Supplementary Materials

Assessing the Purity of IMM-H014 and Its Related Substances for the Treatment of Non-Alcoholic Fatty Liver Disease Using Quantitative Nuclear Magnetic Resonance Method

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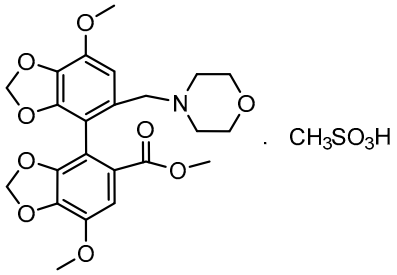
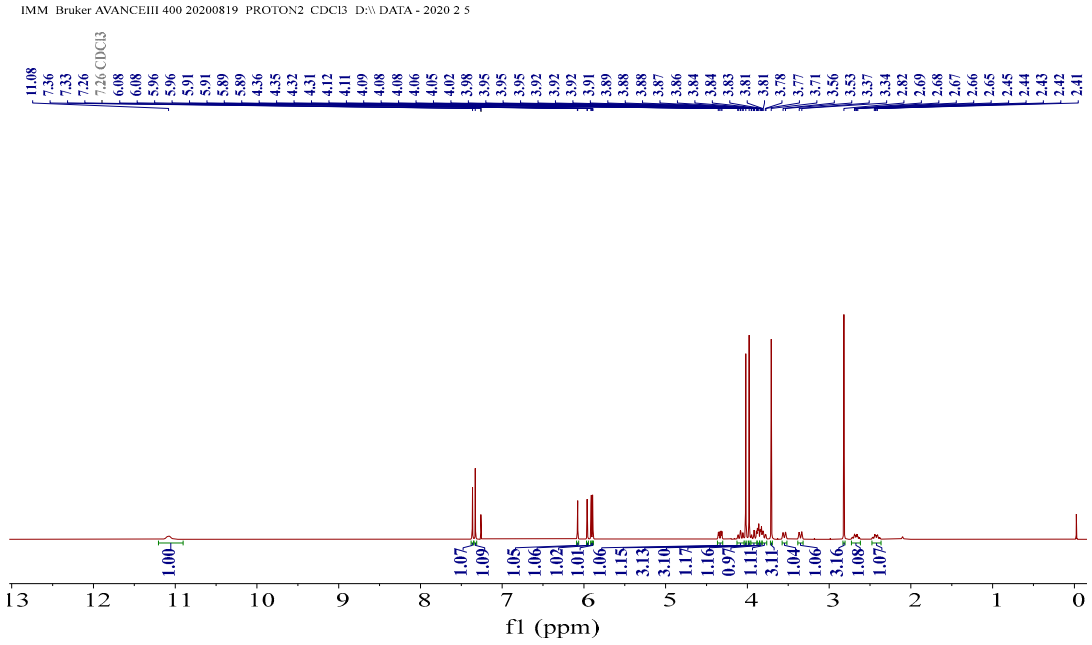
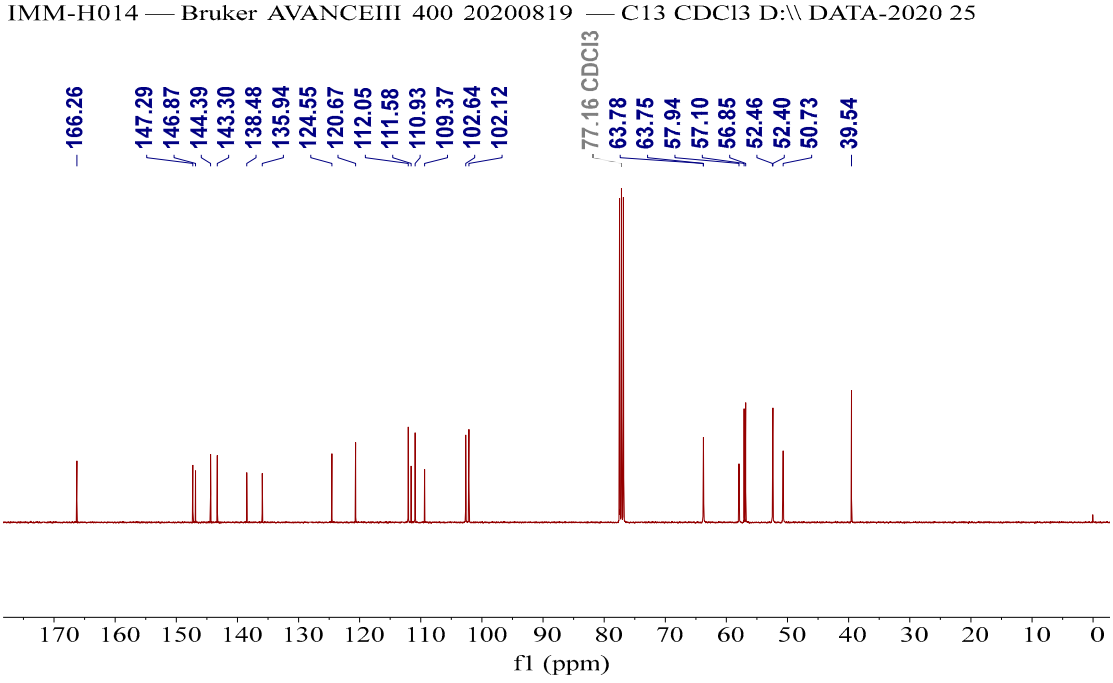
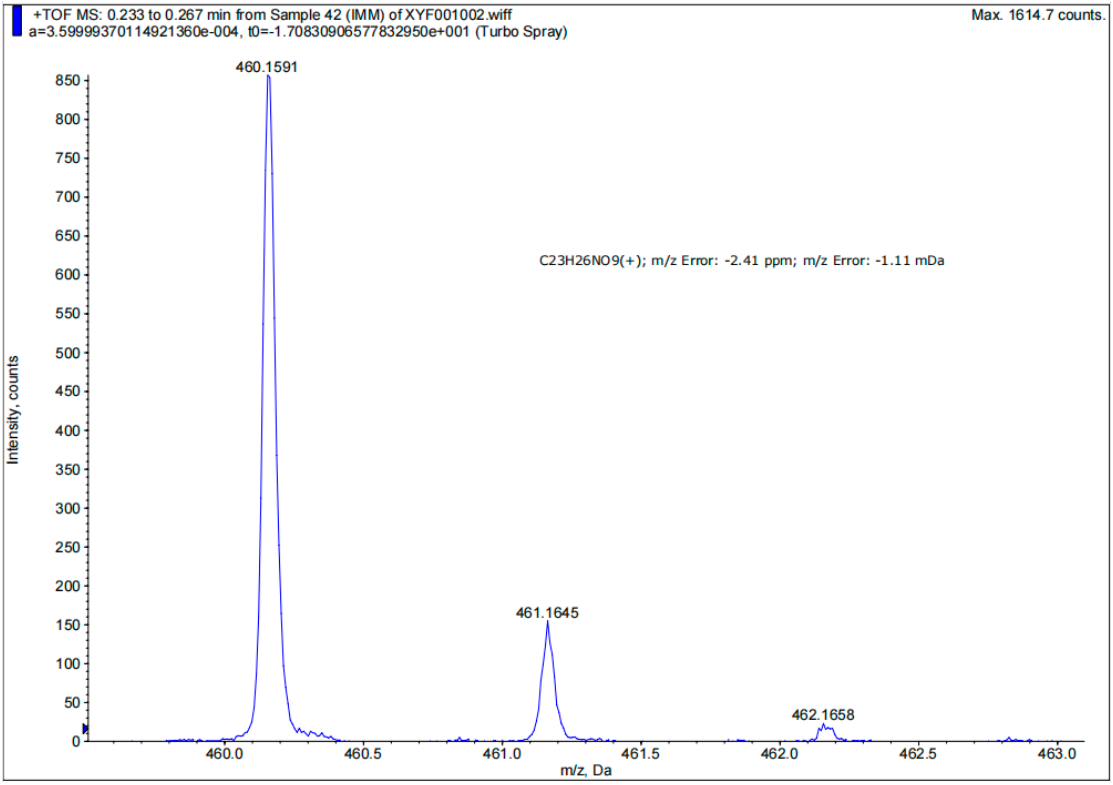
Figure S1. ^{1}H NMR, ^{13}C NMR, and HR-ESI–MS spectral data of IMM-H014

Methyl 7, 7'-dimethoxy-5'-(morpholinomethyl)-[4, 4'-bibenzo[d][1,3]dioxole]-5-carboxylate methanesulfonate





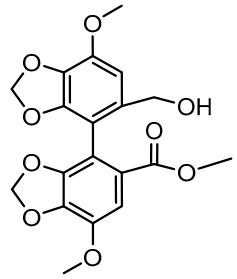




^{1}H NMR (400 MHz, $CDCl_3$) δ 11.08 (s, 1H), 7.36 (s, 1H), 7.33 (s, 1H), 6.08 (d, J = 1.2 Hz, 1H), 5.96 (d, J = 1.2 Hz, 1H), 5.91 (d, J = 1.4 Hz, 1H), 5.89 (d, J = 1.4 Hz, 1H), 4.33 (dd, J = 13.2, 4.2 Hz, 1H), 4.08 (m, J = 13.7, 11.8, 2.2 Hz, 1H), 4.02 (s, 3H), 3.98 (s, 3H), 3.92 (m, J = 12.3, 11.5, 1.8 Hz, 1H), 3.87 (d, J = 6.6 Hz, 1H), 3.85 – 3.82 (m, 1H), 3.79 (dd, J = 13.1, 3.4 Hz, 1H), 3.71 (s, 3H), 3.55 (d, J = 12.1 Hz, 1H), 3.35 (d, J = 12.1 Hz, 1H), 2.82 (s, 3H), 2.67 (m, J = 12.1, 8.8, 3.8 Hz, 1H), 2.48 – 2.36 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.26, 147.29, 146.87, 144.39, 143.30, 138.48, 135.94, 124.55, 120.67, 112.05, 111.58, 110.93, 109.37, 102.64, 102.12, 63.78, 63.75, 57.94, 57.10, 56.85, 52.46, 52.40, 50.73, 39.54. HR-ESI–MS: *m/z* $[M–CH_3SO_3H+H]^+$ (organic base) calcd for $C_{23}H_{25}NO_9$ 460.1529, found 460.1591.

Figure S2. ^{1}H NMR, ^{13}C NMR, and HR-ESI–MS spectral data of impurity I

Methyl 5'-(hydroxymethyl)-7,7'-dimethoxy-[4,4'-bibenzo[d][1,3]dioxole]-5-carboxylate



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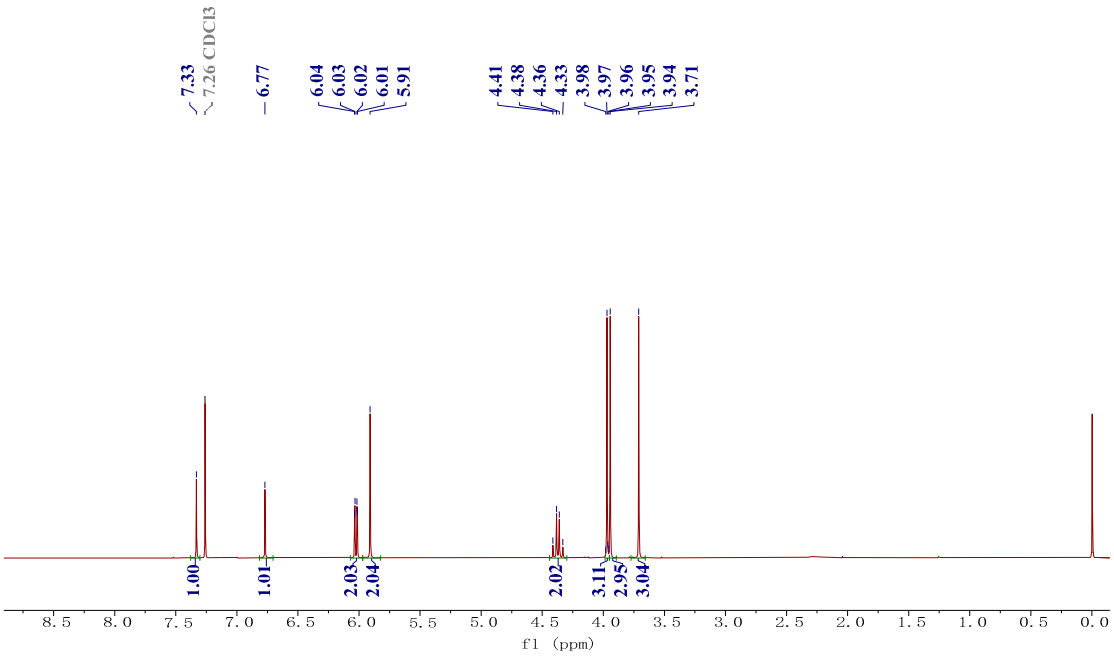
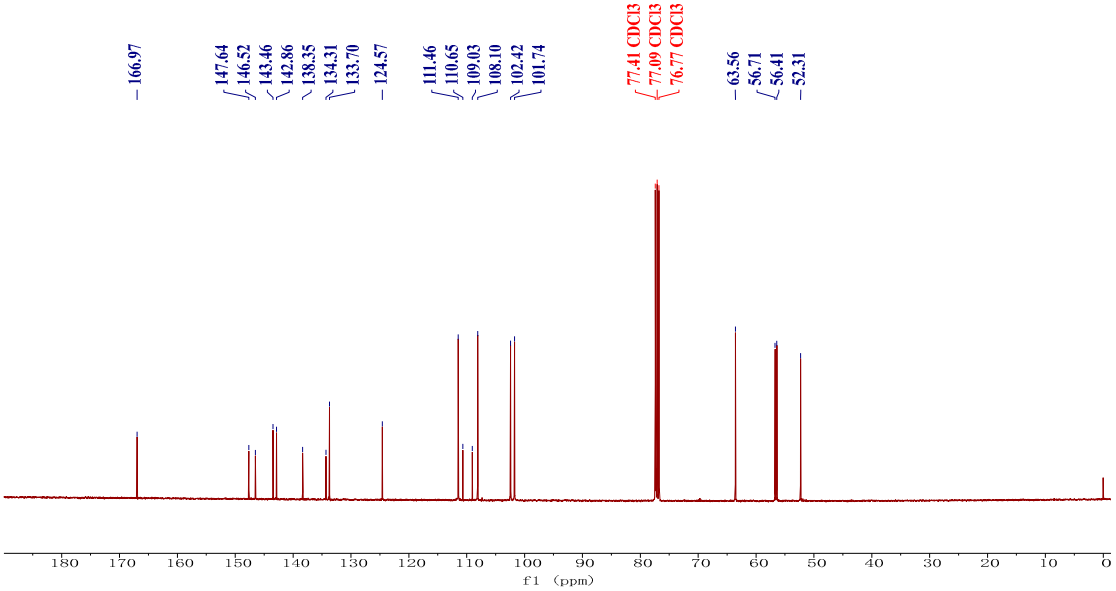
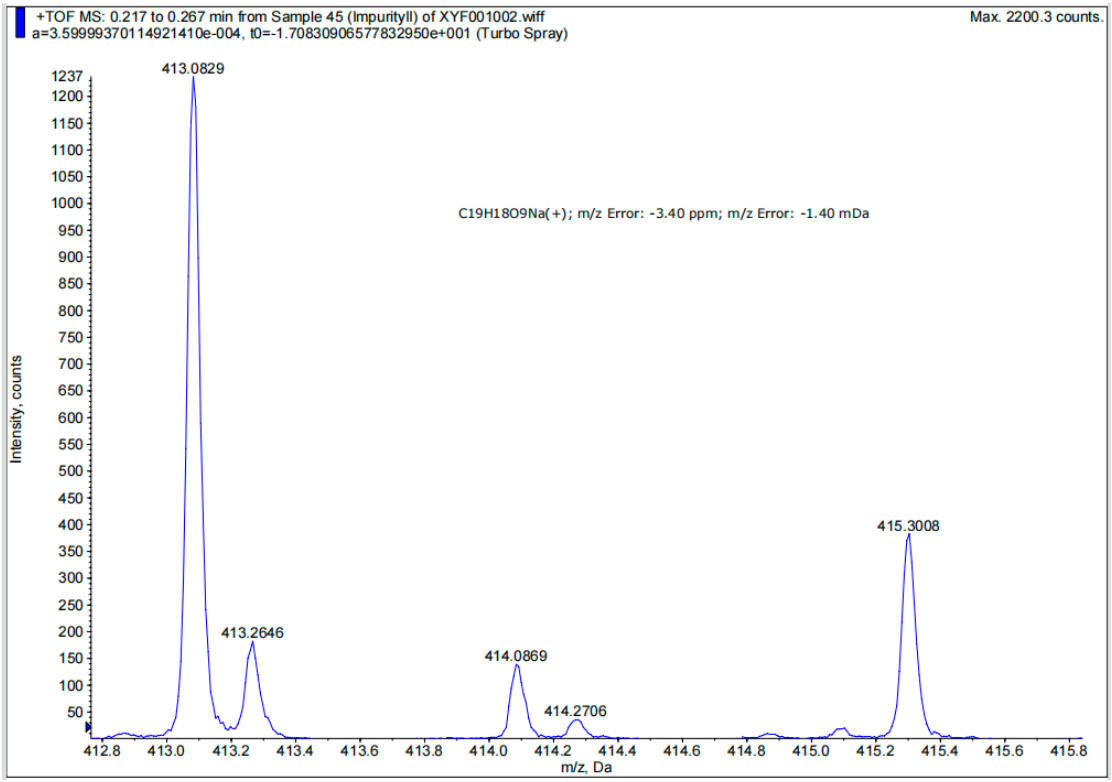
PROTON2 CDCl3 D:\\ DATA-2022 8



20230530 Impurity II.1.fid — Bruker AVANCE III 400 — C13 CDCl3 D:\\ DATA-2023 15

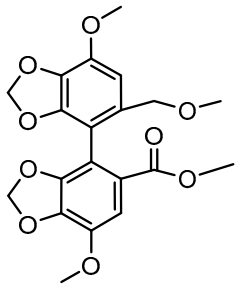


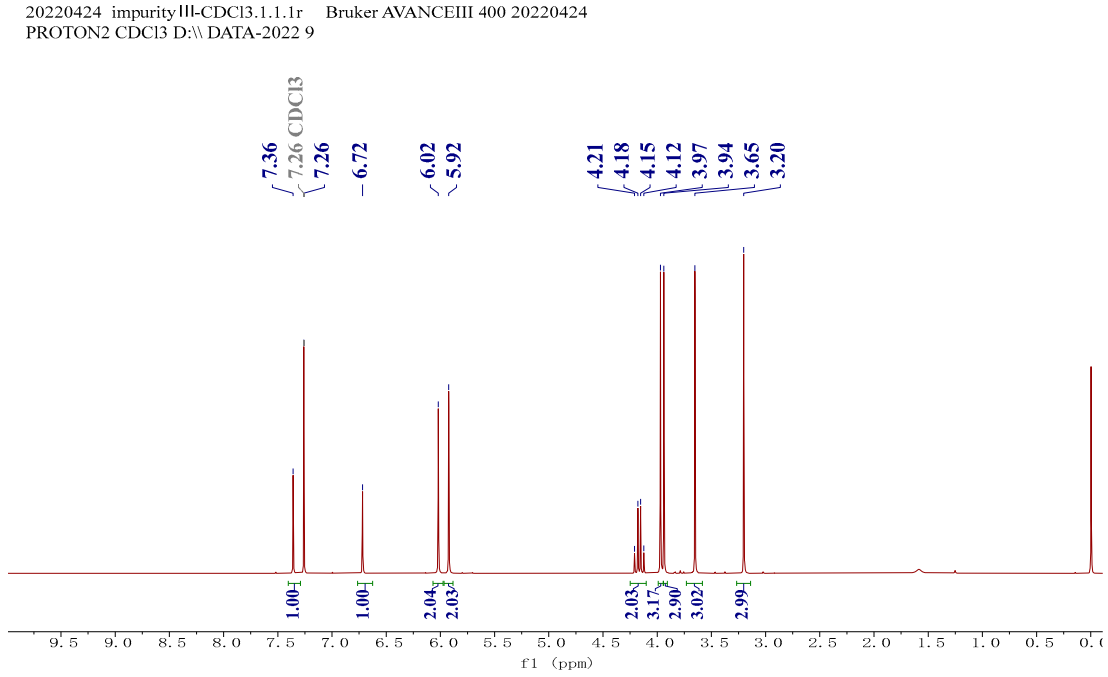


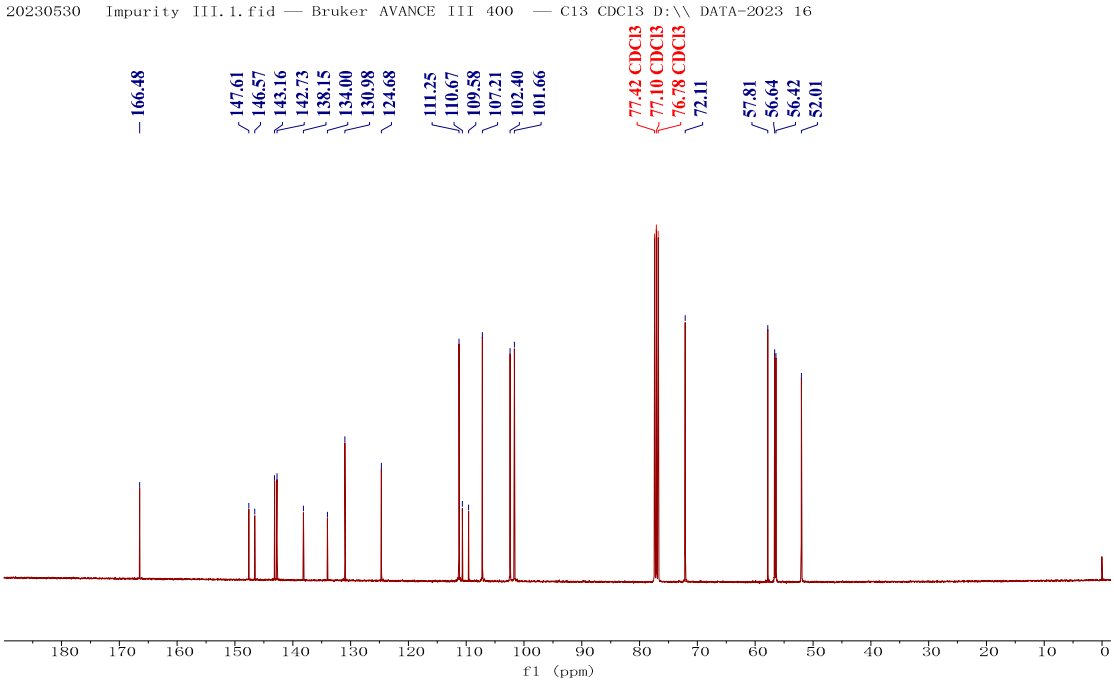
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (s, 1H), 6.77 (s, 1H), 6.03 (q, 2H), 5.91 (s, 2H), 4.37 (q, 2H), 3.97 (s, 3H), 3.94 (s, 3H), 3.71 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.97, 147.64, 146.52, 143.46, 142.86, 138.35, 134.31, 133.70, 124.57, 111.46, 110.65, 109.03, 108.10, 102.42, 101.74, 63.56, 56.71, 56.41, 52.31. HR-ESI–MS: *m/z* $[M+Na]^+$ calcd for $C_{19}H_{18}O_9$ 413.0951, found 413.0829.

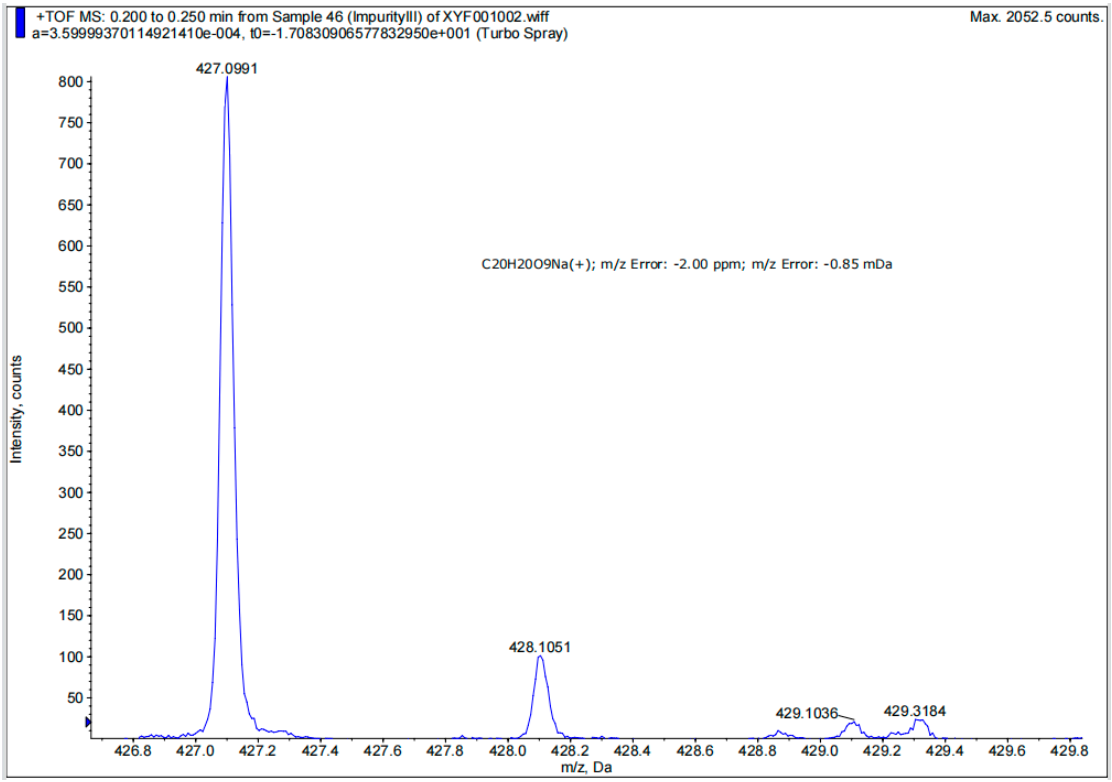
Figure S3. ^{1}H NMR, ^{13}C NMR, and HR-ESI–MS spectral data of impurity II

Methyl 7,7'-dimethoxy-5'-(methoxymethyl)-[4,4'-bibenzo[d][1,3]dioxole]-5-carboxylate





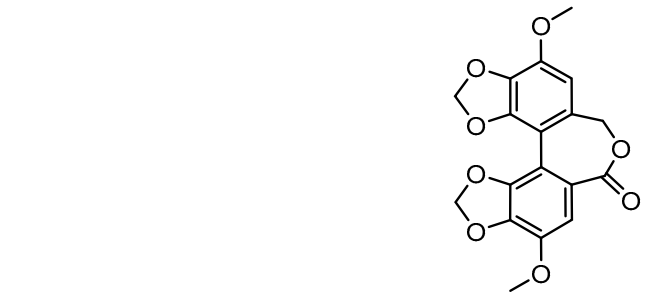


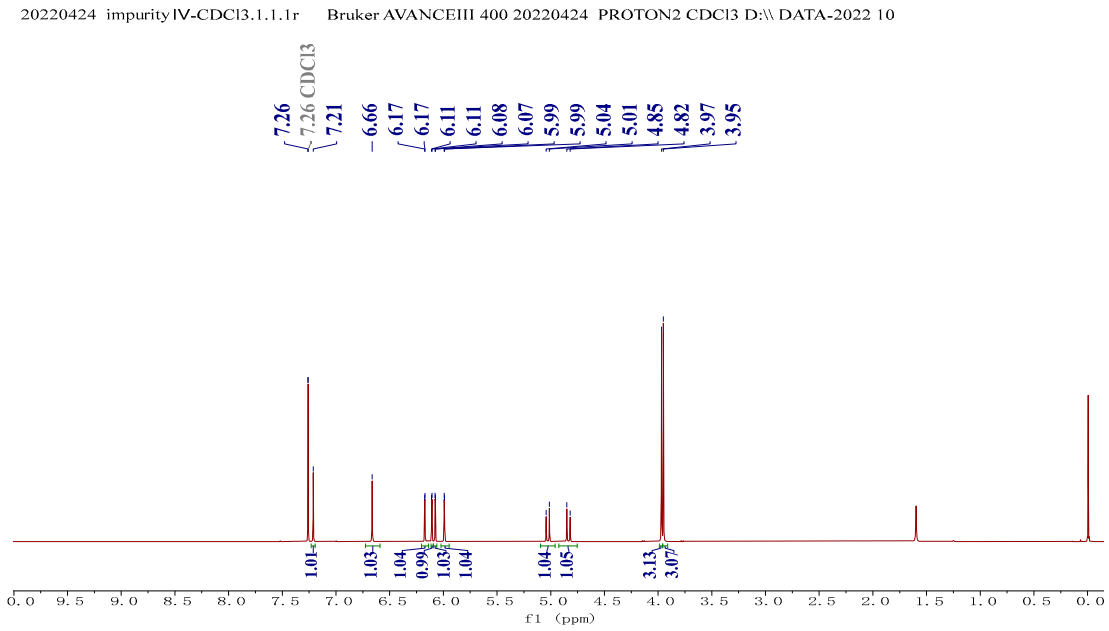


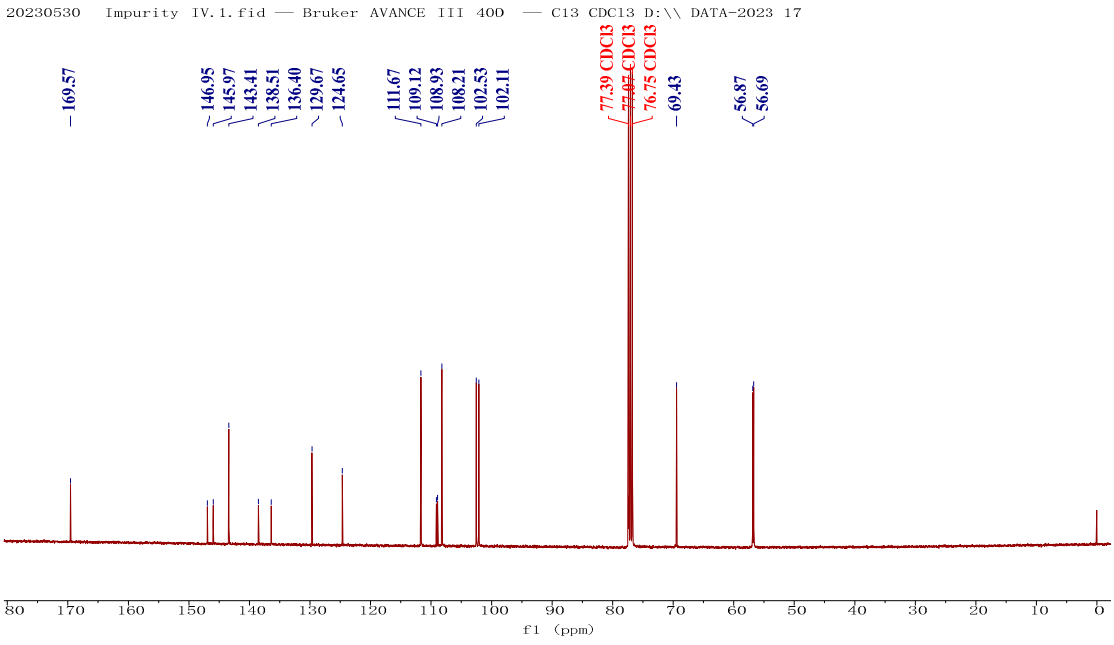
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (s, 1H), 6.72 (s, 1H), 6.02 (s, 2H), 5.92 (s, 2H), 4.16 (q, 2H), 3.97 (s, 3H), 3.94 (s, 3H), 3.65 (s, 3H), 3.20 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.48, 147.61, 146.57, 143.16, 142.73, 138.15, 134.00, 130.98, 124.68, 111.25, 110.67, 109.58, 107.21, 102.40, 101.66, 72.11, 57.81, 56.64, 56.42, 52.01. HR-ESI–MS: *m/z* $[M+Na]^+$ calcd for $C_{20}H_{20}O_9$ 427.1107, found 427.0991.

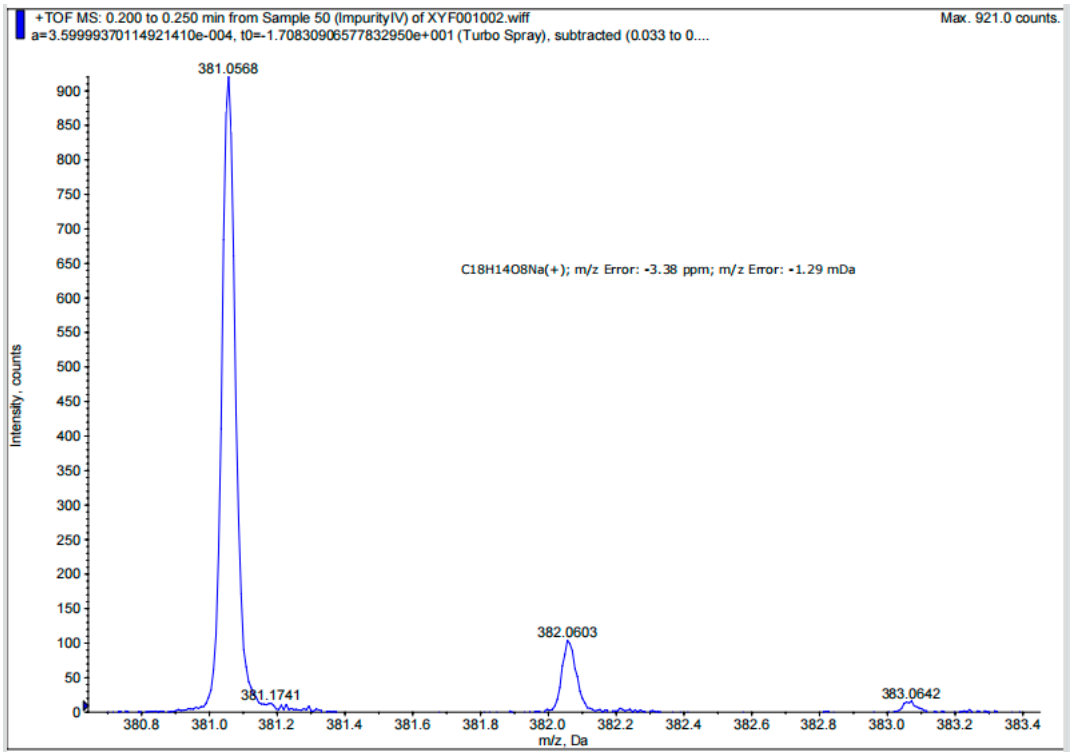
Figure S4. ^{1}H NMR, ^{13}C NMR, and HR-ESI–MS spectral data of impurity Ⅲ

4,10-dimethoxy-[1,3]dioxolo[4',5':3,4]benzo[1,2-*c*][1,3]dioxolo[4',5':5,6]benzo[1,2-*e*]oxepin-6(8H)-one





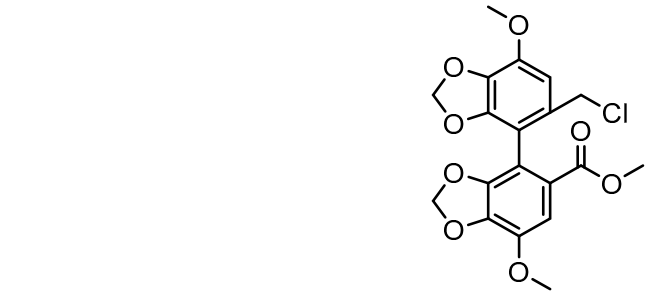


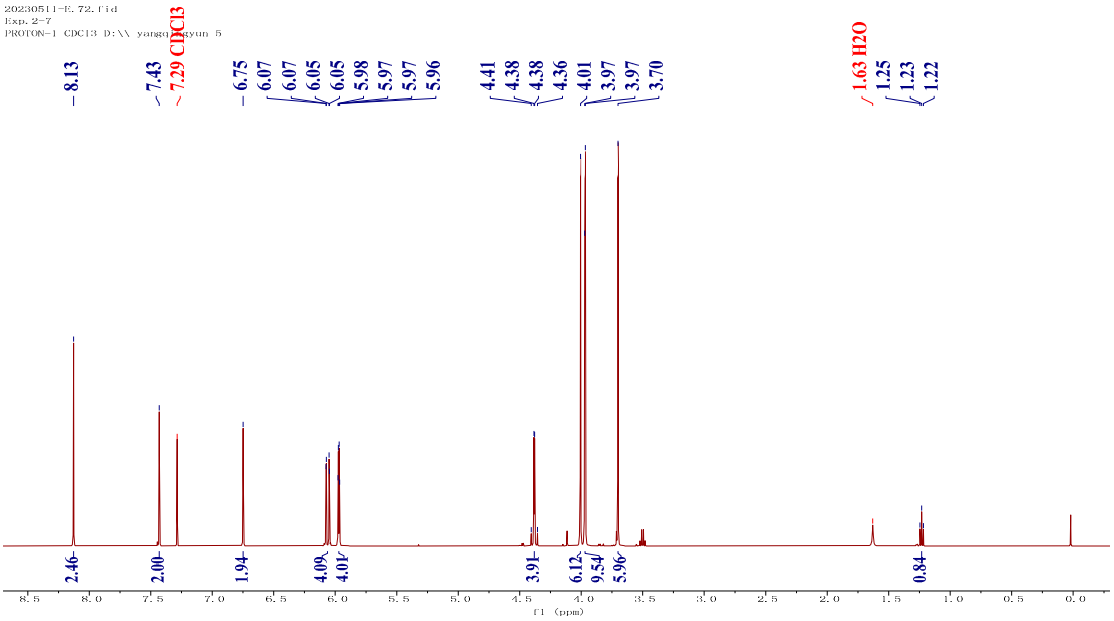


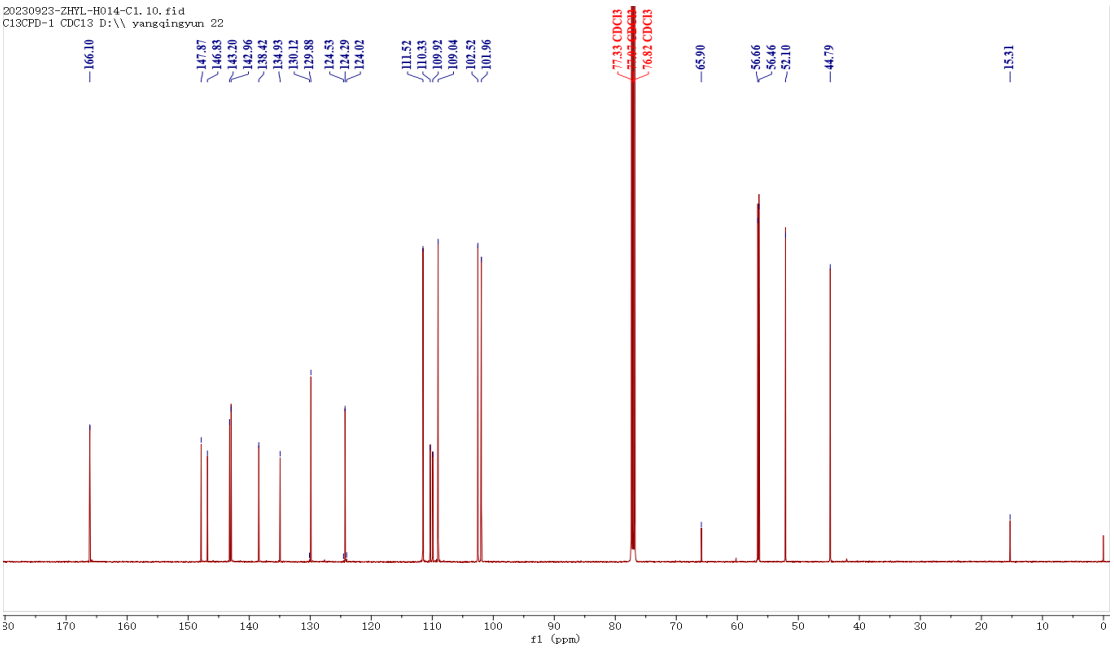
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (s, 1H), 6.67 (s, 1H), 6.17 (d, J = 1.4 Hz, 1H), 6.11 (d, J = 1.5 Hz, 1H), 6.08 (d, J = 1.5 Hz, 1H), 5.99 (d, J = 1.4 Hz, 1H), 5.03 (d, J = 12.2 Hz, 1H), 4.83 (d, J = 12.2 Hz, 1H), 3.97 (s, 3H), 3.95 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.57, 146.95, 145.97, 143.41, 138.51, 136.40, 129.67, 124.65, 111.67, 109.12, 108.93, 108.21, 102.53, 102.11, 69.43, 56.87, 56.69. HR-ESI/APCI–MS: *m/z* $[M+H]^+$ calcd for $C_{18}H_{14}O_8$ 381.0689, found 381.0568.

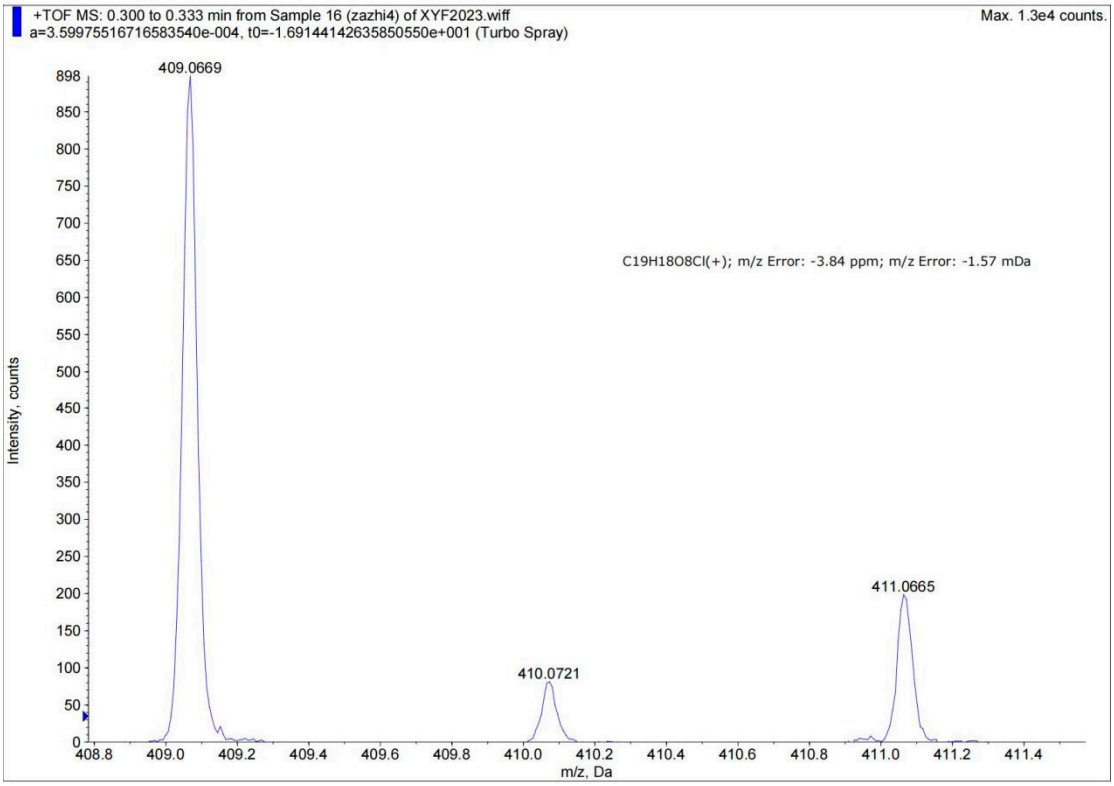
Figure S5. ^{1}H NMR, ^{13}C NMR, and HR-ESI–MS spectral data of Impurity Ⅳ

Methyl 5'-(chloromethyl)-7,7'-dimethoxy-[4,4'-bibenzo[d][1,3]dioxole]-5-carboxylate



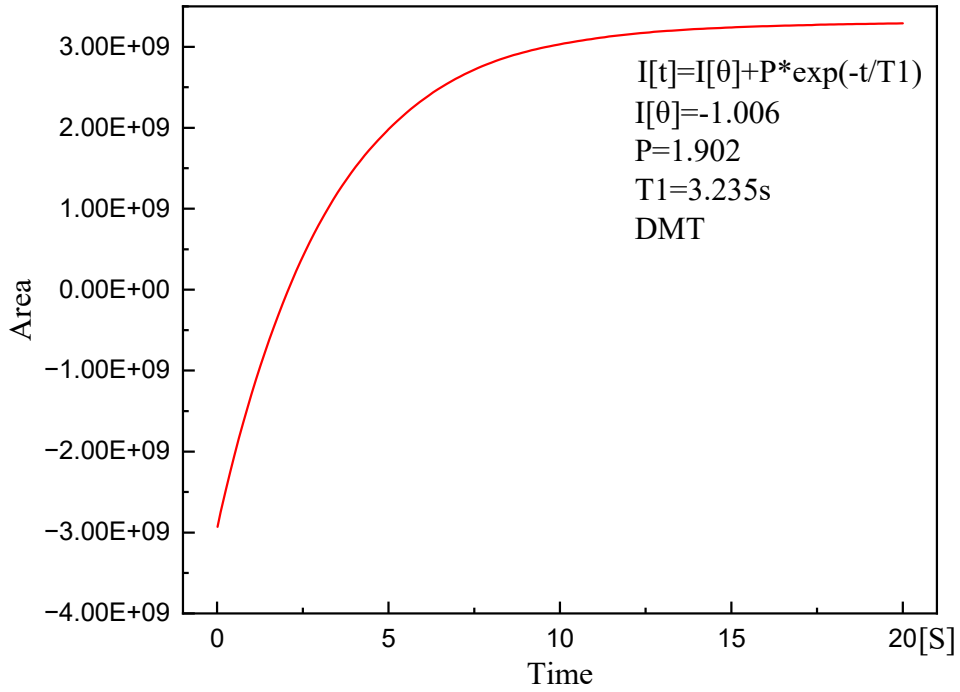


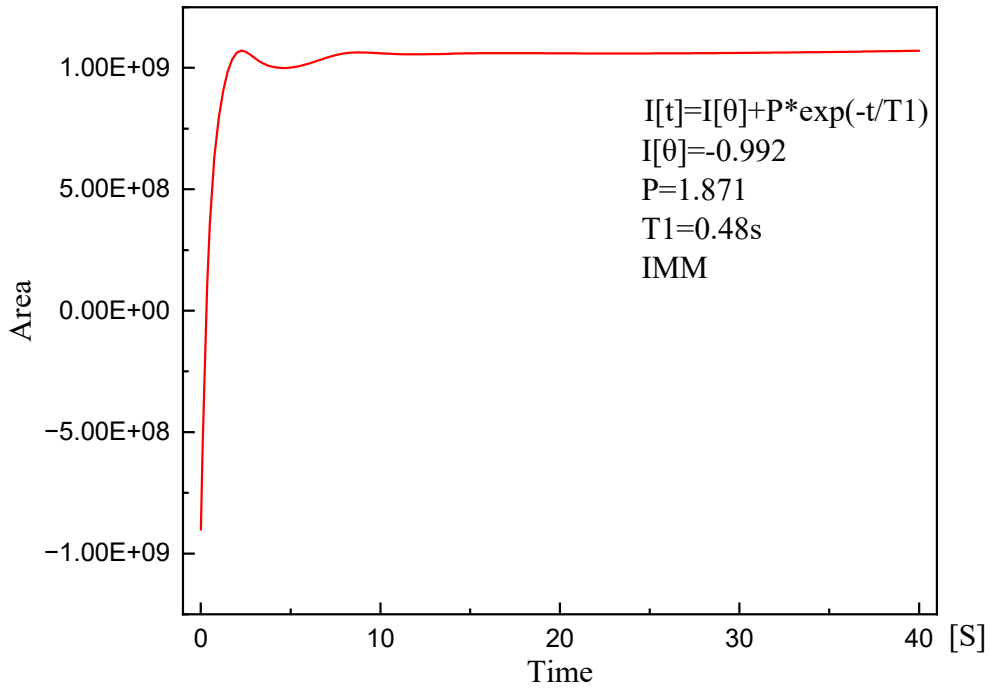


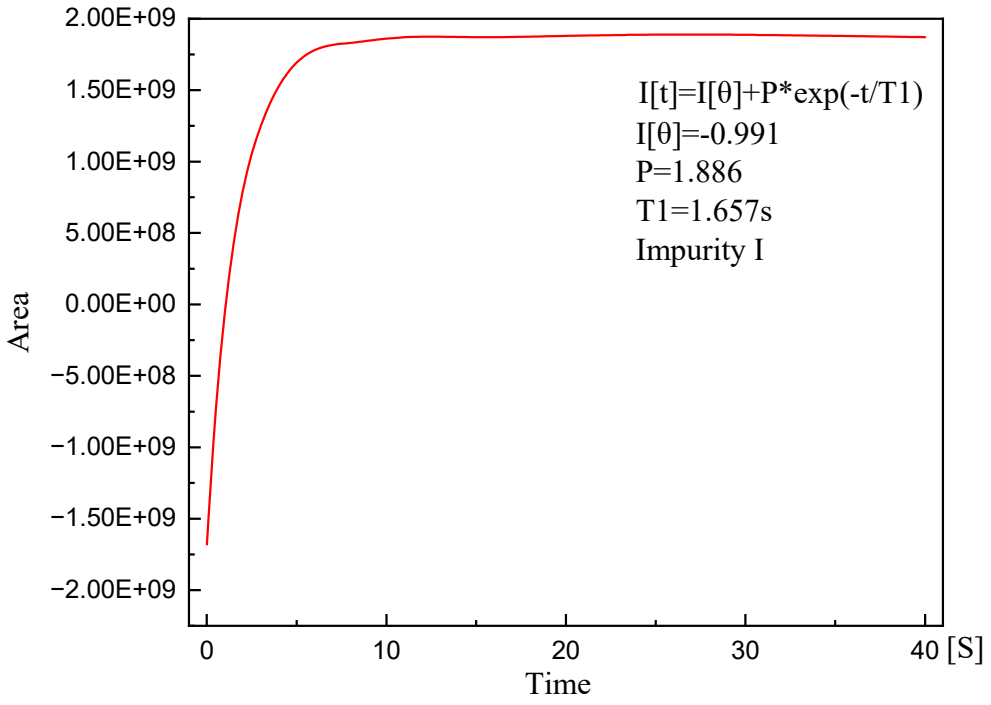


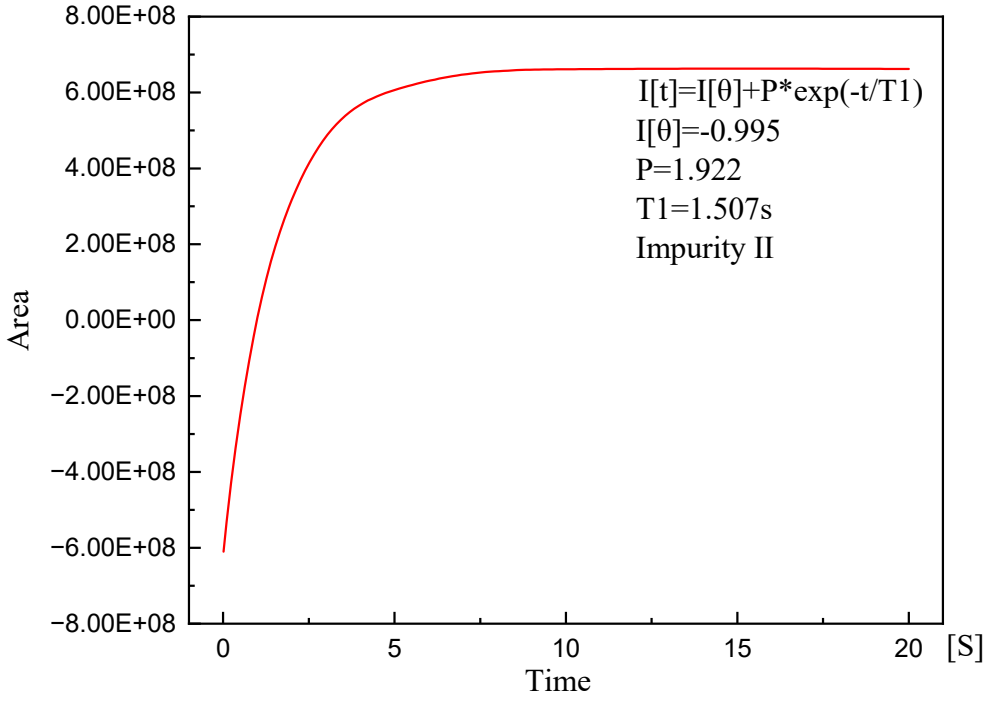
1H NMR (500 MHz, Chloroform-d) δ 8.13 (s, 2H), 7.43 (s, 2H), 6.75 (s, 2H), 6.06 (dd, J = 12.6, 1.4 Hz, 4H), 6.00 – 5.95 (m, 4H), 4.38 (d, J = 2.5 Hz, 4H), 4.01 (s, 6H), 3.97 (d, J = 2.3 Hz, 10H), 3.70 (s, 6H), 1.23 (t, J = 7.0 Hz, 1H). 13C NMR (126 MHz, CDCl3) δ 166.10, 147.87, 146.83, 143.20, 142.96, 138.42, 134.93, 130.12, 129.88, 124.53, 124.29, 124.02, 111.52, 110.33, 109.92, 109.04, 102.52, 101.96, 77.33, 77.07, 76.82, 65.90, 56.66, 56.46, 52.10, 44.79, 15.31. HR-ESI-MS: m/z [M+H]+ calcd for $C_{19}H_{18}ClO_8$ 409.0690, found 409.0669.

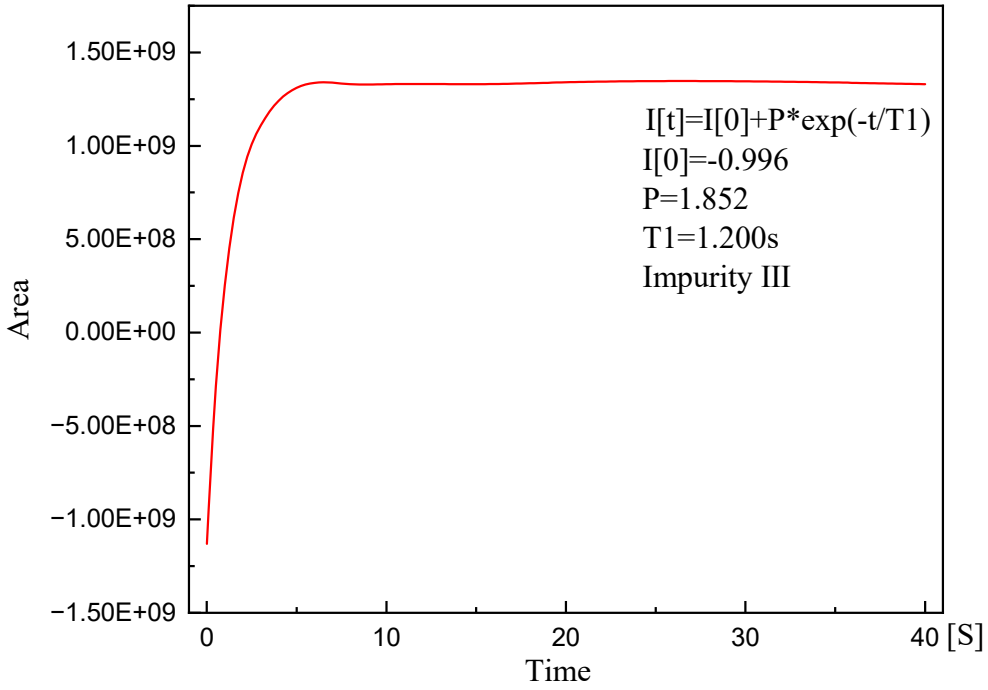
Figure S6. T1 relaxation time of quantitative signal of DMT, IMM-H014 and impurities I–IV






2.00E+09
1.50E+09
1.00E+09
5.00E+08
0.00E+00
−5.00E+08
−1.00E+09
−1.50E+09
−2.00E+09
Area
0
10
20
30
40 [S]
Time
I[t]=I[θ]+P*exp(-t/T1)
I[θ]=-0.991
P=1.886
T1=1.657s
Impurity I


8.00E+08
6.00E+08
4.00E+08
2.00E+08
0.00E+00
−2.00E+08
−4.00E+08
−6.00E+08
−8.00E+08
Area
0
5
10
15
20 [S]
Time
I[t]=I[θ]+P*exp(-t/T1)
I[θ]=-0.995
P=1.922
T1=1.507s
Impurity II


1.50E+09
1.00E+09
5.00E+08
0.00E+00
−5.00E+08
−1.00E+09
−1.50E+09
Area
0
10
20
30
40 [S]
Time
I[t]=I[0]+P*exp(-t/T1)
I[0]=-0.996
P=1.852
T1=1.200s
Impurity III

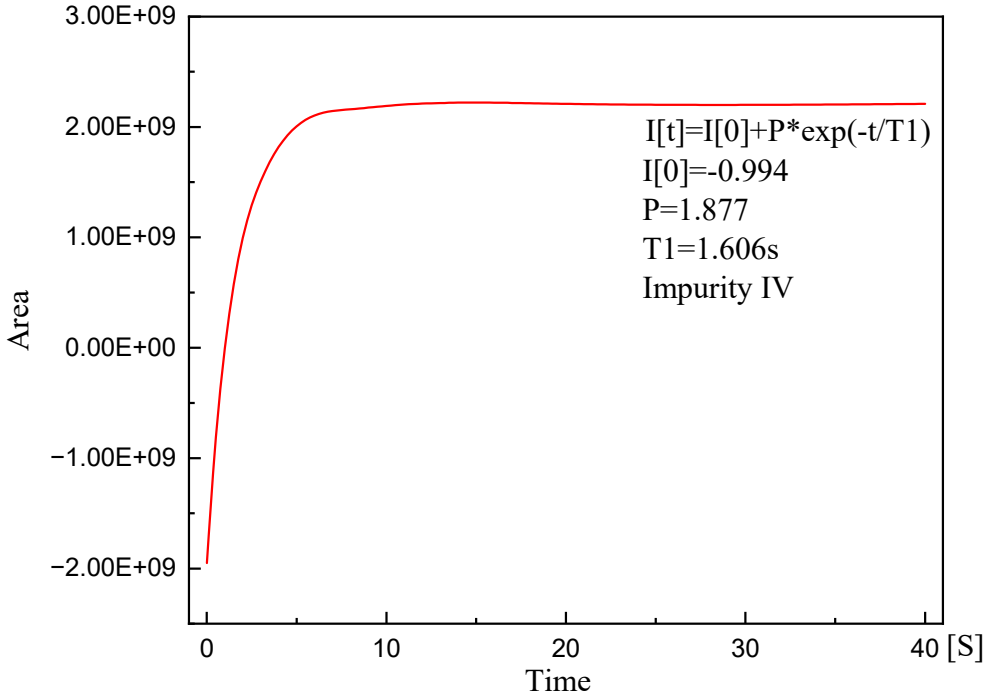
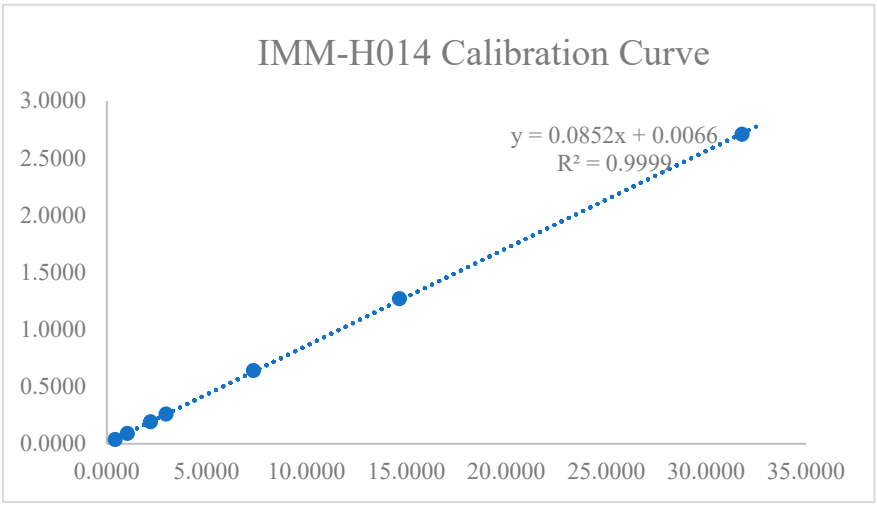
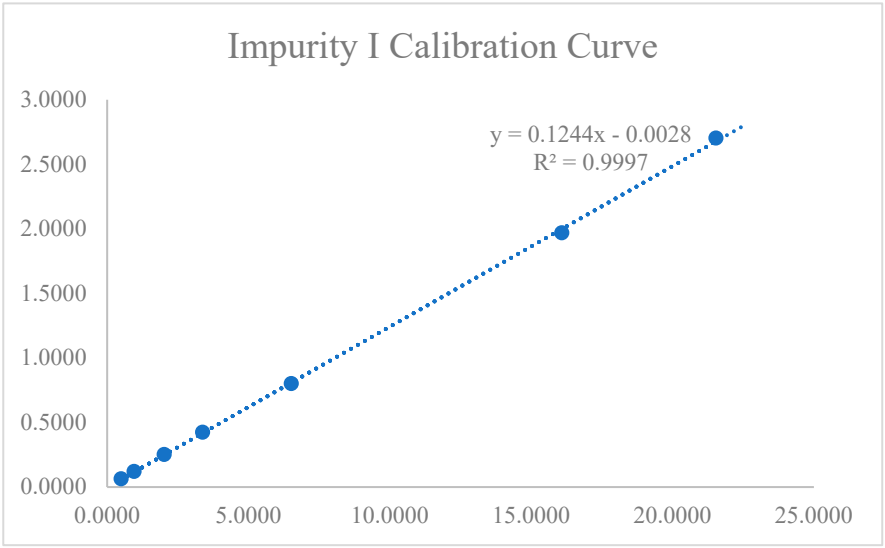
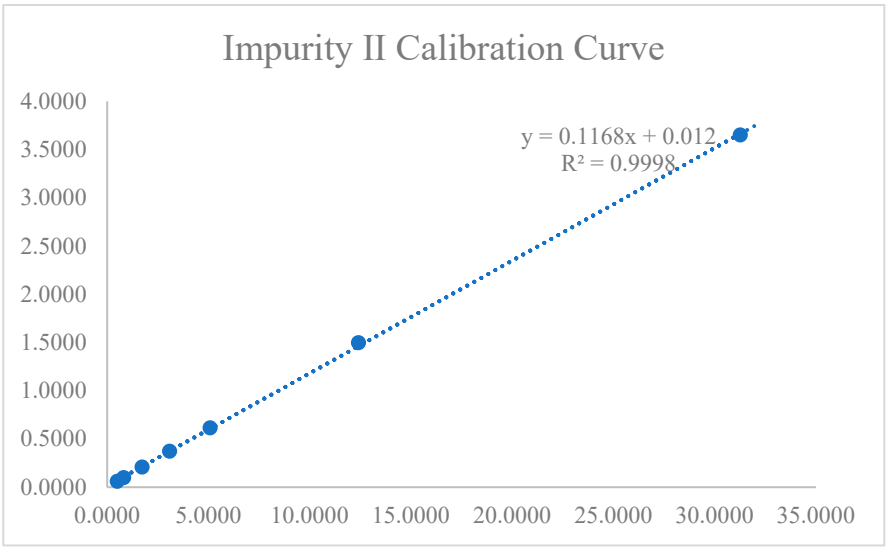
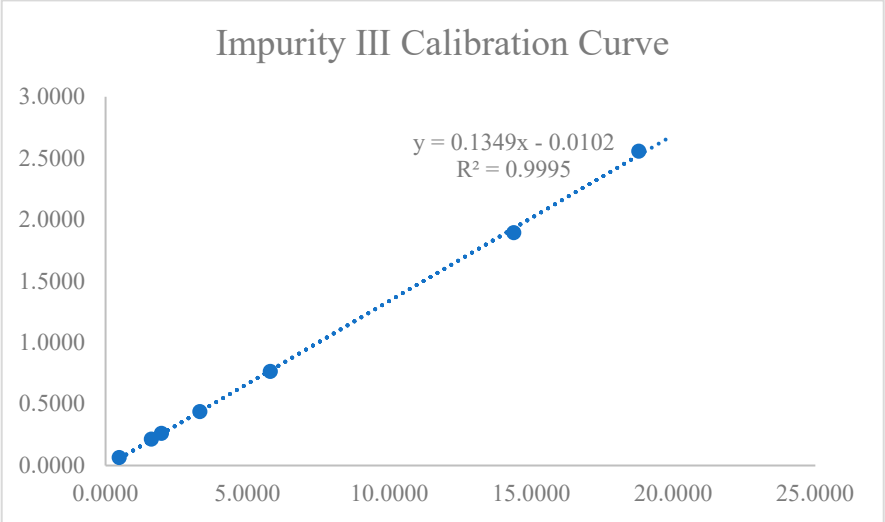

3.00E+09
2.00E+09
1.00E+09
0.00E+00
−1.00E+09
−2.00E+09
Area
0
10
20
30
40 [S]
Time
I[t]=I[0]+P*exp(-t/T1)
I[0]=-0.994
P=1.877
T1=1.606s
Impurity IV

Figure S7. Linearity calibration curves of IMM-H014 and its related substances








Impurity III Calibration Curve
y = 0.1349x - 0.0102
R² = 0.9995
3.0000
2.5000
2.0000
1.5000
1.0000
0.5000
0.0000
0.0000
5.0000
10.0000
15.0000
20.0000
25.0000

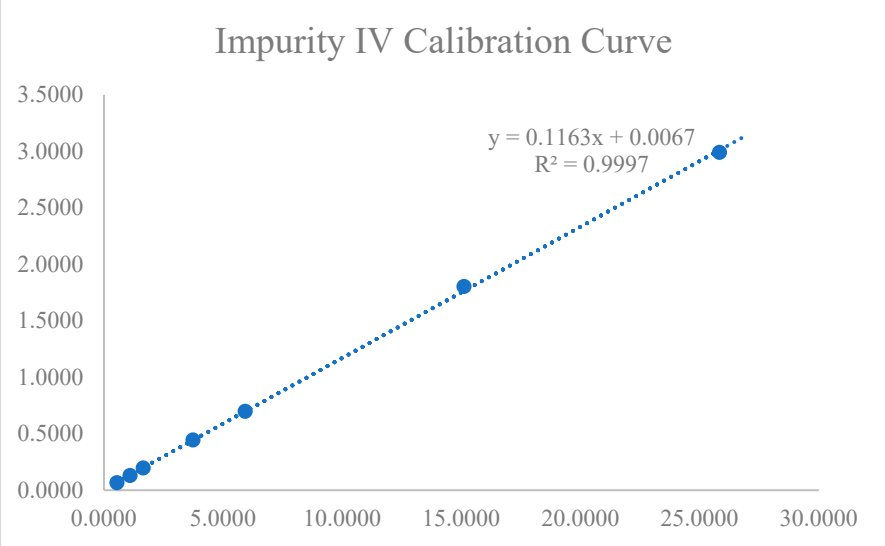
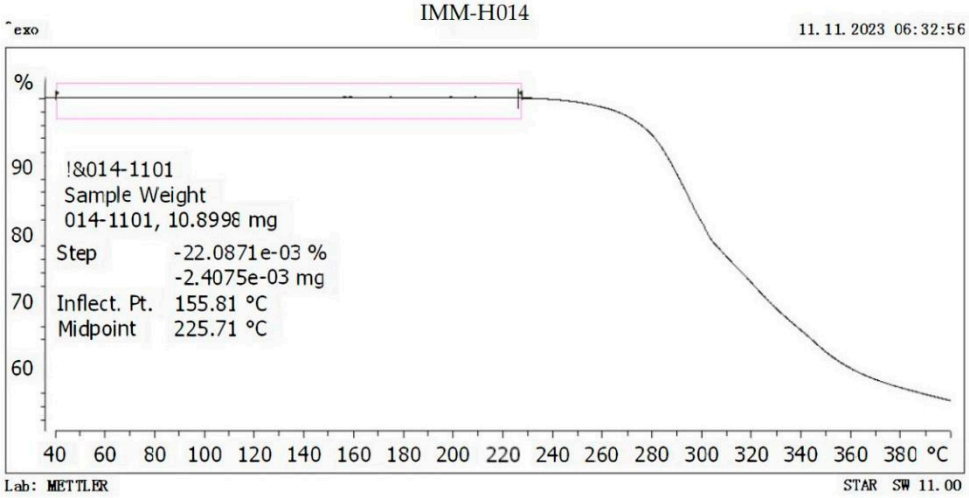
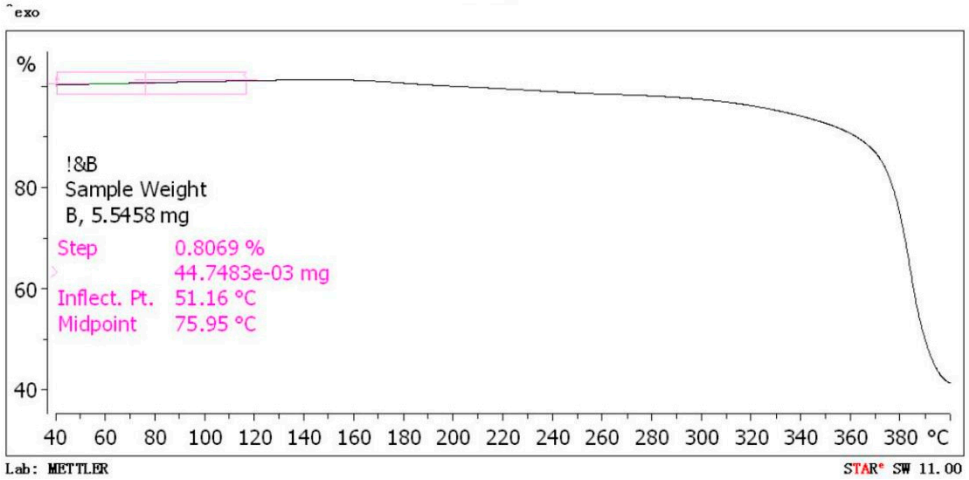

Impurity IV Calibration Curve
y = 0.1163x + 0.0067
R² = 0.9997
3.5000
3.0000
2.5000
2.0000
1.5000
1.0000
0.5000
0.0000
0.0000
5.0000
10.0000
15.0000
20.0000
25.0000
30.0000

Figure S8. TG test of IMM-H014 and Impurity I-IV

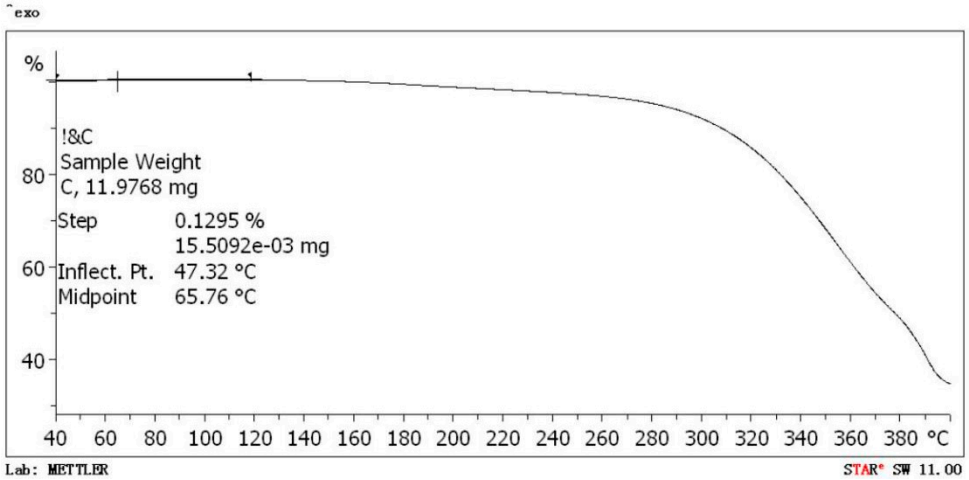
IMM-H014



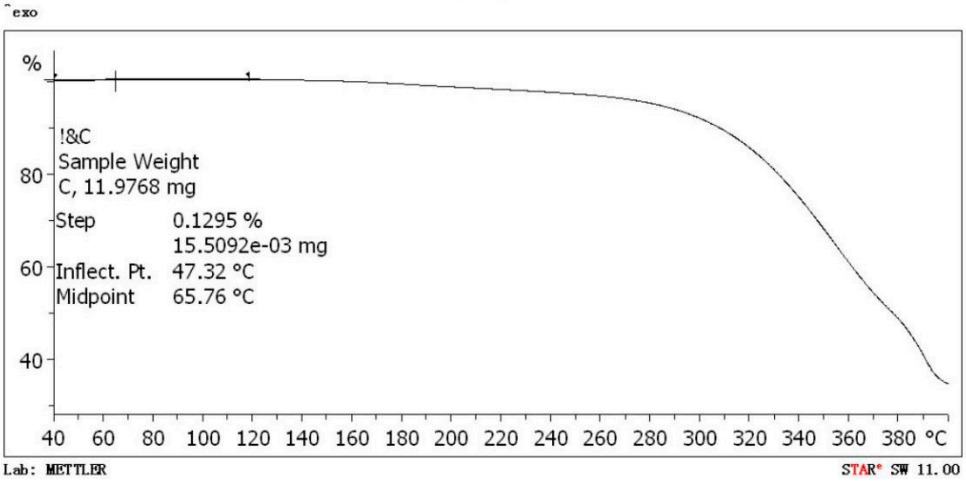
Impurity I



Impurity II



Impurity III



Impurity Ⅳ

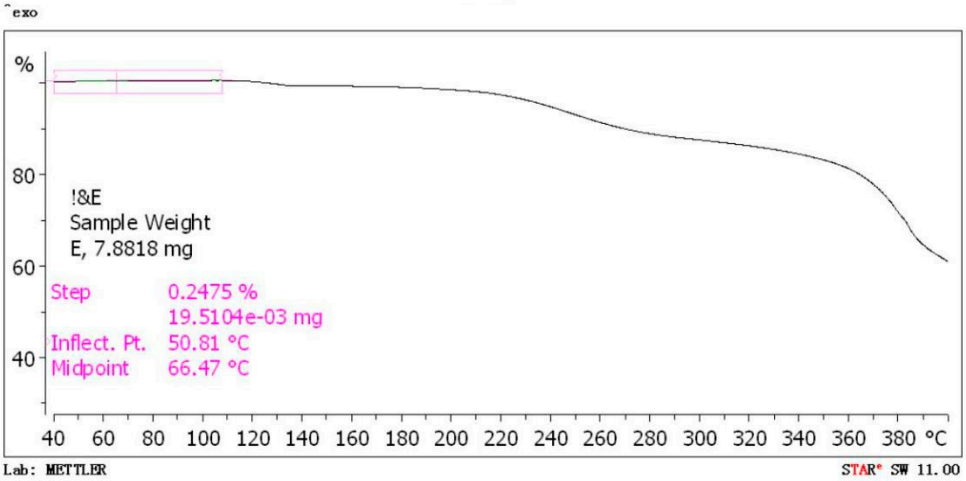


Table S1. Integration using different D1 value

D1/s	DMT	IMM-H014	Impurity I	Impurity II	Impurity III	Impurity IV
1	1.13	0.97	0.96	0.98	0.99	0.97
2	1.17	0.97	0.96	0.97	0.98	0.97
4	1.23	0.96	0.96	0.97	0.98	0.97
8	1.28	0.95	0.96	0.97	0.98	0.97
16	1.31	0.96	0.96	0.97	0.98	0.97
32	1.30	0.95	0.96	0.97	0.98	0.97
64	1.30	0.95	0.96	0.97	0.98	0.97

Table S2. Precision, accuracy and stability test of IMM-H014

	No.	mx	mstd	Px%	Average	RSD
Precision (n = 6)	1	18.138	2.469	99.27%	99.55%	0.23%
	2	18.138	2.469	99.80%		
	3	18.138	2.469	99.61%		
	4	18.138	2.469	99.61%		
	5	18.138	2.469	99.27%		
	6	18.138	2.469	99.76%		
Repeatability (n = 6)	1	18.138	2.469	99.67%	99.81%	0.38%
	2	17.378	2.970	99.90%		
	3	16.686	2.097	99.85%		
	4	16.223	2.755	99.60%		
	5	17.654	2.182	100.00%		
	6	17.129	3.507	99.82%		
Accuracy (recovery)	80%	16.223	2.755	99.82%	99.82%	0.52%
		16.223	2.755	99.82%		
		16.223	2.755	99.82%		
	100%	16.686	2.097	99.90%	99.81%	0.19%
		16.686	2.097	99.60%		
		16.686	2.097	99.82%		
	120%	17.129	3.507	99.67%	99.82%	0.61%
		17.129	3.507	99.85%		
		17.129	3.507	100.00%		
Stability	0h	18.138	2.469	99.27%	99.59%	0.20%
	1h	18.138	2.469	99.80%		
	2h	18.138	2.469	99.61%		
	4h	18.138	2.469	99.61%		
	8h	18.138	2.469	99.27%		
	12h	18.138	2.469	99.76%		
	24h	18.138	2.469	99.80%		
	36h	18.138	2.469	99.61%		
	48h	18.138	2.469	99.61%		

Table S3. Precision, accuracy and stability test of Impurity I

	No.	mx	mstd	Px%	Average	RSD
Precision (n=6)	1	8.673	1.330	99.20%	98.64%	0.44%
	2	8.673	1.330	98.27%		
	3	8.673	1.330	98.27%		
	4	8.673	1.330	98.46%		
	5	8.673	1.330	98.46%		
	6	8.673	1.330	99.20%		
Repeatability (n = 6)	1	8.673	1.330	98.58%	99.63%	0.70%
	2	8.17	1.686	99.33%		
	3	8.289	2.134	99.46%		
	4	8.697	1.119	99.86%		
	5	7.982	1.185	100.35%		
	6	7.917	1.313	100.17%		
Accuracy (recovery)	80%	6.402	1.208	99.62%	99.67%	0.59%
		6.402	1.208	99.71%		
		6.402	1.208	99.69%		
	100%	8.673	1.330	98.58%	98.54%	0.57%
		8.673	1.330	98.71%		
		8.673	1.330	98.34%		
	120%	10.153	1.286	100.53%	100.75%	0.33%
		10.153	1.286	100.86%		
		10.153	1.286	100.86%		
Stability	0h	8.673	1.330	99.20%	98.54%	0.57%
	1h	8.673	1.330	98.27%		
	2h	8.673	1.330	98.27%		
	4h	8.673	1.330	98.46%		
	8h	8.673	1.330	98.46%		
	12h	8.673	1.330	99.20%		
	24h	8.673	1.330	97.54%		
	36h	8.673	1.330	98.27%		
	48h	8.673	1.330	99.20%		

Table S4. Precision, accuracy and stability test of Impurity II

	No.	mx	mstd	Px%	Average	RSD
Precision (n=6)	1	8.459	1.662	100.27%	99.64%	1.15%
	2	8.459	1.662	101.22%		
	3	8.459	1.662	100.27%		
	4	8.459	1.662	99.33%		
	5	8.459	1.662	98.38%		
	6	8.459	1.662	98.38%		
Repeatability (n = 6)	1	8.459	1.662	100.59%	100.25%	0.91%
	2	8.170	1.686	99.49%		
	3	8.289	1.134	101.82%		
	4	8.697	1.119	99.52%		
	5	7.982	1.185	99.58%		
	6	7.917	1.313	100.51%		
Accuracy (recovery)	80%	6.625	1.127	100.59%	99.37%	1.02%
		6.625	1.127	98.70%		
		6.625	1.127	98.82%		
	100%	8.459	1.662	99.07%	99.33%	0.38%
		8.459	1.662	99.45%		
		8.459	1.662	99.48%		
	120%	9.704	1.206	99.14%	99.42%	0.92%
		9.704	1.206	99.44%		
		9.704	1.206	99.68%		
Stability	0h	8.459	1.662	100.27%	99.37%	1.02%
	1h	8.459	1.662	101.22%		
	2h	8.459	1.662	100.27%		
	4h	8.459	1.662	99.33%		
	8h	8.459	1.662	98.38%		
	12h	8.459	1.662	98.38%		
	24h	8.459	1.662	98.38%		
	36h	8.459	1.662	99.13%		
	48h	8.459	1.662	98.95%		

Table S5. Precision, accuracy and stability test of Impurity III

	No.	mx	mstd	Px%	Average	RSD
Precision (n=6)	1	7.553	1.300	97.64%	97.18%	0.44%
	2	7.553	1.300	97.42%		
	3	7.553	1.300	97.20%		
	4	7.553	1.300	97.42%		
	5	7.553	1.300	96.92%		
	6	7.553	1.300	96.47%		
Repeatability (n = 6)	1	7.553	1.300	97.42%	97.19%	0.33%
	2	7.553	1.940	97.00%		
	3	7.553	1.430	97.01%		
	4	7.553	1.941	97.16%		
	5	7.553	1.412	97.41%		
	6	7.553	1.681	97.17%		
Accuracy (recovery)	80%	7.553	1.942	97.16%	97.06	0.20%
		7.553	1.942	97.05%		
		7.553	1.942	96.97%		
	100%	7.553	1.300	97.42%	97.15%	0.37%
		7.553	1.300	96.94%		
		7.553	1.300	97.09%		
	120%	7.553	0.983	97.72%	97.55%	0.53%
		7.553	0.983	97.57%		
		7.553	0.983	97.37%		
Stability	0h	7.553	1.300	97.64%	97.15%	0.37%
	1h	7.553	1.300	97.42%		
	2h	7.553	1.300	97.20%		
	4h	7.553	1.300	97.42%		
	8h	7.553	1.300	96.92%		
	12h	7.553	1.300	96.47%		
	24h	7.553	1.300	97.36%		
	36h	7.553	1.300	96.99%		
	48h	7.553	1.300	96.92%		

Table S6. Precision, accuracy and stability test of Impurity IV

	No.	mx	mstd	Px%	Average	RSD
Precision (n=6)	1	8.215	1.384	98.49%	98.67%	0.33%
	2	8.215	1.384	99.12%		
	3	8.215	1.384	98.81%		
	4	8.215	1.384	98.49%		
	5	8.215	1.384	98.90%		
	6	8.215	1.384	98.21%		
Repeatability (n = 6)	1	8.215	1.384	98.81%	99.26%	0.67%
	2	7.908	1.018	99.58%		
	3	8.756	1.043	99.27%		
	4	8.320	1.643	99.97%		
	5	8.071	1.061	98.95%		
	6	8.605	1.044	98.99%		
Accuracy (recovery)	80%	6.726	1.572	99.79%	99.43%	0.56%
		6.726	1.572	99.54%		
		6.726	1.572	98.97%		
	100%	8.215	1.384	98.81%	98.94%	0.50%
		8.215	1.384	98.53%		
		8.215	1.384	99.48%		
	120%	9.576	0.973	99.31%	99.18%	0.80%
		9.576	0.973	98.70%		
		9.576	0.973	99.54%		
Stability	0h	8.215	1.384	98.49%	98.94%	0.50%
	1h	8.215	1.384	99.12%		
	2h	8.215	1.384	98.81%		
	4h	8.215	1.384	98.49%		
	8h	8.215	1.384	98.90%		
	12h	8.215	1.384	98.21%		
	24h	8.215	1.384	99.25%		
	36h	8.215	1.384	99.60%		
	48h	8.215	1.384	99.59%		