

Supplemental Data for

Theophylline acetaldehyde as the initial product in doxophylline metabolism in human liver

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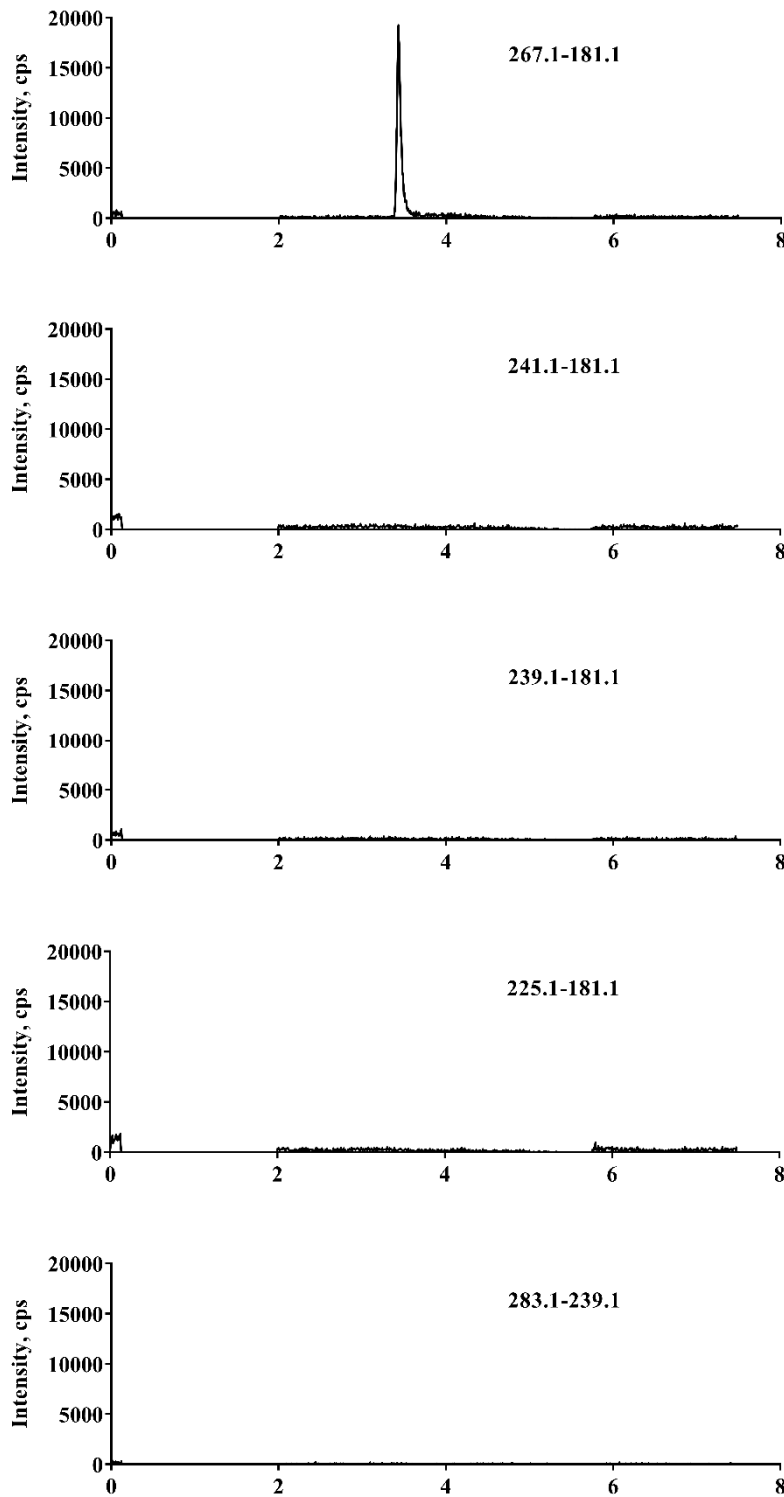
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Supplemental Figure

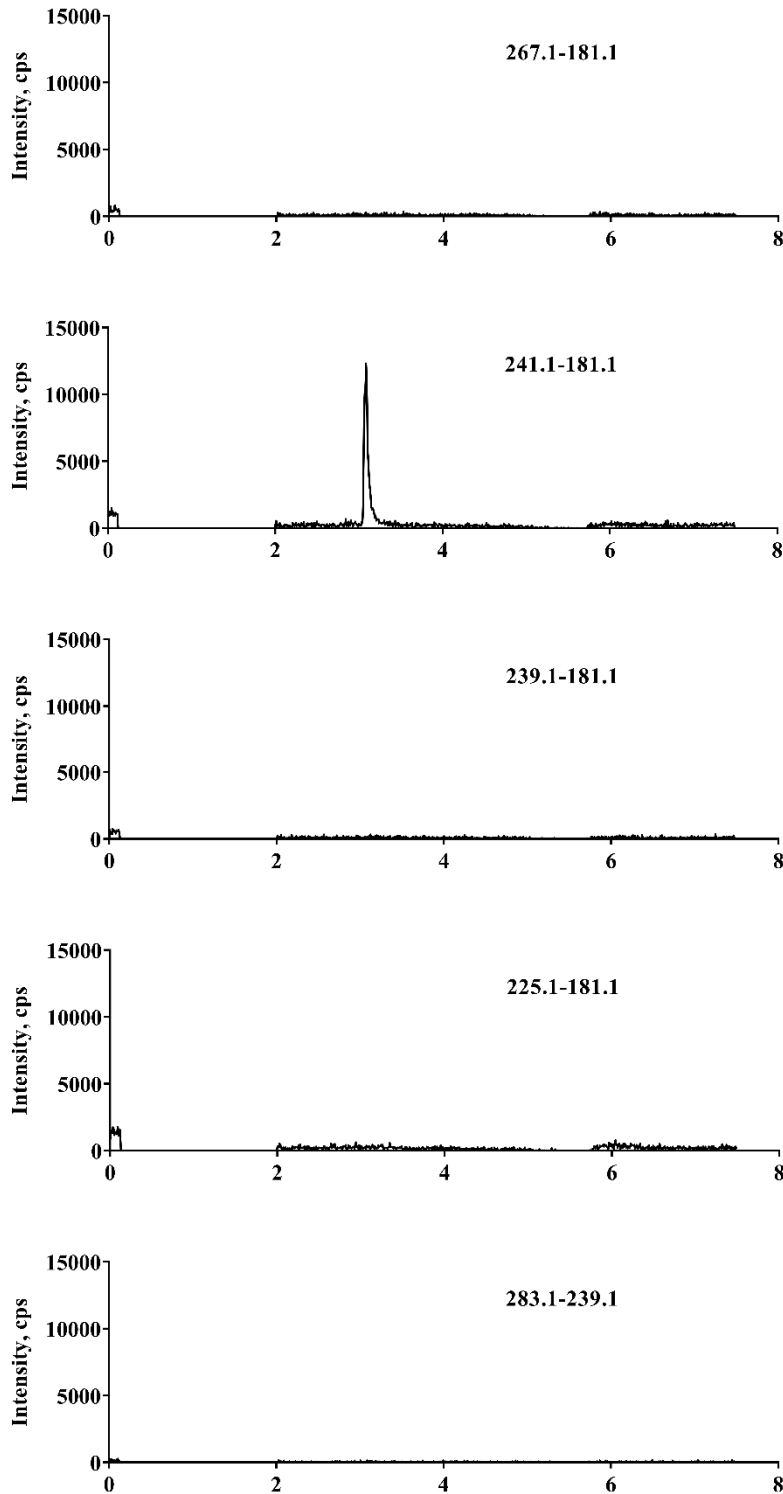
A



SFig. 1 Representative MRM chromatograms for DOXO and metabolites.

(A) DOXO solution (100 ng/mL)

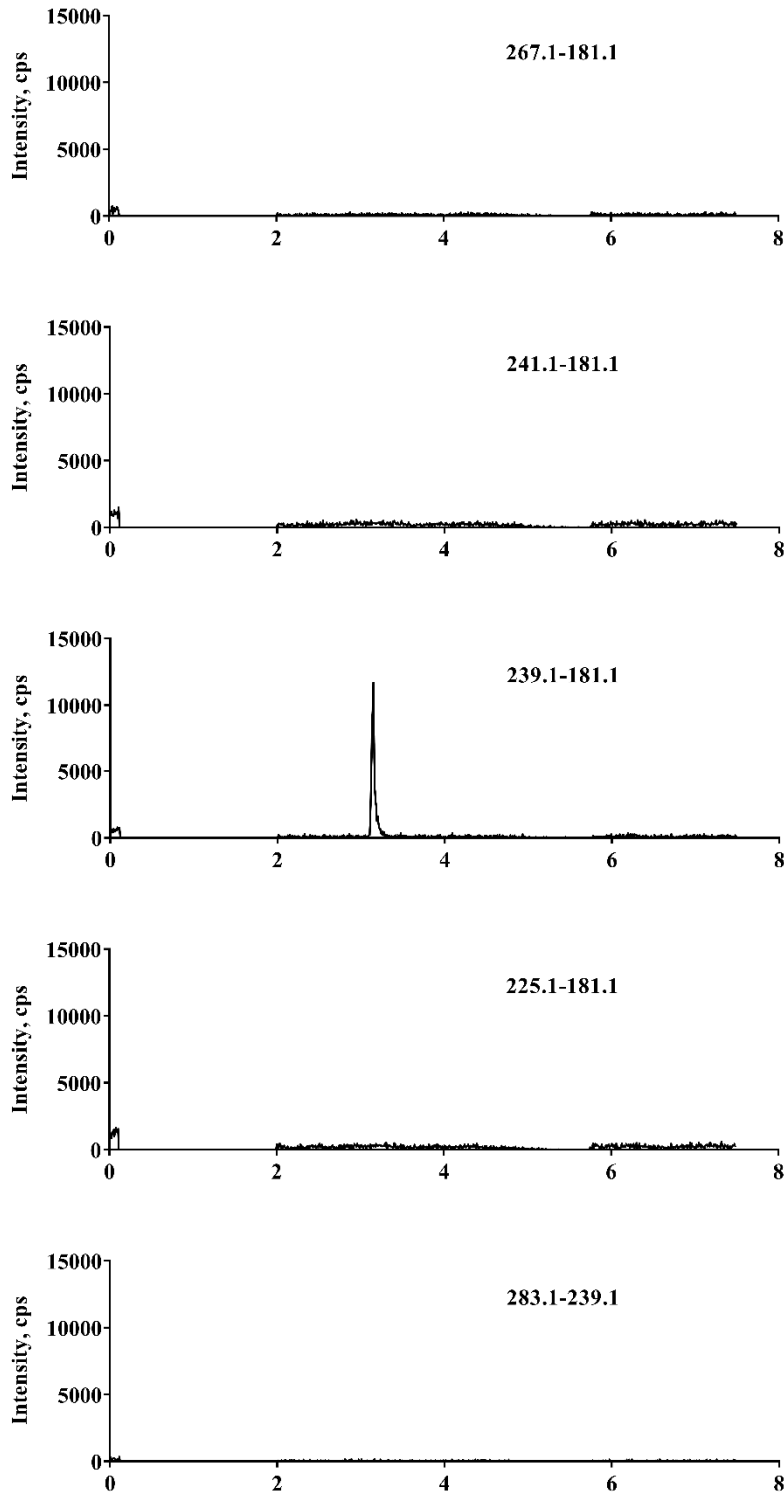
B



SFig. 1 Representative MRM chromatograms for DOXO and metabolites.

(B) M1 solution (100 ng/mL)

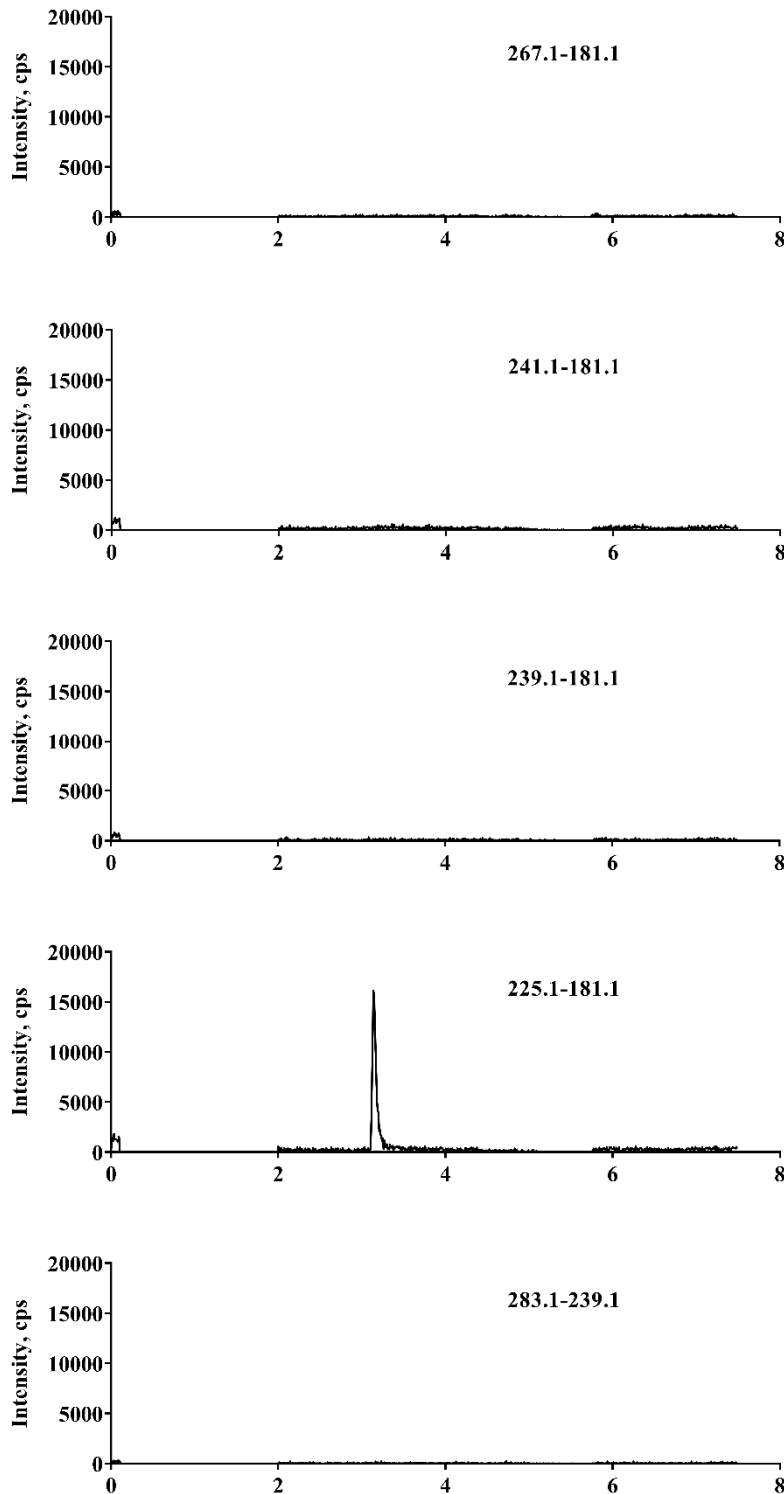
C



SFig. 1 Representative MRM chromatograms for DOXO and metabolites.

(C) M2 solution (100 ng/mL)

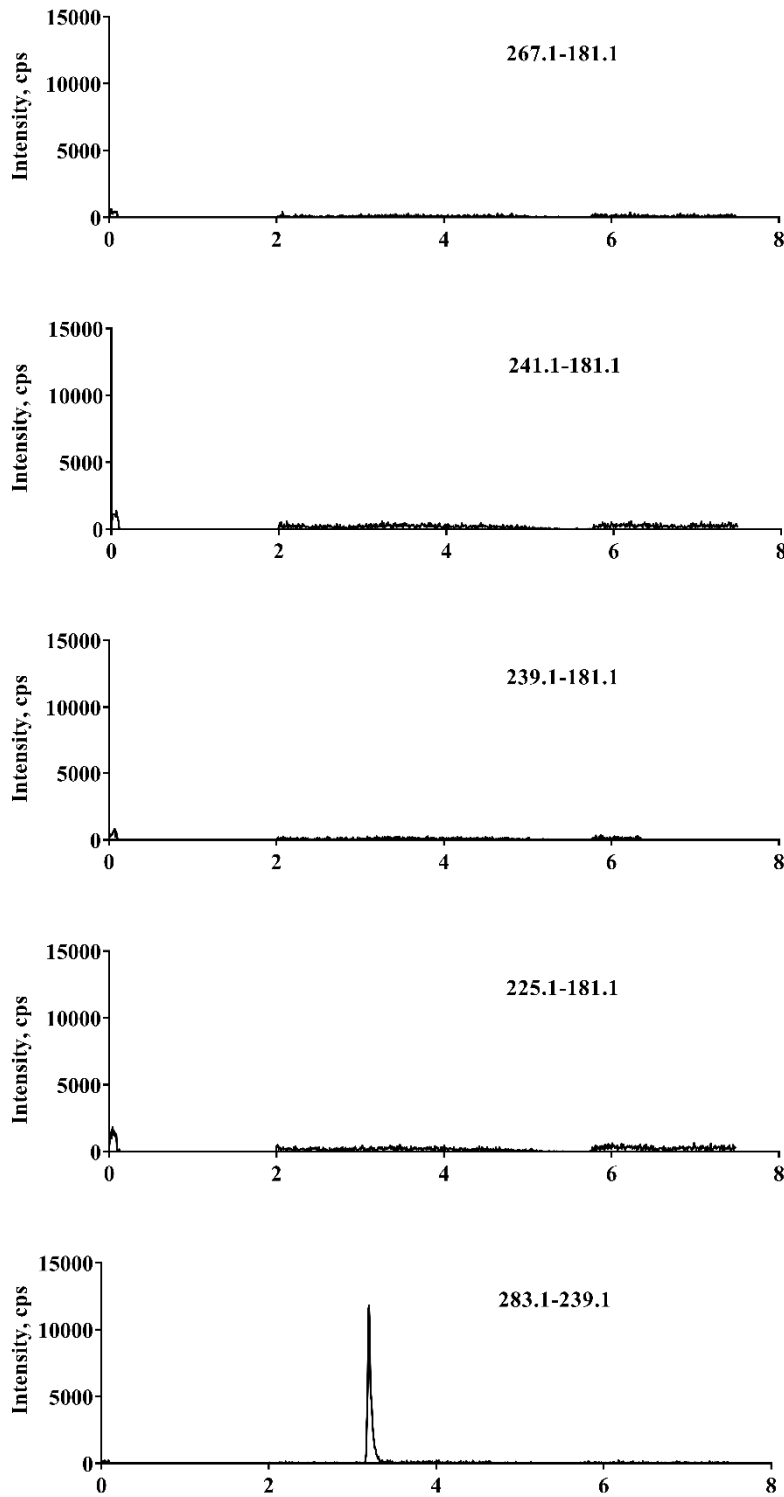
D



SFig. 1 Representative MRM chromatograms for DOXO and metabolites.

(D) M4 solution (100 ng/mL)

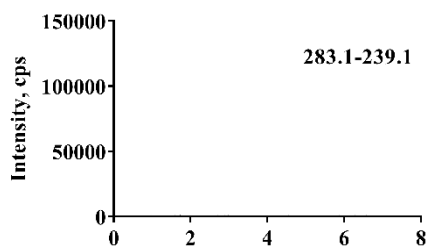
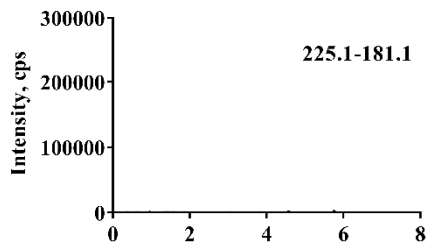
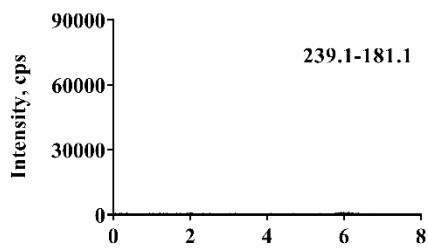
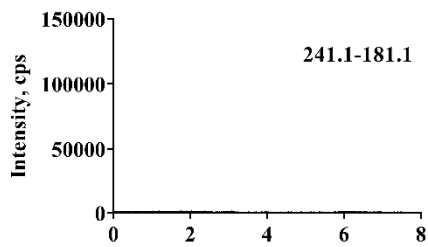
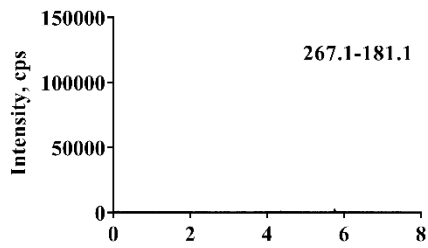
E



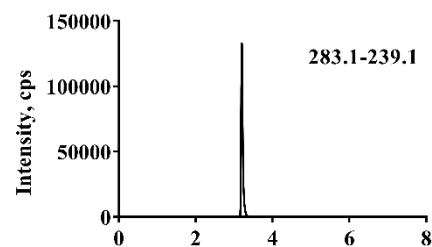
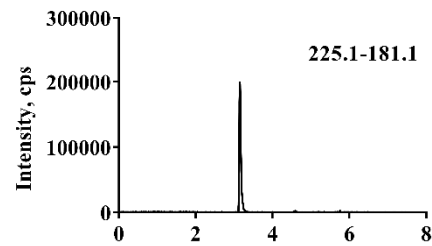
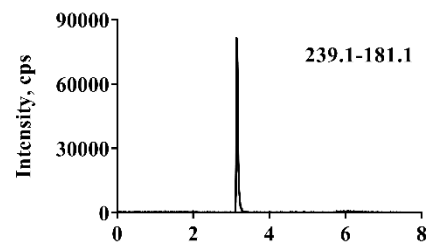
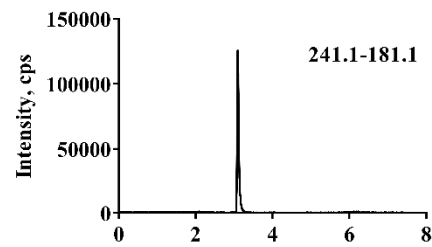
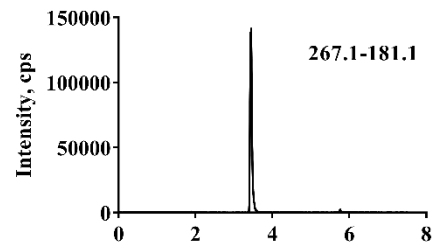
SFig. 1 Representative MRM chromatograms for DOXO and metabolites.

(E) M5 solution (100 ng/mL)

F



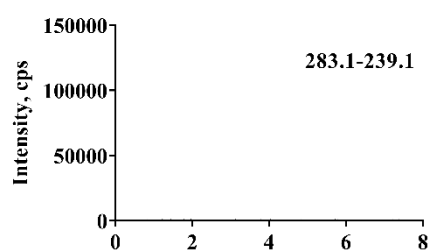
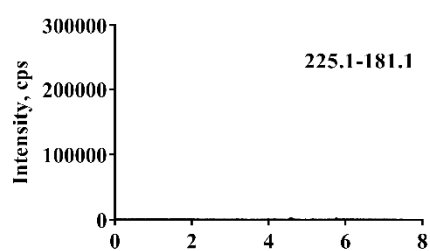
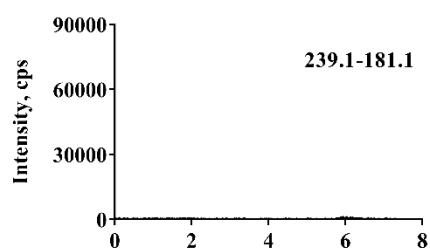
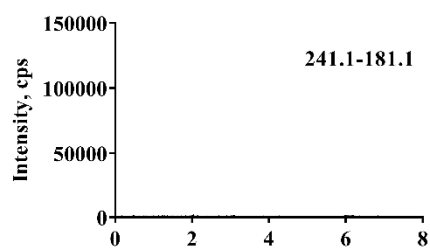
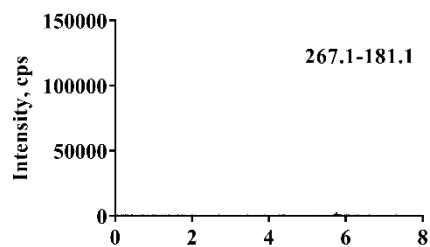
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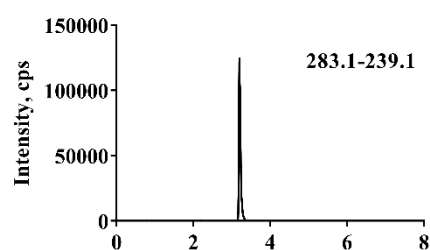
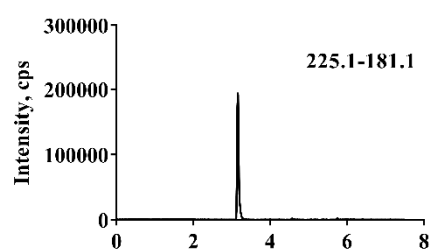
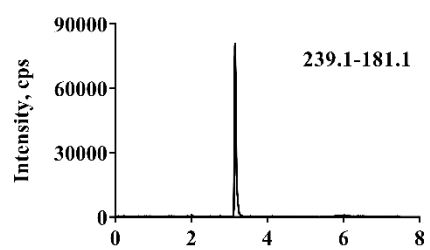
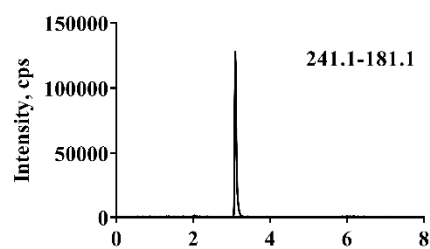
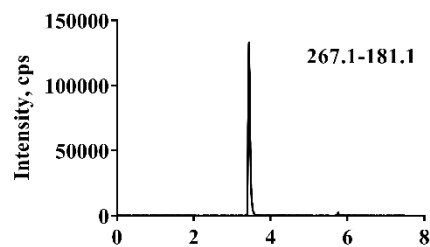
SFig. 1 Representative MRM chromatograms for DOXO and metabolites.

(F) HLM incubation, blank sample (without DOXO); (G) HLM incubation, blank sample spiked with mixture of DOXO, M1, M2, M4 and M5 (final concentration: 100 ng/ml each);

H



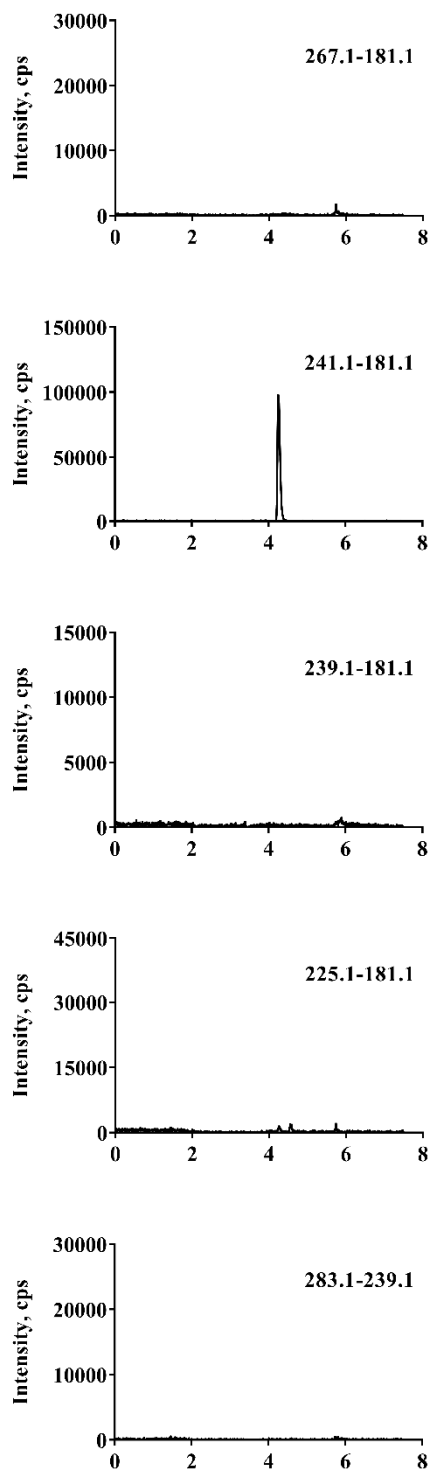
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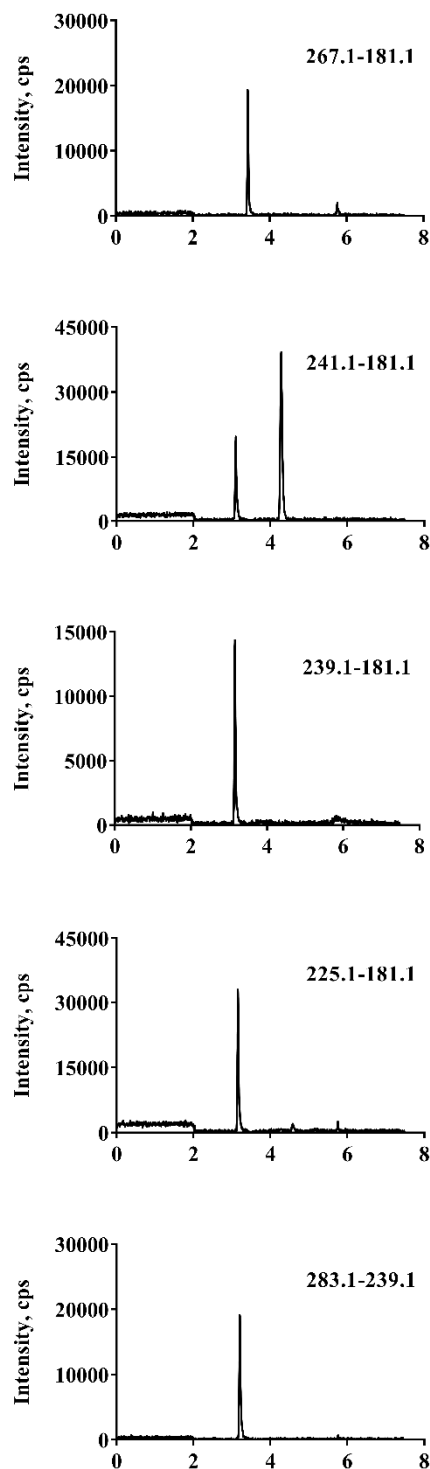
SFig. 1 Representative MRM chromatograms for DOXO and metabolites.

(H) HLS9 incubation, blank sample (without DOXO); (I) HLS9 incubation, blank sample spiked with mixture of DOXO, M1, M2, M4 and M5 (final concentration: 100 ng/ml each);

J



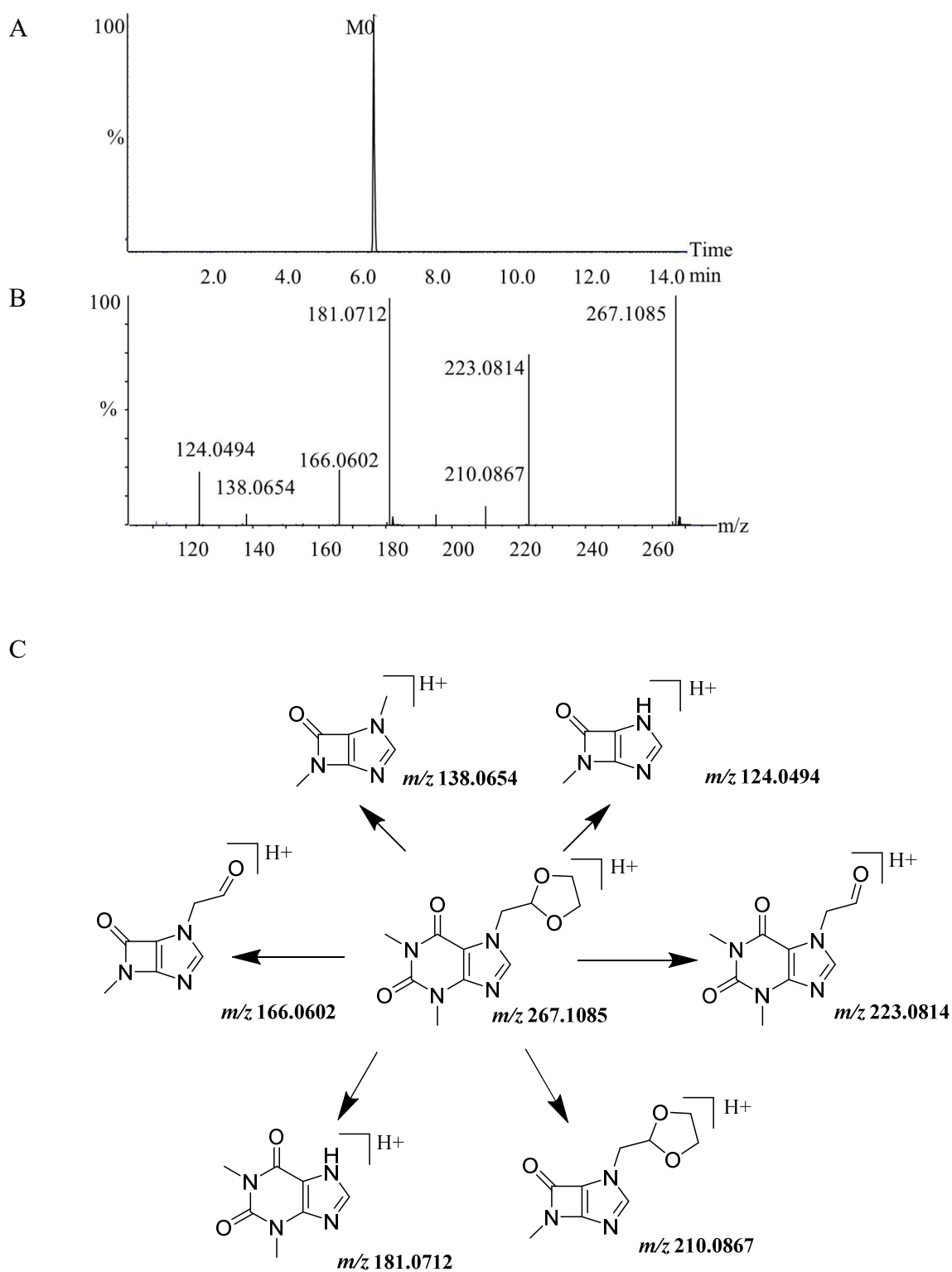
K



SFig. 1 Representative MRM chromatograms for DOXO and metabolites.

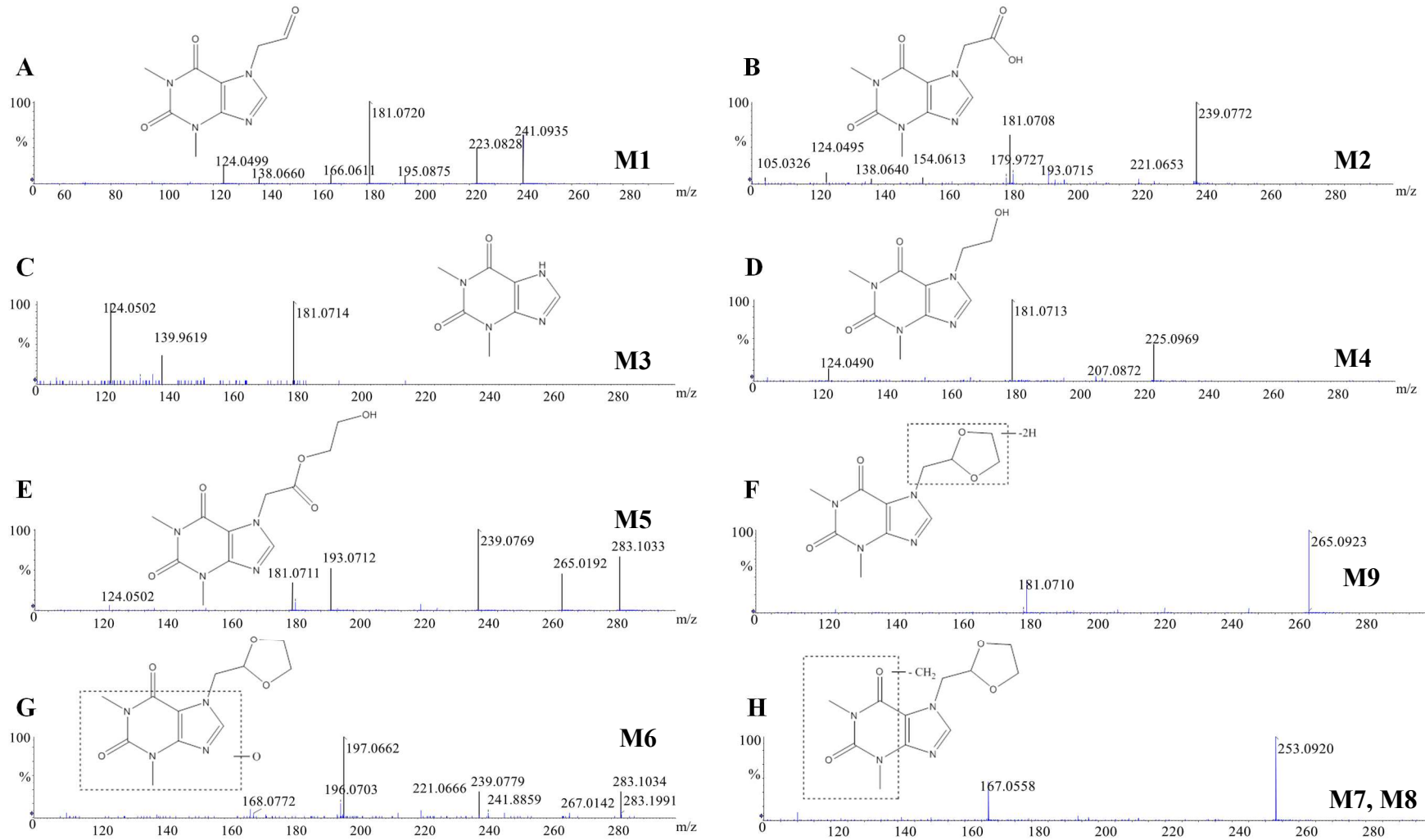
(J) Plasma, blank sample (non-DOXO treated),

(K) Plasma, blank sample spiked with mixture standards of DOXO, M1, M2, M4 and M5 (final plasma concentration: 100 ng/ml each).

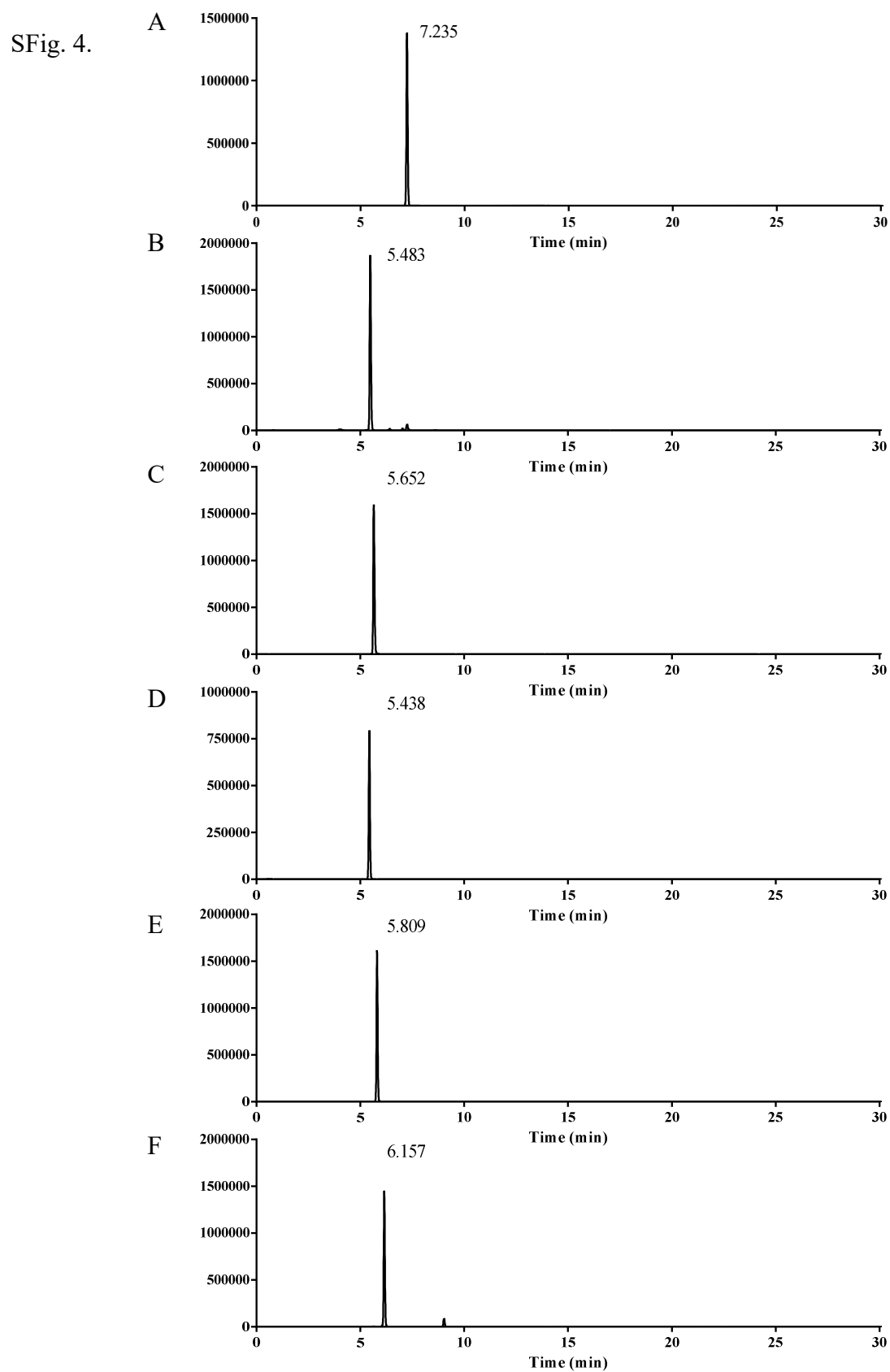


SFig. 2. Chromatography - Mass Spectrometry of DOXO obtained on LC-Triple TOF.
(A) Chromatographic graph of DOXO;
(B) MS² spectrum of DOXO in the positive ion mode.
(C) Tentative structural illustration of the most informative fragment ions for DOXO.

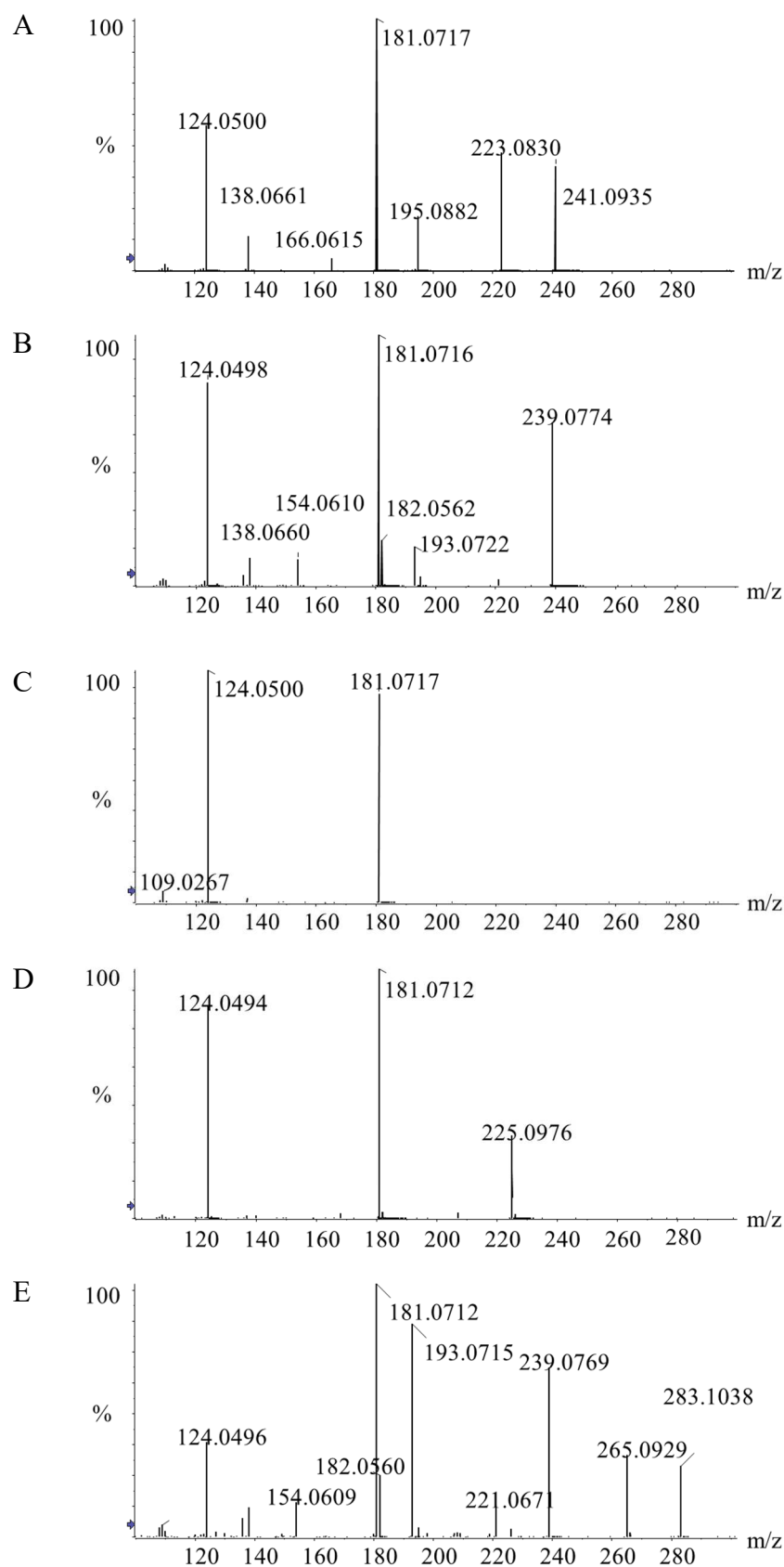
Theophylline acetaldehyde as the initial product in doxophylline metabolism in human liver



Sfig. 3. The MS2 spectra of representative metabolites in HLM. (A) M1; (B) M2; (C) M3; (D) M4; (E) M5; (F) M9; (G) M6; (H) M7 and M8.

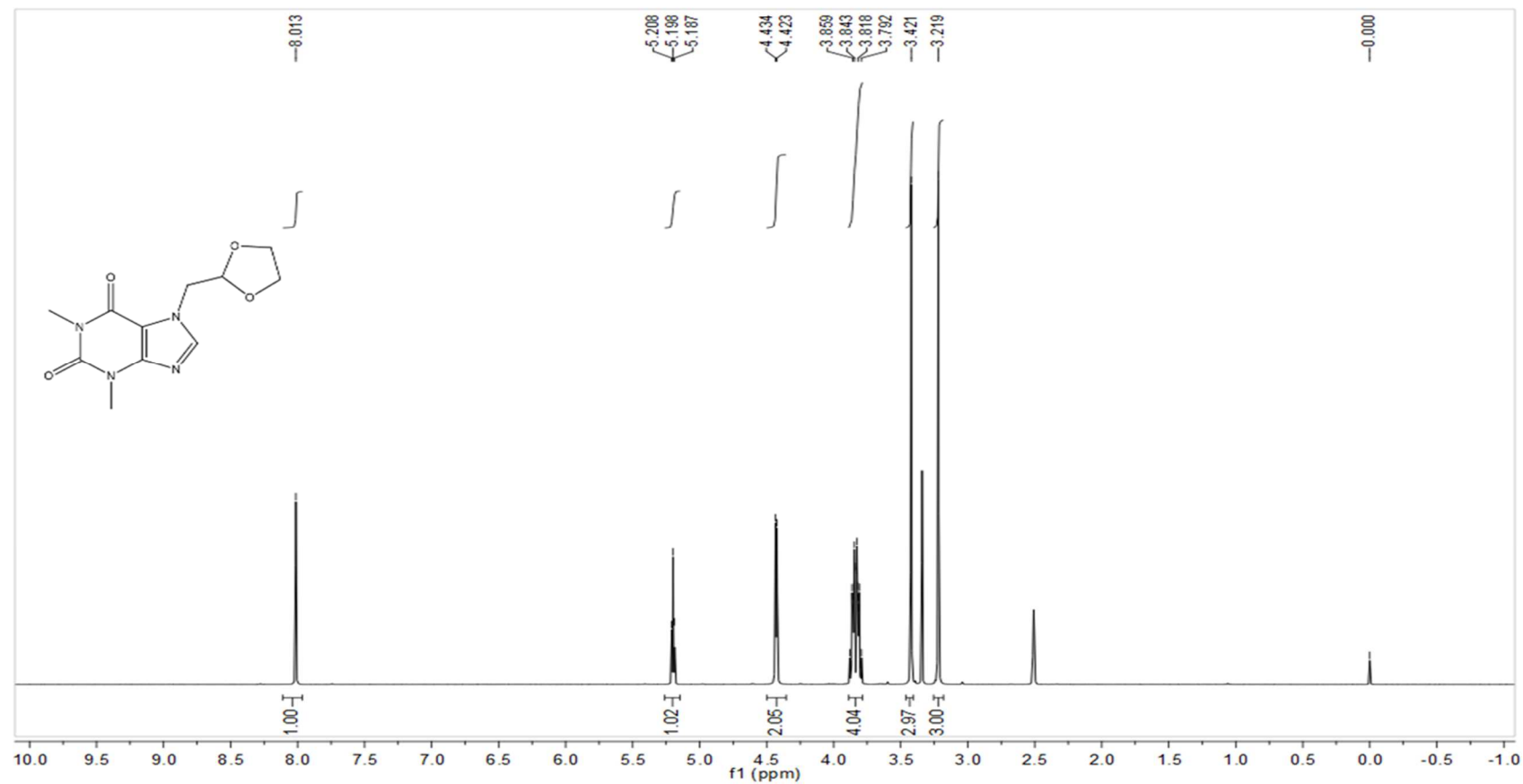


SFig. 4. Chromatography of purity test for reference standards used in the study. (A) DOXO; (B) M1; (C) M2; (D) M3; (E) M4; (F) M5.



SFig. 5. The MS² spectra for reference standards obtained on LC-Triple TOF. (A) M1; (B) M2; (C) M3; (D) M4; (E) M5.

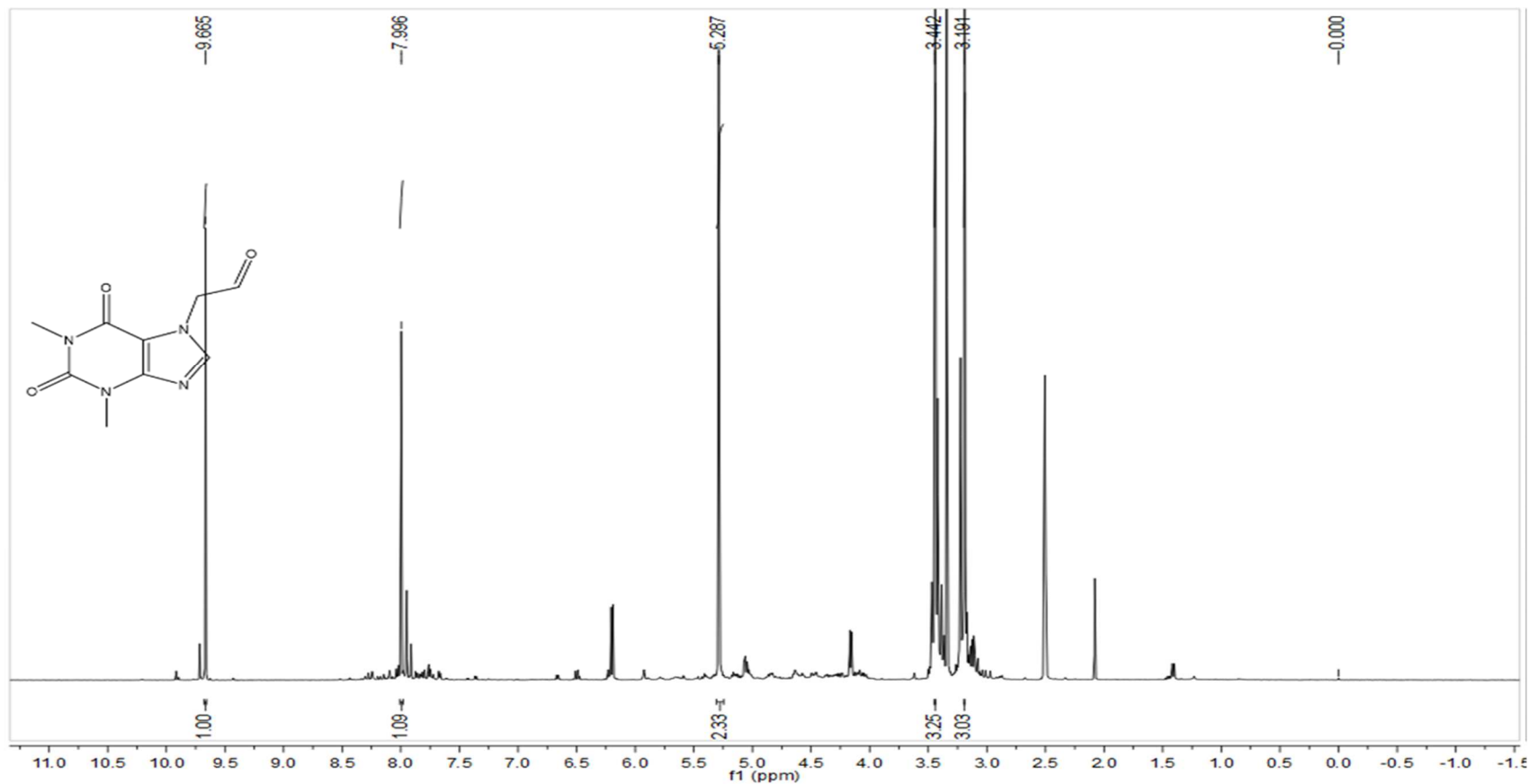
A



^1H NMR (400 MHz, DMSO) δ 8.01 (s, 1H), 5.20 (t, $J = 4.2$ Hz, 1H), 4.43 (d, $J = 4.2$ Hz, 2H), 3.84 (m, 4H), 3.42 (s, 3H), 3.22 (s, 3H).

SFig. 6. The ^1H -NMR for reference standards. (A) DOXO

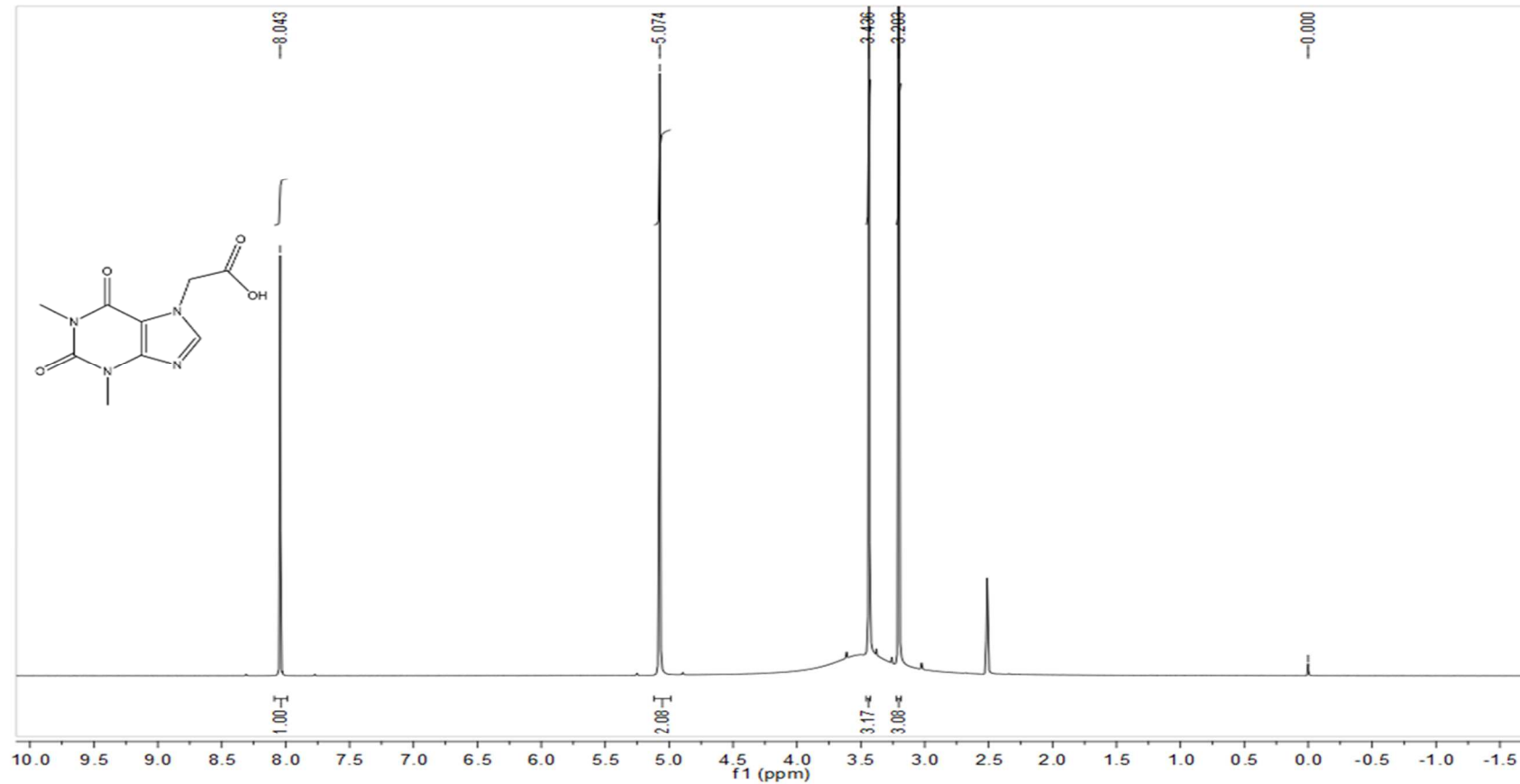
B



^1H NMR (400 MHz, DMSO) δ 9.67 (s, 1H), 8.00 (s, 1H), 5.29 (s, 2H), 3.44 (s, 3H), 3.19 (s, 3H).

SFig. 6. The ^1H -NMR for reference standards. (B) M1;

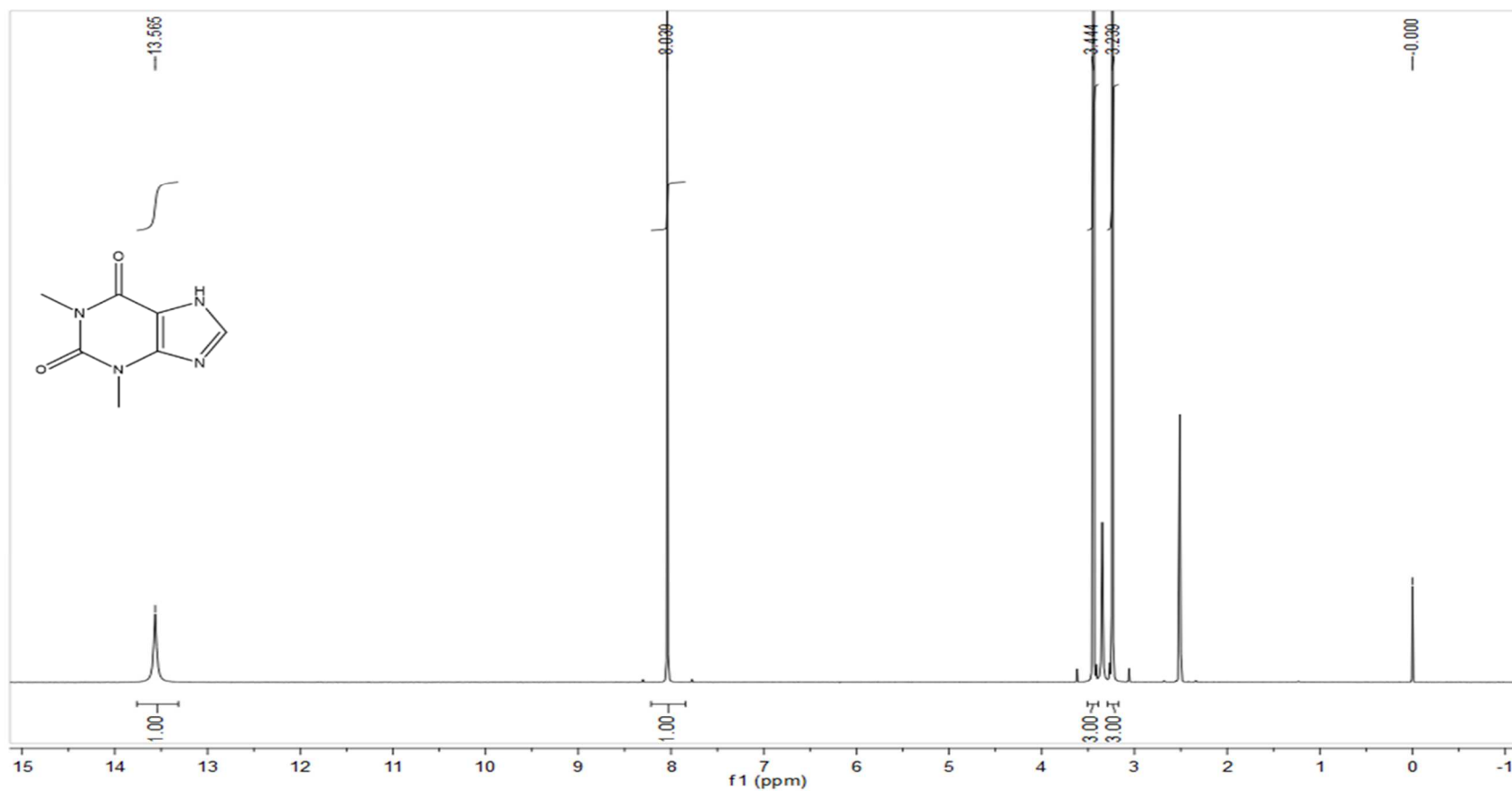
C



^1H NMR (400 MHz, DMSO) δ 8.04 (s, 1H), 5.07 (s, 2H), 3.44 (s, 3H), 3.20 (s, 3H).

SFig. 6. The ^1H -NMR for reference standards. (C) M2;

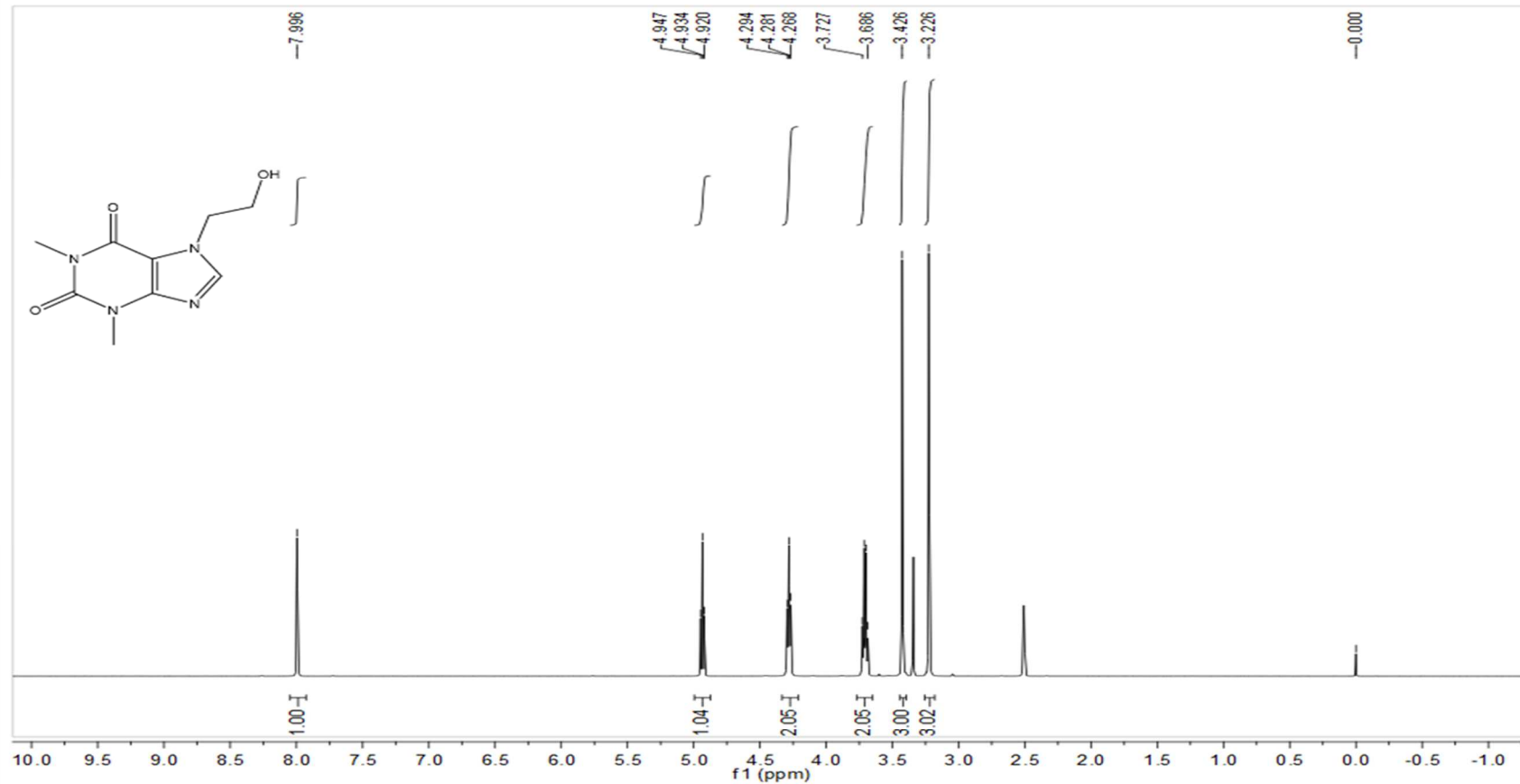
D



^1H NMR (400 MHz, DMSO) δ 13.56 (s, 1H), 8.04 (s, 1H), 3.44 (s, 3H), 3.24 (s, 3H).

SFig. 6. The ^1H -NMR for reference standards. (D) M3;

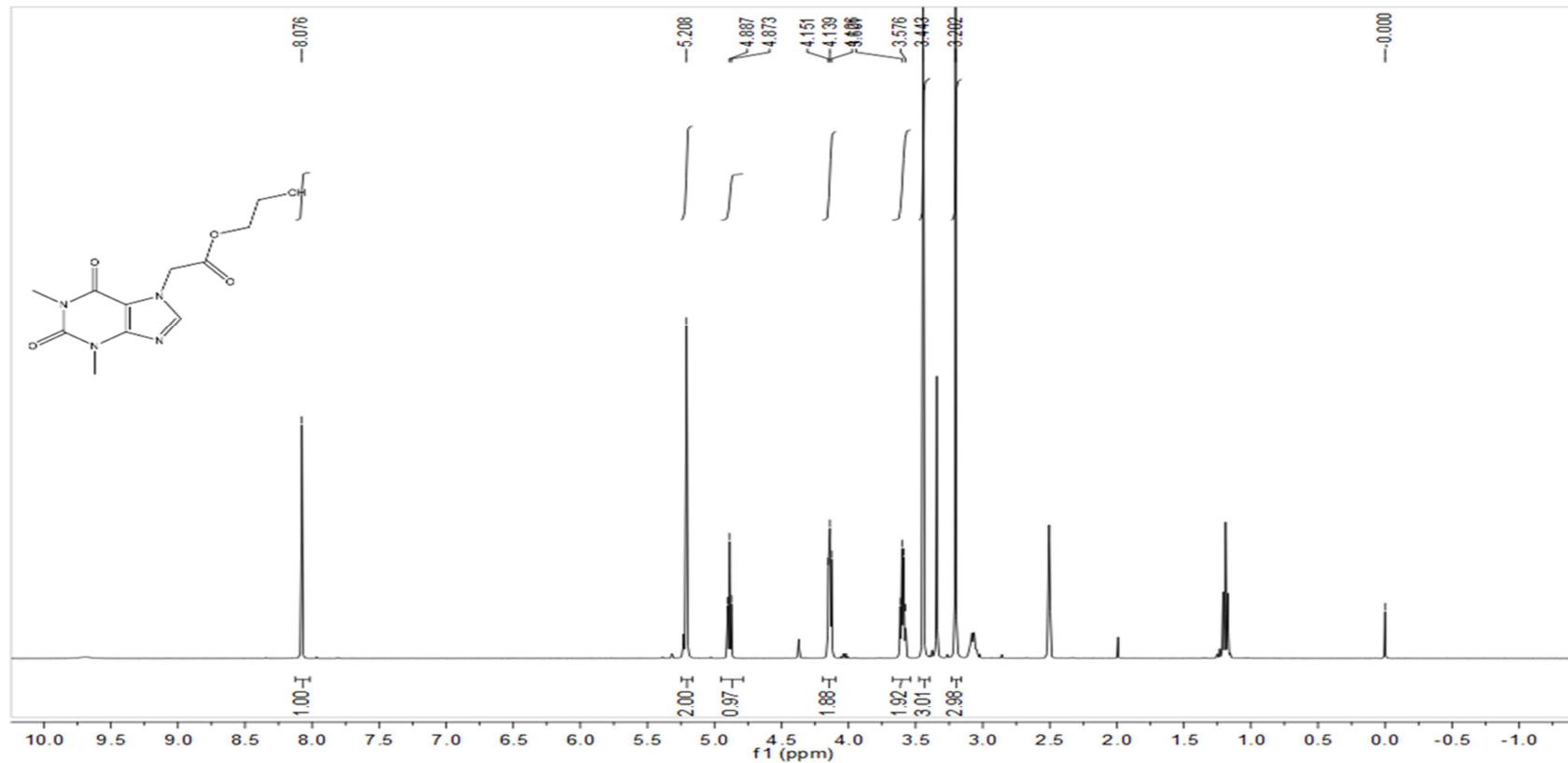
E



¹H NMR (400 MHz, DMSO) δ 8.00 (s, 1H), 4.93 (t, $J = 5.4$ Hz, 1H), 4.28 (t, $J = 5.3$ Hz, 2H), 3.71 (m, 2H), 3.43 (s, 3H), 3.23 (s, 3H).

SFig. 6. The ¹H-NMR for reference standards. (E) M4;

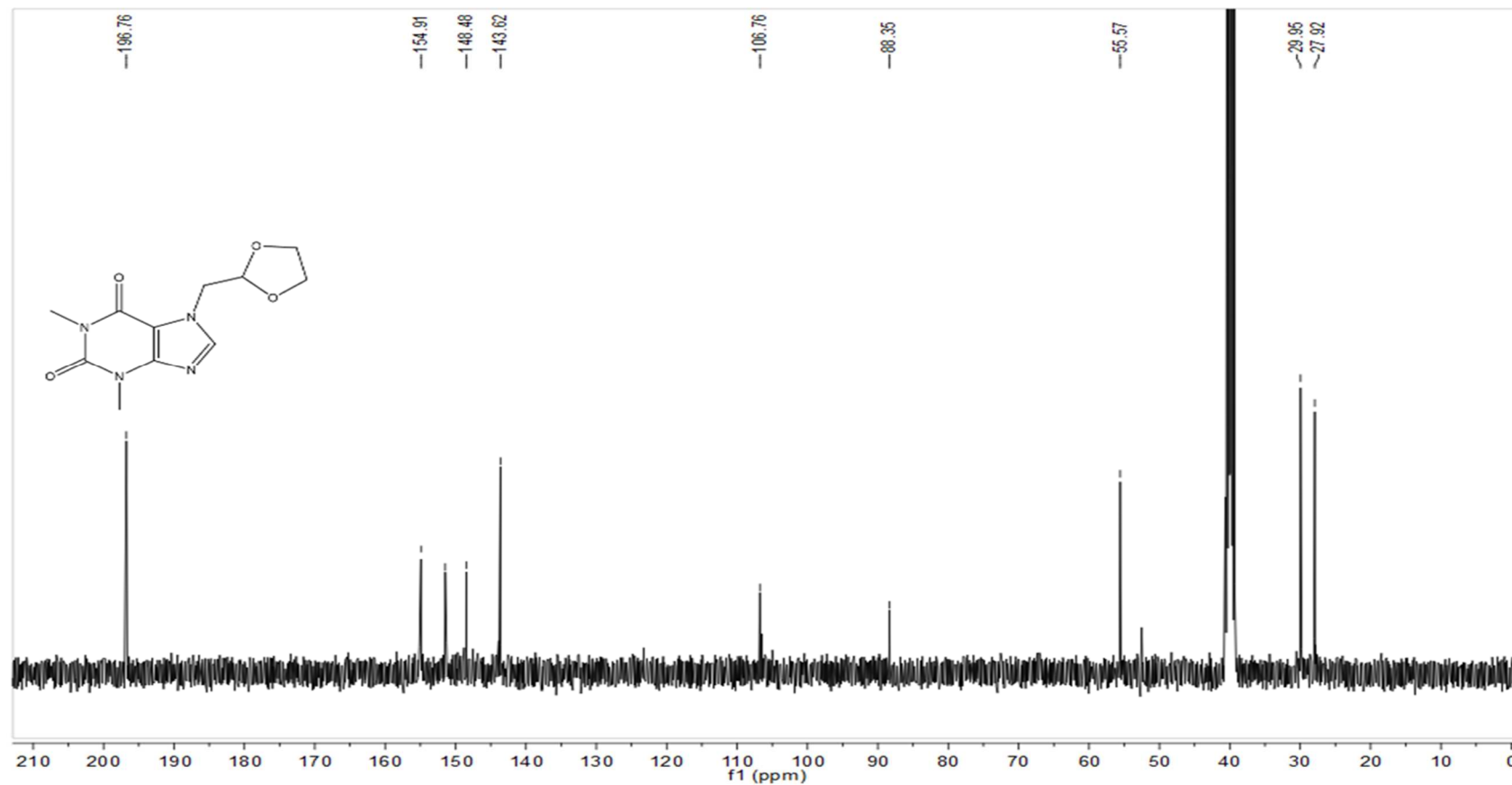
F



¹H NMR (400 MHz, DMSO) δ 8.08 (s, 1H), 5.21 (s, 2H), 4.89 (t, $J = 5.5$ Hz, 1H), 4.14 (m, 2H), 3.60 (dd, $J = 10.2, 5.4$ Hz, 2H), 3.44 (s, 3H), 3.20 (s, 3H).

SFig. 6. The ¹H-NMR for reference standards. (F) M5.

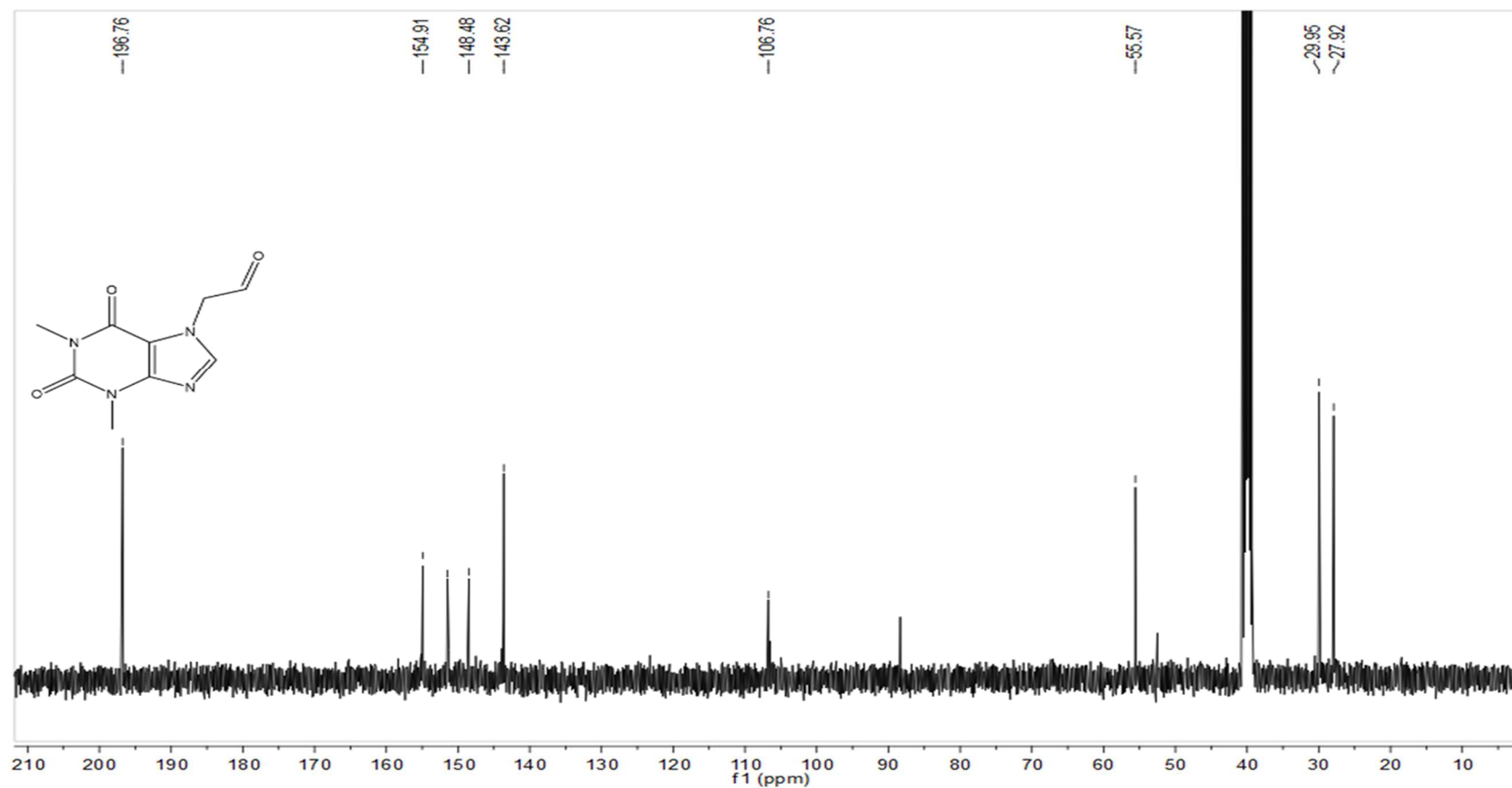
A



^{13}C NMR (101 MHz, DMSO) δ 196.76, 154.91, 151.47, 148.48, 143.62, 106.76, 88.35, 55.57, 29.95, 27.92.

SFig. 7. The ^{13}C -NMR for reference standards. (A) DOXO;

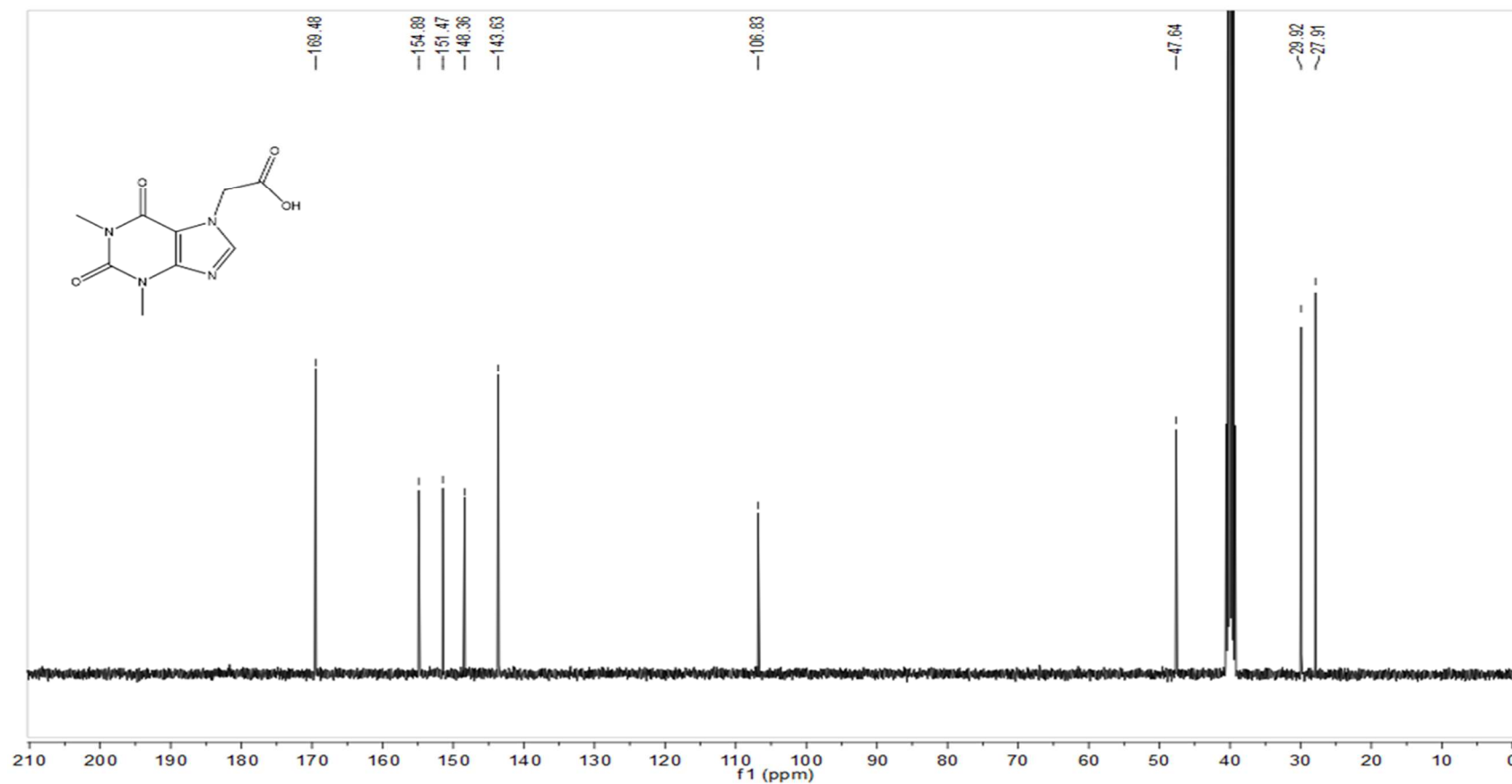
B



^{13}C NMR (101 MHz, DMSO) δ 196.76, 154.91, 151.47, 148.48, 143.62, 106.76, 55.57, 29.95, 27.92.

SFig. 7. The ^{13}C -NMR for reference standards. (B) M1;

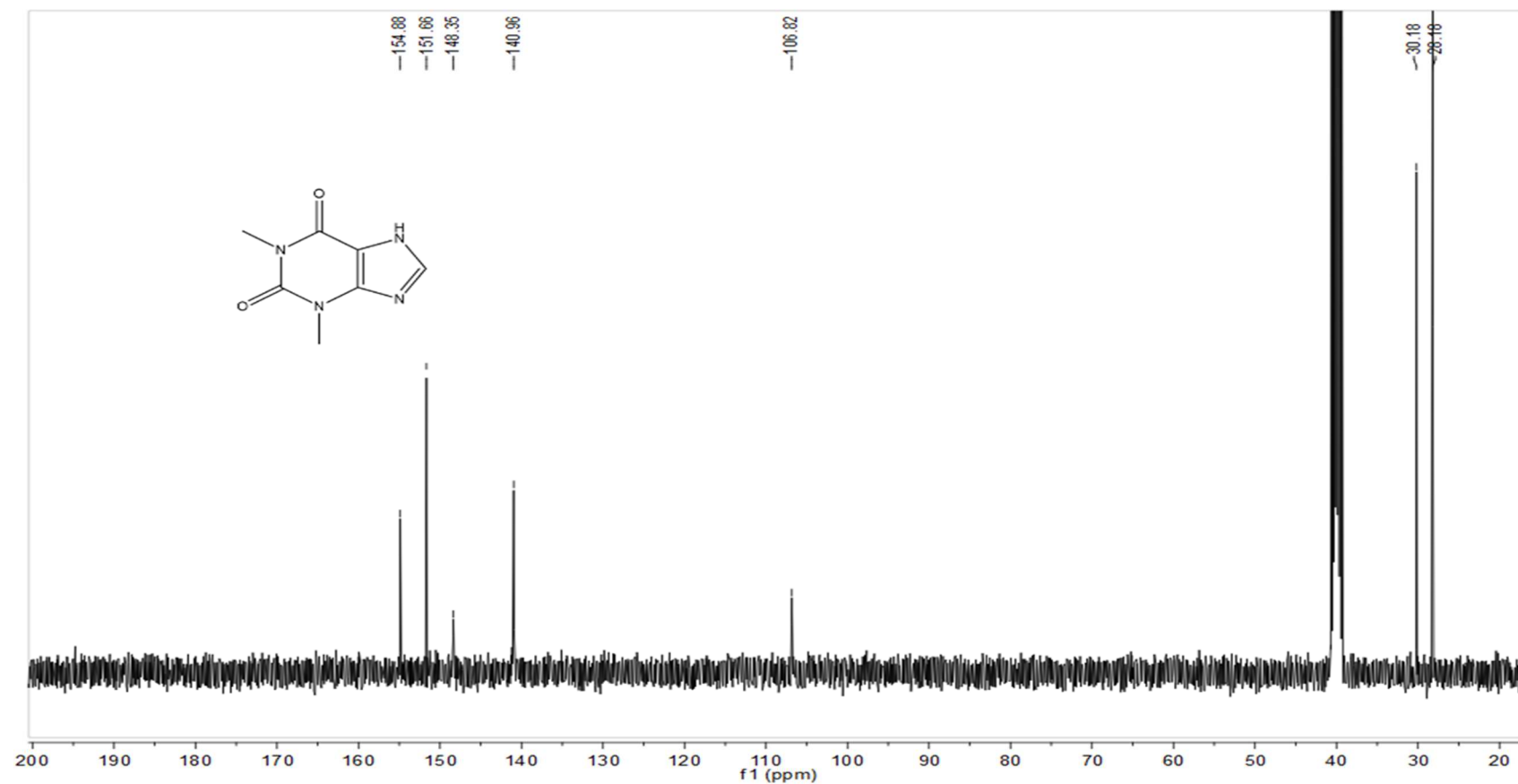
C



^{13}C NMR (101 MHz, DMSO) δ 169.48, 154.89, 151.47, 148.36, 143.63, 106.83, 47.64, 29.92, 27.91.

SFig. 7. The ^{13}C -NMR for reference standards. (C) M2;

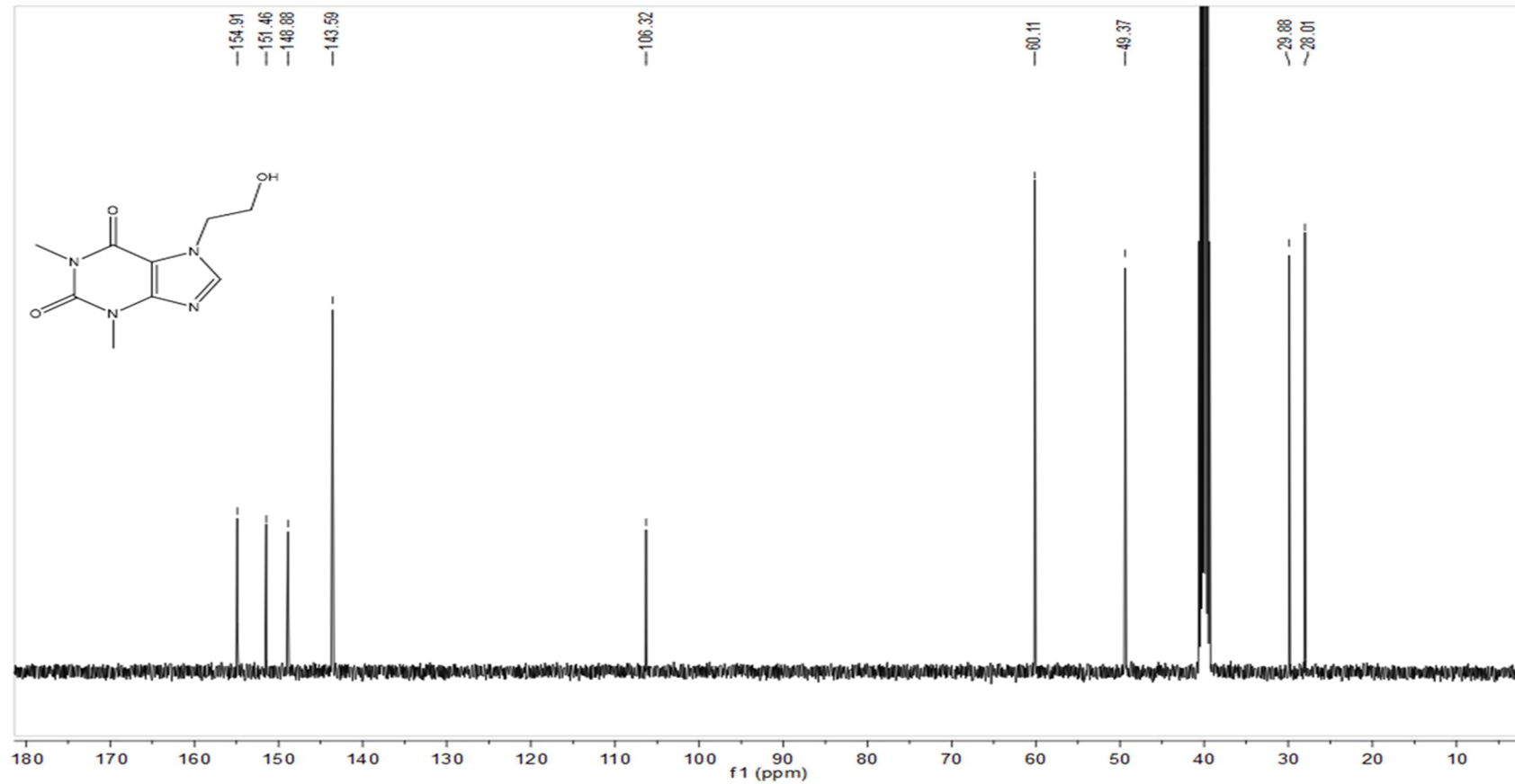
D



^{13}C NMR (101 MHz, DMSO) δ 154.88, 151.66, 148.35, 140.96, 106.82, 30.18, 28.18.

SFig. 7. The ^{13}C -NMR for reference standards. (D) M3;

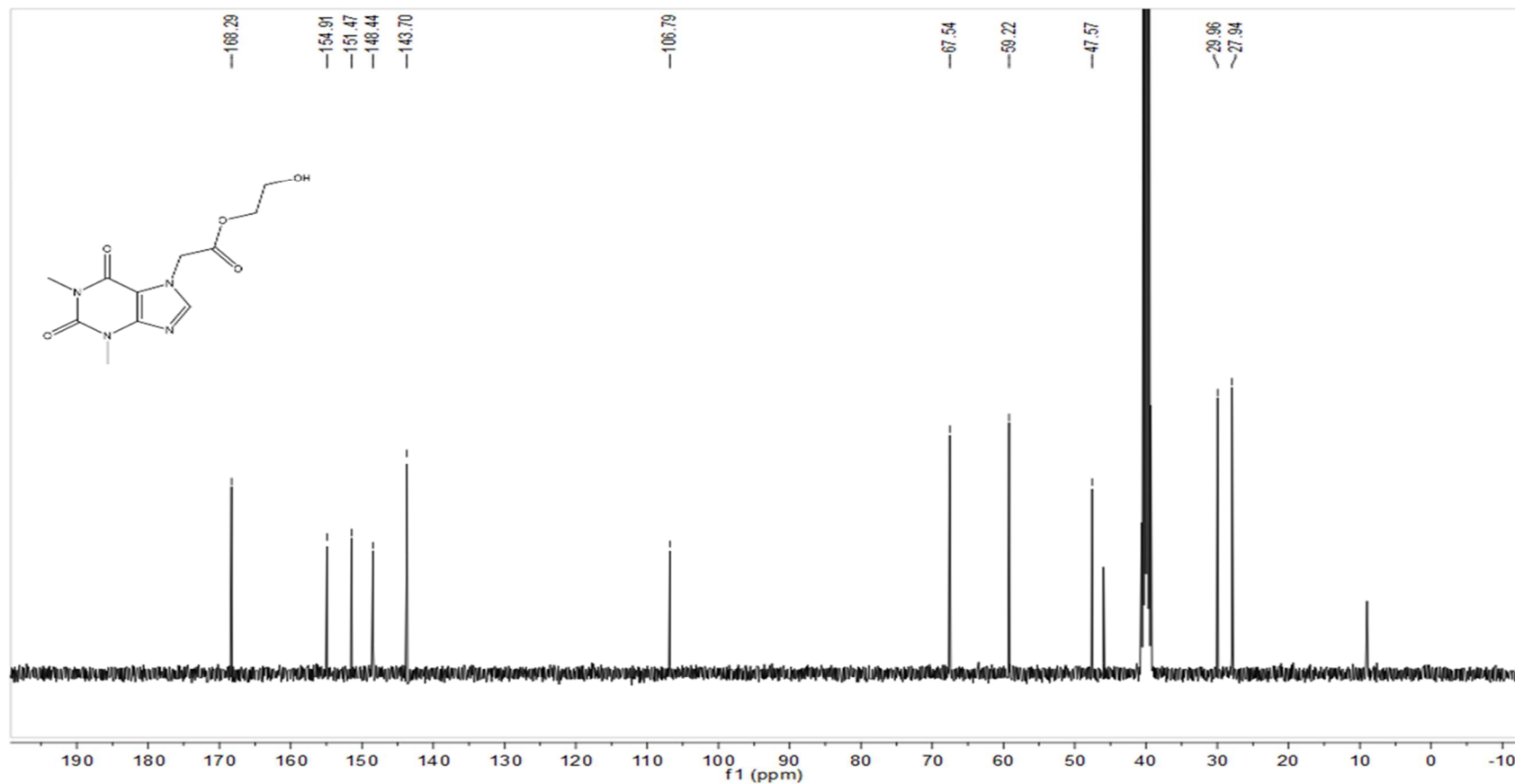
E



^{13}C NMR (101 MHz, DMSO) δ 154.91, 151.46, 148.88, 143.59, 106.32, 60.11, 49.37, 29.88, 28.01.

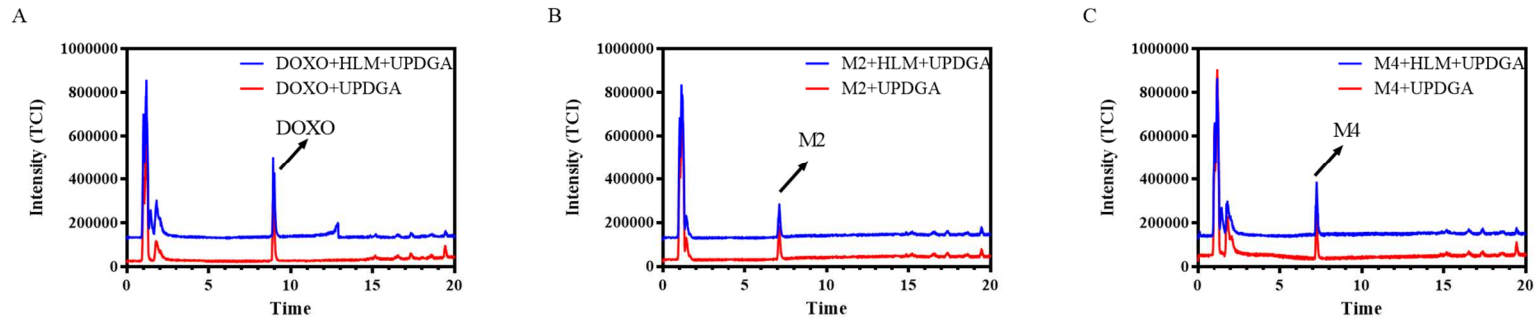
SFig. 7. The ^{13}C -NMR for reference standards. (E) M4;

F



^{13}C NMR (101 MHz, DMSO) δ 168.29, 154.91, 151.47, 148.44, 143.70, 106.79, 67.54, 59.22, 47.57, 29.96, 27.94.

SFig. 7. The ^{13}C -NMR for reference standards. (F) M5.



SFig. 8. TIC chromatogram of different substrates in phase II metabolism incubation system in the presences or absence of HLM determined by LC-Triple TOF. (A) DOXO; (B) M2; (C) M4.

Supplemental Table

STable 1. The ion transitions monitored for DOXO and metabolites.

analyte	Q1 Mass (Da)	Q3 Mass (Da)	Dwell (msec)
DOXO	267.1	181.1	60
M1	241.1	181.1	60
M2	239.1	181.1	60
M4	225.1	181.1	60
M5	283.1	239.1	60
M8	253.1	167.1	60
M9	265.1	181.1	60

STable 2. Metabolites of DOXO detected by LC-Triple TOF in HLM.

	Rt		Ion	Formula	Error ppm	Product ions (m/z)					
	min	Measured					HLM	MLM	RaLM	RLM	CyLM
M0	6.68	267.1085	[M+H] ⁺	C ₁₁ H ₁₄ N ₄ O ₄	-1.0	223.0814, 210.0867, 181.0712, 166.0602, 138.0654, 124.0494					
M1	5.10	241.0935	[M+H ₂ O+H] ⁺	C ₉ H ₁₀ N ₄ O ₃	1.5	223.0828, 195.0875, 181.0720, 166.0611, 138.0660, 124.0499	+++	+++	+++	+++	+++
M2	4.65	239.0772	[M+H] ⁺	C ₉ H ₁₀ N ₄ O ₄	-1.2	221.0653, 181.0708, 138.0640, 124.0495	+	++	+	+	+
M3	4.67	181.0714	[M+H] ⁺	C ₇ H ₈ N ₄ O ₂	-3.3	124.0502, 139.9619	+	+	+	+	+
M4	5.24	225.0969	[M+H] ⁺	C ₉ H ₁₂ N ₄ O ₃	-5.9	207.0872, 181.0713, 124.0490	+	+++	+++	+	++
M5	5.58	283.1033	[M+H] ⁺	C ₁₁ H ₁₄ N ₄ O ₅	-1.4	265.0192, 239.0712, 181.0711, 124.0502	+	+++	++	+++	++
M6	5.84	283.1034	[M+H] ⁺	C ₁₁ H ₁₄ N ₄ O ₅	-1.1	267.0142, 239.0779, 197.0662	+	+	+	+	+
M7	5.18	253.0928	[M+H] ⁺	C ₁₀ H ₁₂ N ₄ O ₄	-1.3	167.0558	+	+	+	+	+
M8	5.72	253.0920	[M+H] ⁺	C ₁₀ H ₁₂ N ₄ O ₄	-4.9	167.0558	+	+	+	+	+
M9	6.36	265.0923	[M+H] ⁺	C ₁₁ H ₁₂ N ₄ O ₄	-3.1	181.0710	+	+	+	+	+

STable. 3. The HPLC condition for purity test.

column	VP-ODS 3.0×100 mm 4.6 μm
mobile phase	A: 0.2% formic acid; B acetonitrile 0~2 min: 2%B 2~28 min: 2%B~90%B 28~30 min: 90%B 30.1min: 2%B
Flow:	0.8 mL/min
Temperature:	30°C
Detector:	UV@273
Injection volume:	10 μL

STable. 4. Representative calibration curves for DOXO, M1, M2, M4, and M5.

analyte	curve	r^2
DOXO	$y=4034.84212 x + 117.49016$	0.9936
M1	$y=1260.46153 x + 174.46048$	0.9952
M2	$y=1205.52800 x + 97.24184$	0.9969
M4	$y=2683.56612 x + 654.08111$	0.9943
M5	$y=1803.35266 x + 1165.32597$	0.9917