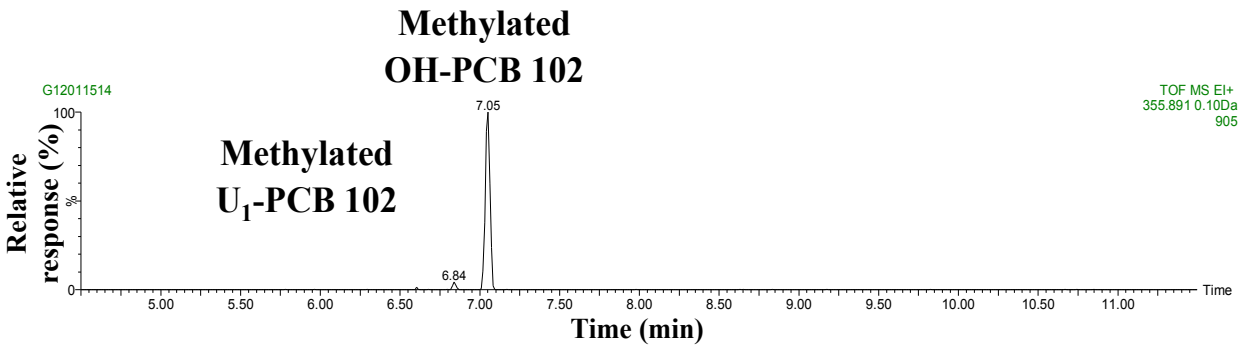


**Figure S20A. Representative gas chromatogram showing the formation of OH-PCB 102 (RT 7.05 min; RRT 0.810; *m/z* 355.9) and one unknown metabolite, $U_1$-PCB 102 (RT 6.84 min; RRT 0.784; *m/z* 355.9), from prochiral PCB 102 in incubations with male hamster liver microsomes (analyzed as the corresponding methylated derivative).** The methylated derivative of OH-PCB 102 was tentatively identified based on its RRT and mass spectrum (Figure S20B). $U_1$-PCB 102 was tentatively identified based on its RRT. In addition, diOH-PCB 102 (RT 8.34 min; RRT 0.958), as dimethylated derivative, was tentatively identified based on its RRT (data not shown). The incubation was performed in a shaking water bath at 37 °C for 2 hours and, after extraction with hexane-MTBE (1:1, *v/v*) and derivatization with diazomethane, the organic extract was analyzed on a Waters GCT Premier gas chromatograph-mass spectrometer as described in the Experimental Section.

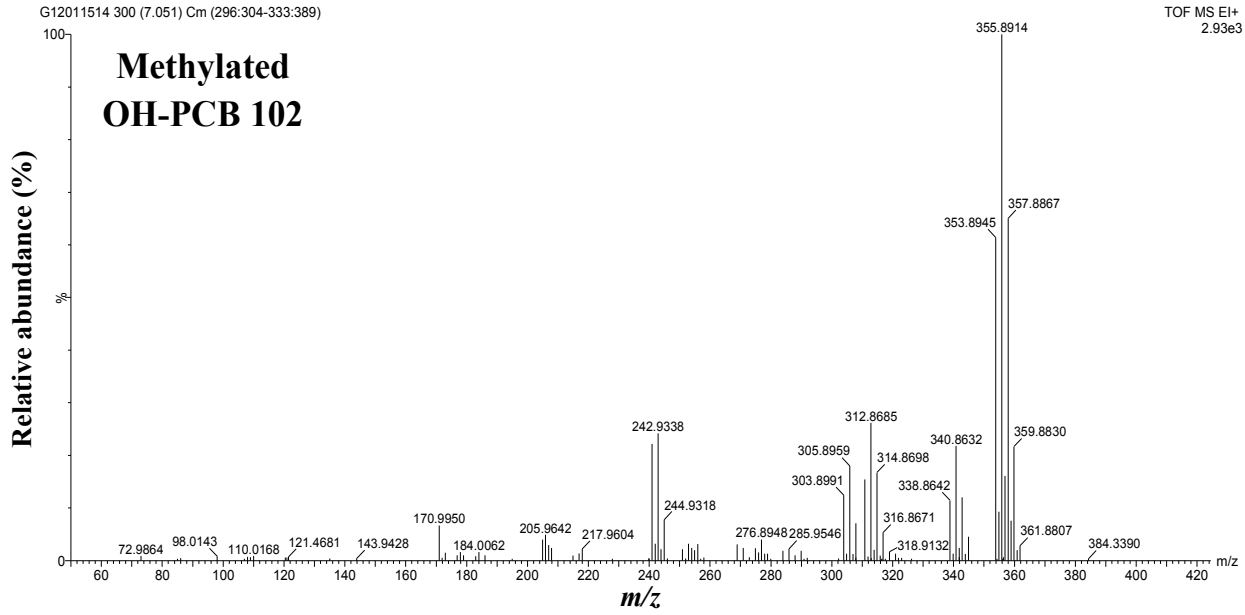


**Figure S20B. Mass spectrum of methylated OH-PCB 102 (RT 7.05 min; RRT 0.810) obtained from incubations of PCB 102 with male hamster liver microsomes after derivatization.** The accurate mass determination of the monoisotopic $[M]^+$ (*m/z* 353.8945) versus the *m/z* calculated for $C_{13}H_7O_1{}^{35}Cl_5$ (*m/z* 353.8940); the isotope pattern of the molecular ion (1:1.7:1.1:0.3) compared to the theoretical ratio (1:1.6:1:0.3); and the fragmentation pattern were consistent with a monohydroxylated pentachlorobiphenyl (as the corresponding methylated derivative). See the manuscript for a discussion of the chemical structure of this metabolite.

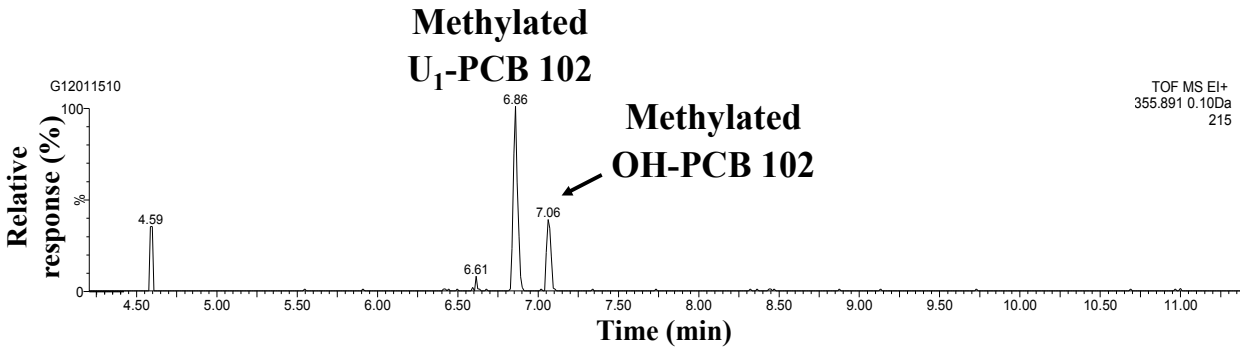


**Figure S21A. Representative gas chromatogram showing the formation of OH-PCB 102 (RT 7.06 min; RRT 0.808; *m/z* 355.9) and an unknown metabolite, $U_1$-PCB 102 (RT 6.86 min; RRT 0.785; *m/z* 355.9), from prochiral PCB 102 in incubations with male monkey liver microsomes (analyzed as the corresponding methylated derivative).** The methylated derivative of OH-PCB 102 was tentatively identified based on its RRT. The mass spectrum of the unidentified metabolite $U_1$-PCB 102, a monohydroxylated pentachlorobiphenyl (as methylated derivative), is shown in Figure S21B. diOH-PCB 102, a metabolite observed in microsomal incubations with other rat liver microsomes or liver microsomes prepared from other mammalian species, was not detected. The incubation was performed in a shaking water bath at 37 °C for 2 hours and, after extraction with hexane-MTBE (1:1, *v/v*) and derivatization with diazomethane, the organic extract was analyzed on a Waters GCT Premier gas chromatograph-mass spectrometer as described in the Experimental Section.

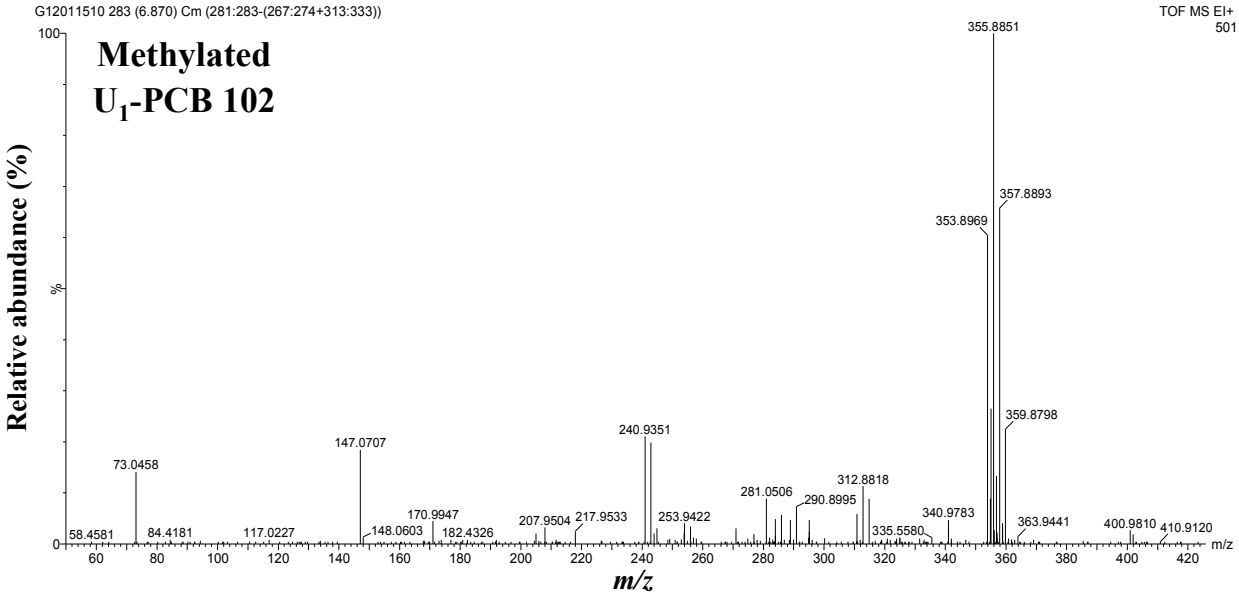


**Figure S21B. Mass spectrum of methylated $U_1$-PCB 102 (RT 6.86 min; RRT 0.785) obtained from incubations of PCB 102 with male monkey liver microsomes after derivatization.** The accurate mass determination of the monoisotopic $[M]^+$ (*m/z* 353.8969) versus the *m/z* calculated for $C_{13}H_7O_1{}^{35}Cl_5$ (*m/z* 353.8940); the isotope pattern of the molecular ion (1:1.7:1.1:0.4) compared to the theoretical ratio (1:1.6:1:0.3); and the fragmentation pattern were consistent with a monohydroxylated pentachlorobiphenyl (as the corresponding methylated derivative). See the manuscript for a discussion of the chemical structure of this metabolite.

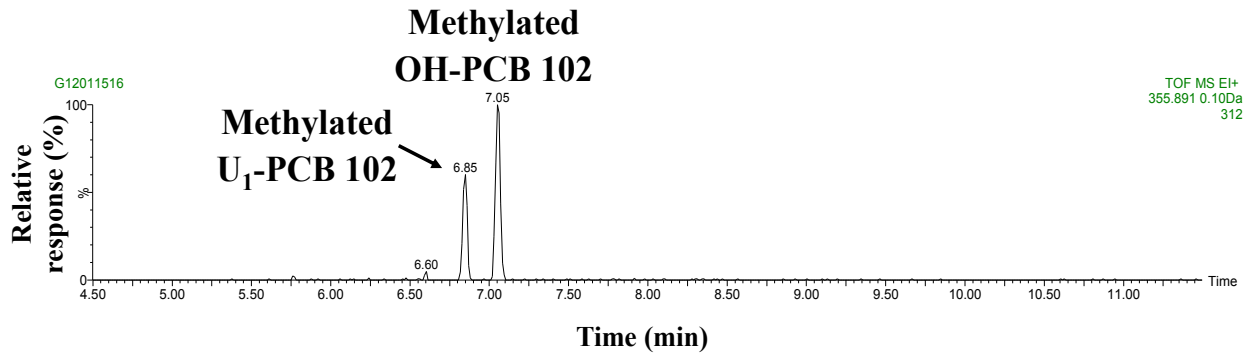


**Figure S22A. Representative gas chromatogram showing the formation of OH-PCB 102 (RT 7.05 min; RRT 0.809; *m/z* 355.9) and an unknown metabolite, $U_1$-PCB 102 (RT 6.85 min; RRT 0.786; *m/z* 355.9), from prochiral PCB 102 in incubations with male rabbit liver microsomes (analyzed as the corresponding methylated derivative).** The methylated derivative of OH-PCB 102 was tentatively identified based on its RRT and mass spectrum (Figure S22B). $U_1$-PCB 102 was tentatively identified based on its RRT. diOH-PCB 102, a metabolite observed in microsomal incubations with other rat liver microsomes or liver microsomes prepared from other mammalian species, was not detected. The incubation was performed in a shaking water bath at 37 °C for 2 hours and, after extraction with hexane-MTBE (1:1, *v/v*) and derivatization with diazomethane, the organic extract was analyzed on a Waters GCT Premier gas chromatograph-mass spectrometer as described in the Experimental Section.

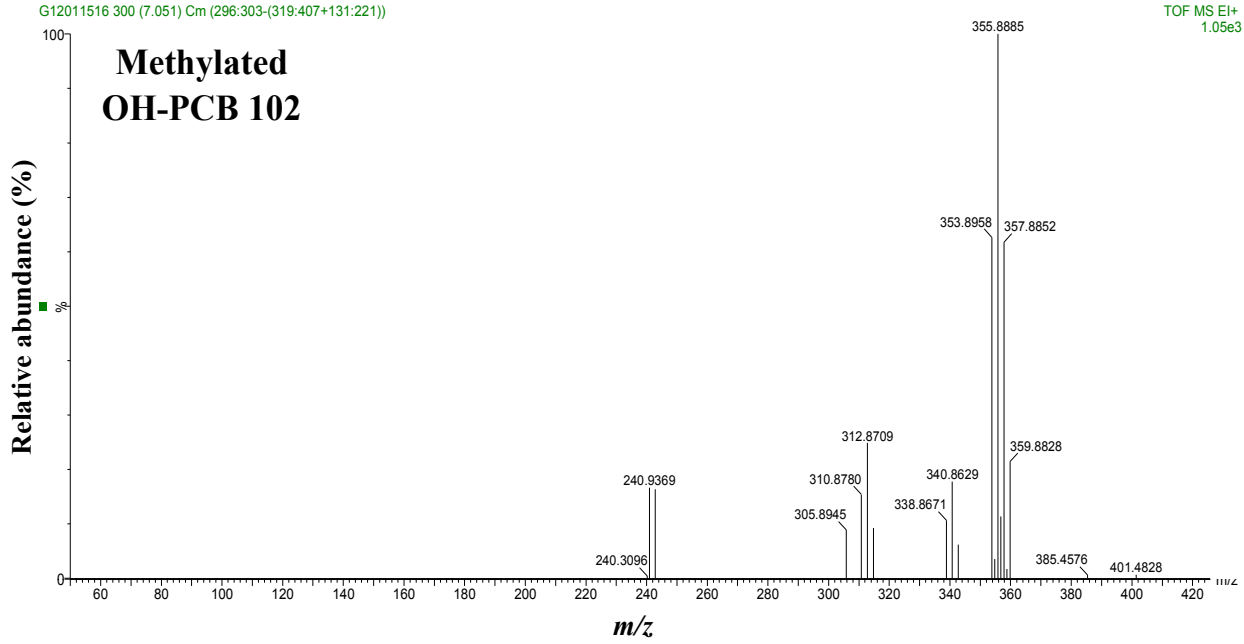


**Figure S22B. Mass spectrum of methylated OH-PCB 102 (RT 7.05 min; RRT 0.809) obtained from incubations of PCB 102 with male rabbit liver microsomes after derivatization.** The accurate mass determination of the monoisotopic $[M]^+$ (*m/z* 353.8958) versus the *m/z* calculated for $C_{13}H_7O_1{}^{35}Cl_5$ (*m/z* 353.8940); the isotope pattern of the molecular ion (1:1.6:1:0.3) compared to the theoretical ratio (1:1.6:1:0.3); and the fragmentation pattern were consistent with a monohydroxylated pentachlorobiphenyl (as the corresponding methylated derivative). See the manuscript for a discussion of the chemical structure of this metabolite.

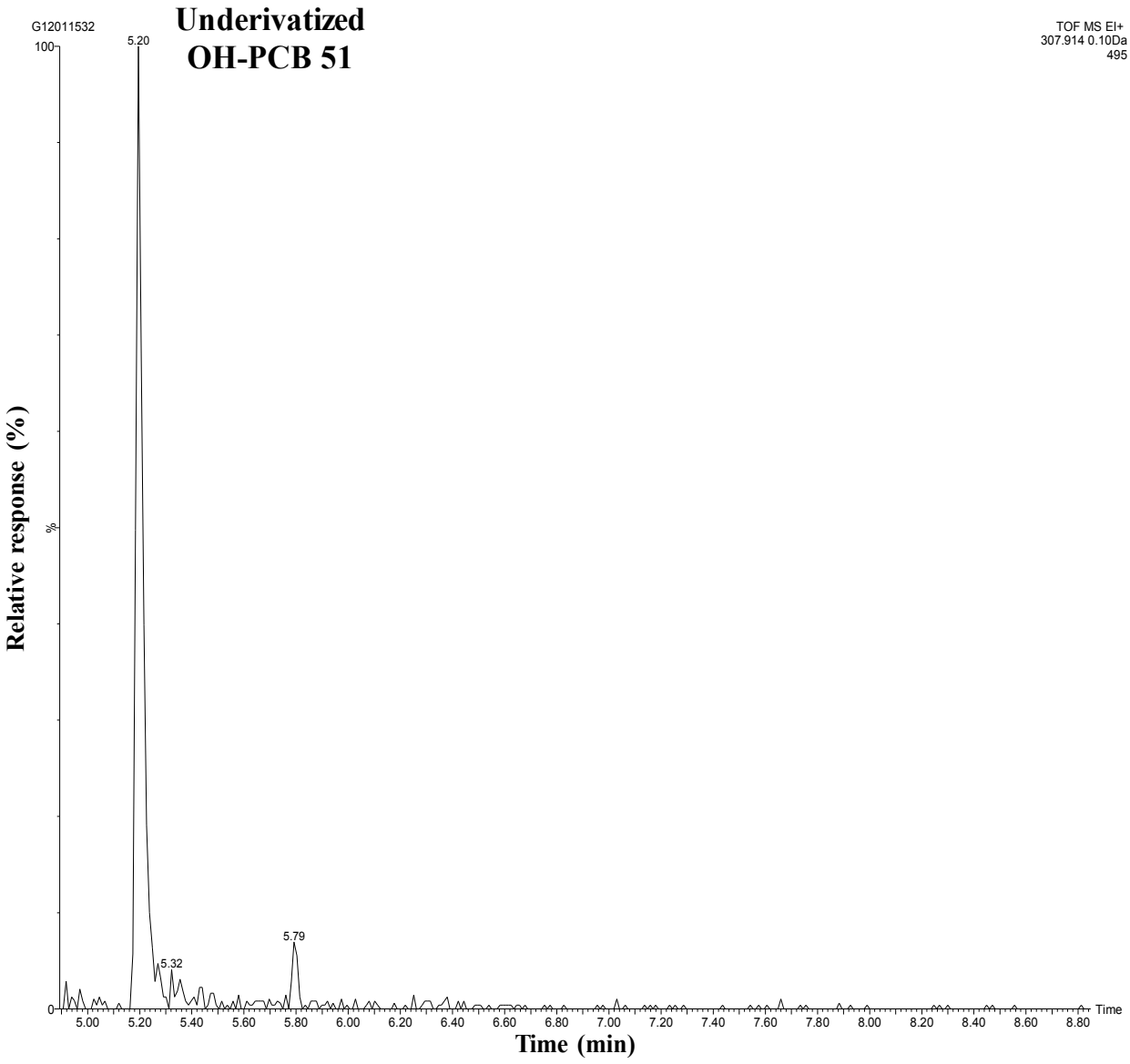


**Figure S23A. Representative gas chromatogram showing traces of an underivatized, monohydroxylated metabolite (RT 5.20 min; RRT 0.596; *m/z* 307.9) present in extracts from incubations of PCB 51 with male rabbit liver microsomes.** The metabolite was tentatively identified as underivatized OH-PCB 51 based on its mass spectrum (Figure S23B). The presence of this monohydroxylated PCB 51 may be due to incomplete derivatization of OH-PCB 51 or could be a formed during the sulfuric acid clean-up step from an epoxide metabolite of PCB 51.[9]

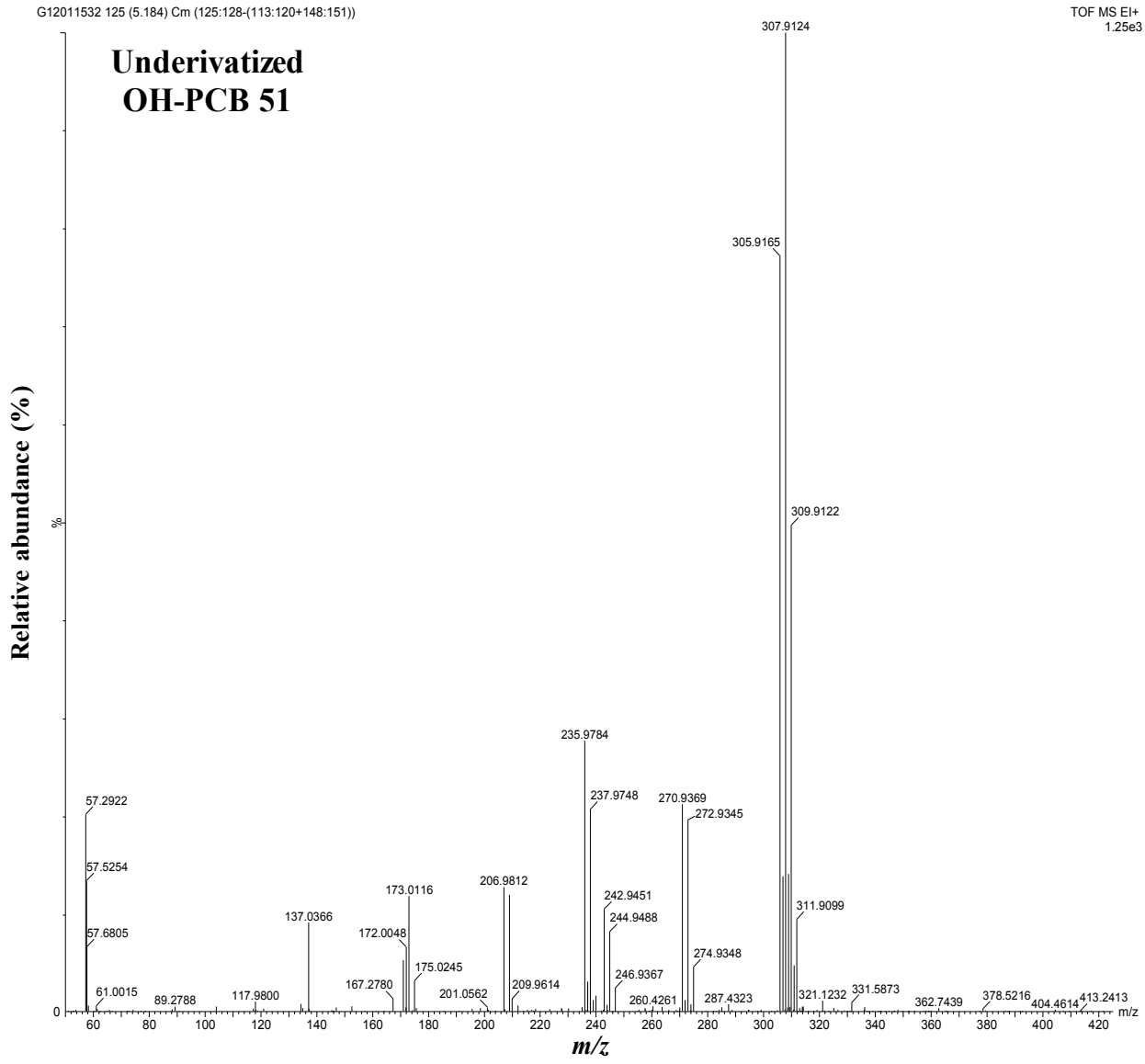


**Figure S23B. Mass spectrum of an underivatized, monohydroxylated PCB 51 metabolite (RT 5.20 min; RRT 0.596) present in extracts from incubations of PCB 51 with male rabbit liver microsomes.** The isotope pattern of the molecular ion and the fragmentation pattern are consistent with a monohydroxylated tetrachlorobiphenyl.

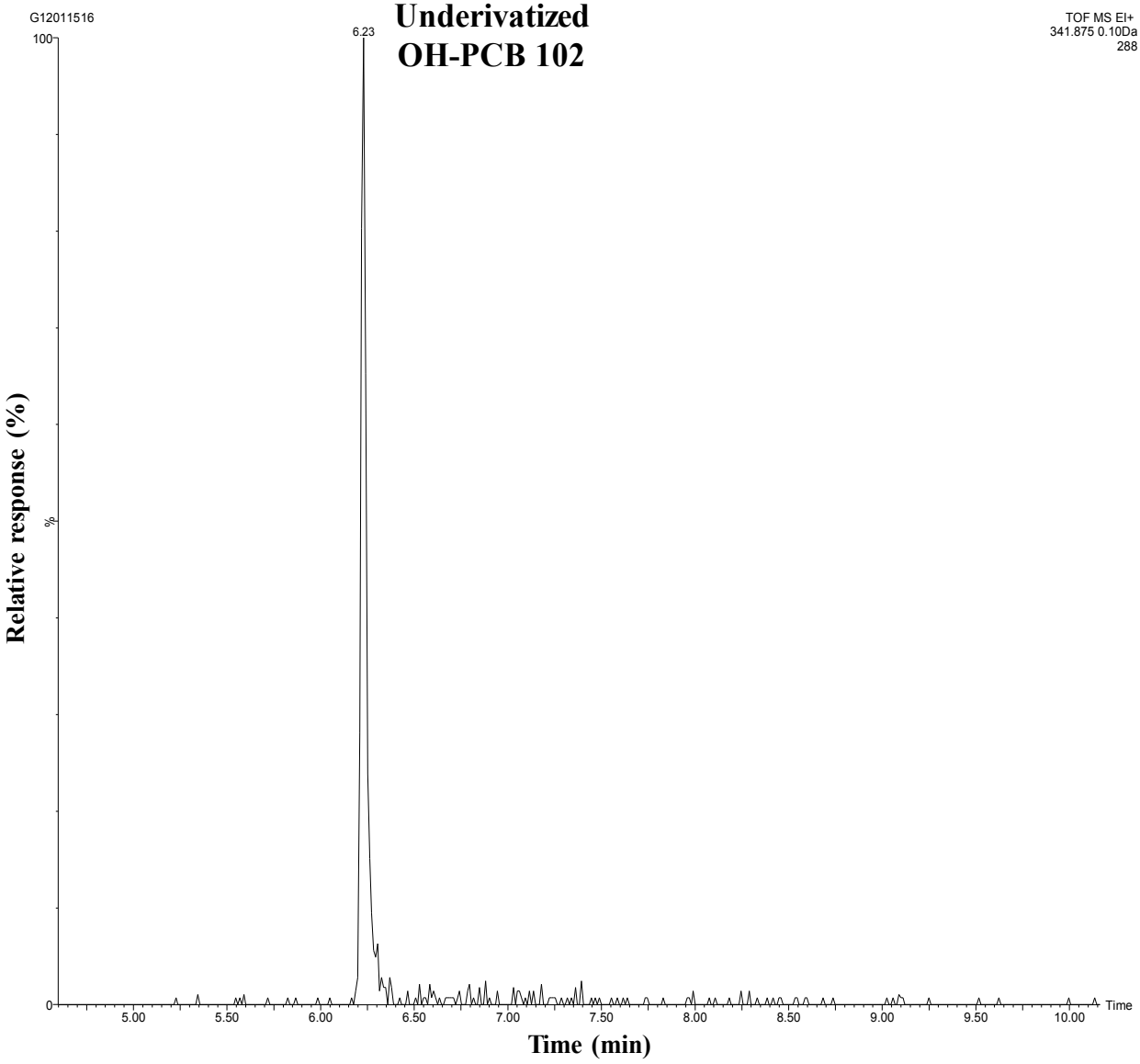


**Figure S24A. Representative gas chromatogram showing traces of an underivatized, monohydroxylated metabolite (RT 6.23 min; RRT 0.714; *m/z* 341.9) present in extracts from incubations of PCB 102 with male rabbit liver microsomes.** The metabolite was tentatively identified as underivatized OH-PCB 102 based on its mass spectrum (Figure S24B). The presence of this monohydroxylated PCB 102 may be due to incomplete derivatization of OH-PCB 102 or could be a formed during the sulfuric acid clean-up step from an epoxide metabolite of PCB 102.[9]

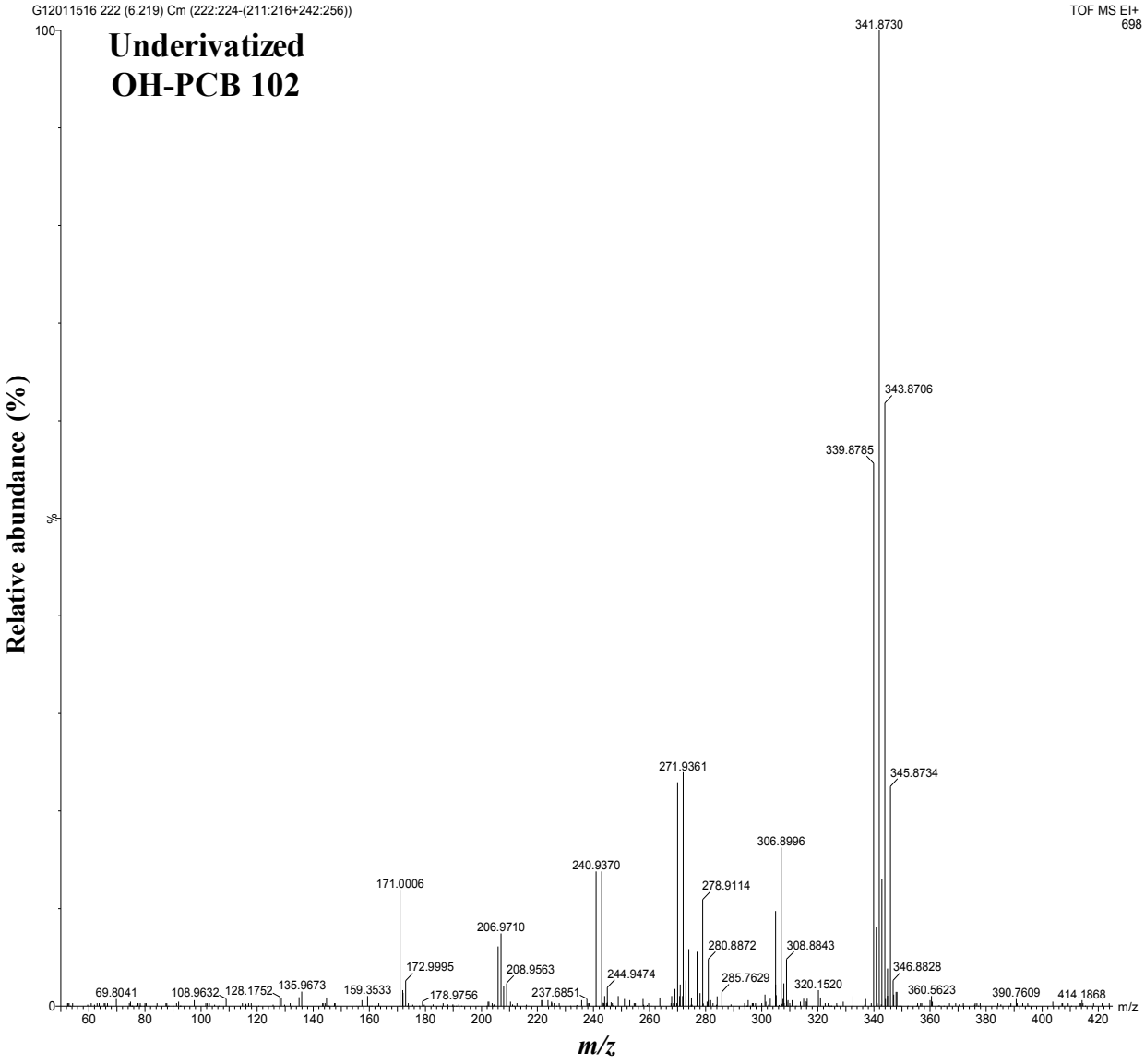


**Figure S24B. Mass spectrum of an underivatized, monohydroxylated PCB 102 metabolite (RT 6.23 min; RRT 0.714) present in extracts from incubations of PCB 102 with male rabbit liver microsomes.** The isotope pattern of the molecular ion and the fragmentation pattern are consistent with monohydroxylated pentachlorobiphenyl.

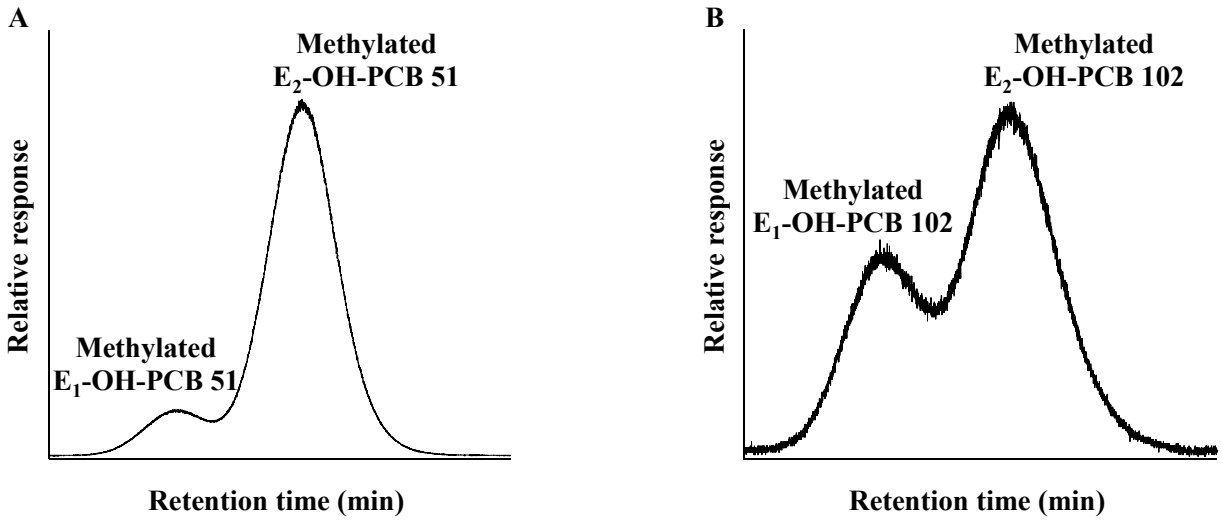


**Figure S25. Representative gas chromatogram showing the separation of the atropisomers of (A) OH-PCB 51 and (B) OH-PCB 102 formed in incubation of the respective prochiral PCBs with microsomes prepared from male dexamethasone pretreated rats (analyzed as the corresponding methylated derivative).** Large-scale incubations were performed with rat liver microsomes prepared from dexamethasone-pretreated rats and contained phosphate buffer (0.1 M, pH 7.4), liver microsomes (0.1 mg/mL) and NADPH (0.5 mM). PCB 51 or PCB 102 in DMSO (0.5% of the incubation volume) was added to give a final concentration of 50 μM in a 16 mL incubation as described in the Experimental Section. After extraction with hexane-MTBE (1:1, *v/v*) and derivatization with diazomethane, the organic extract was analyzed on a GC-MS equipped with a BDM capillary column (30 m length, 250 μm inner diameter, 0.12 μm film thickness; Supelco, St Louis, MO, USA) at 130 °C or 140 °C for OH-PCB 51 or OH-PCB 102, respectively. The helium flow rate was 3 mL/min. The injector temperature was 280 °C and the temperatures of the mass selective detector were as follow: transfer line, 280 °C; source, 230 °C; and quadrupole, 150 °C. Ions were created by negative electron impact ionization. Data were acquired in the selected ion monitoring (SIM) mode for *m/z* 320, 322 (for methylated OH-PCB 51) and *m/z* 356, 358 (for methylated OH-PCB 102).

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