

Supplementary materials

Rapid preparation of a large sulfated metabolite library for structure validation in human samples

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Supplementary figures

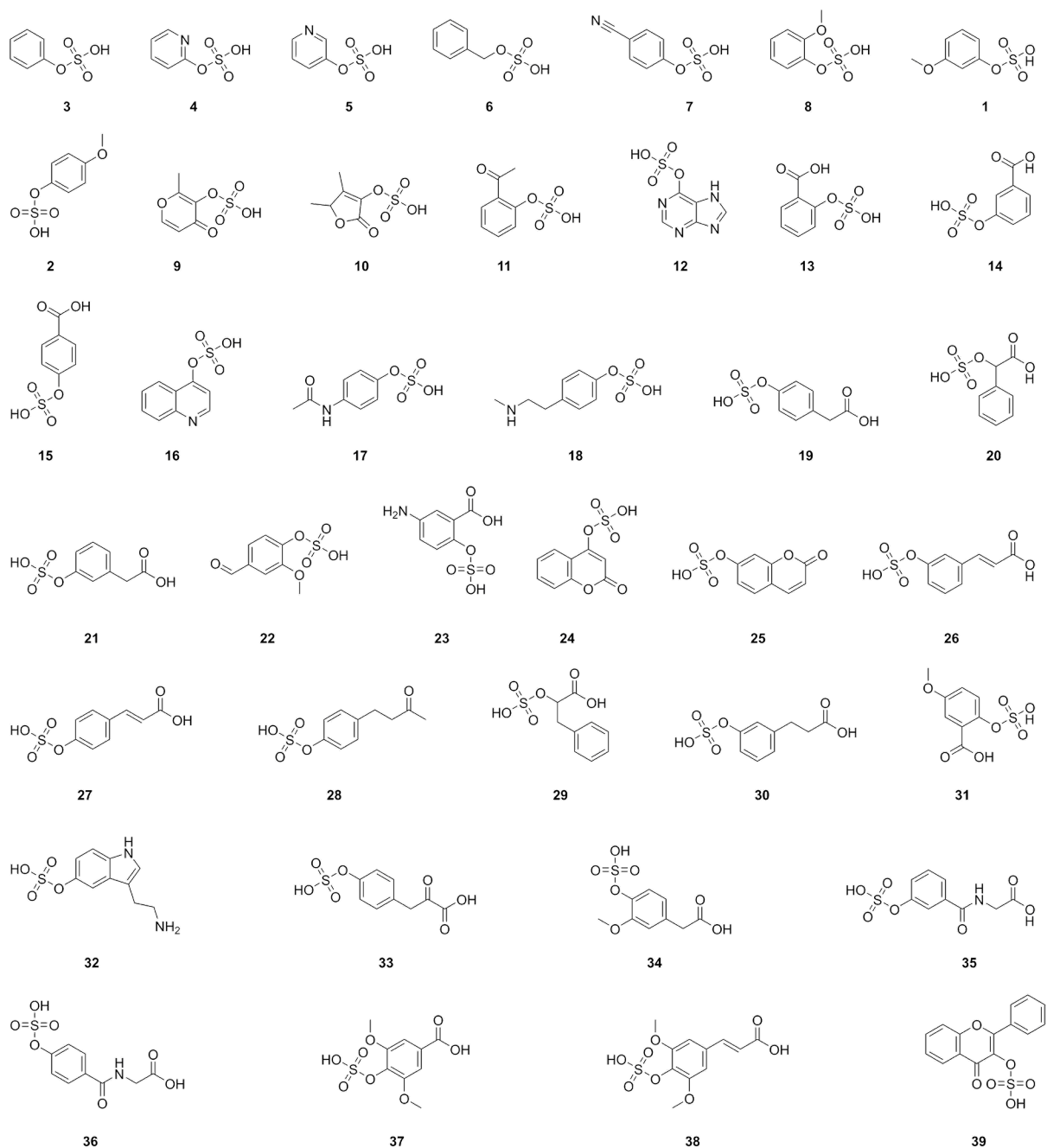


Figure S1 – Overview of the structures of the molecules synthesized. Molecules organized by m/z ratio.

Supplementary tables

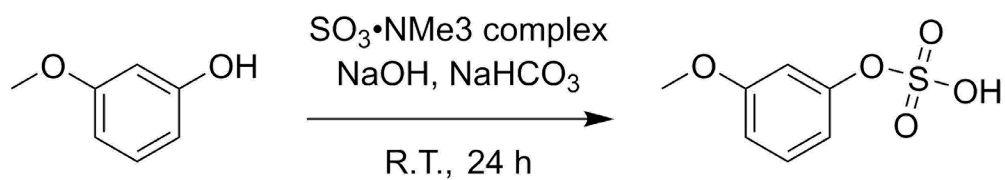
Table S1 – All sulfated metabolites

Newly synthesized sulfated metabolites	In-house sulfated metabolites
Phenol sulfate (3)	p-Cresol sulfate
2-Hydroxypyridine sulfate (4)	Ferulic acid sulfate
3-Hydroxypyridine sulfate (5)	Mannose-6-sulfate
Benzyl alcohol sulfate (6)	4-Hydroxy-3-methoxyphenylglycol sulfate
4-Cyanophenol sulfate (7)	4-Nitrophenol sulfate
2-Methoxyphenol sulfate (8)	N-Acetylserotonin sulfate
3-Methoxyphenol sulfate (1)	Tyrosine sulfate
4-Methoxyphenol sulfate (2)	Resorcinol sulfate
3-Hydroxy-2-methyl-4-pyrone sulfate (9)	Vanillic acid sulfate
4,5-Dimethyl-3-hydroxy-2,5-dihydrofuran-2-one sulfate (10)	N-Acetyltyramine sulfate
2-Hydroxyacetophenone sulfate (11)	Isovanillic acid sulfate
Hypoxanthine sulfate (12)	Indoxyl sulfate
2-Hydroxybenzoic acid sulfate (13)	p-Coumaric acid sulfate
3-Hydroxybenzoic acid sulfate (14)	4-Methylumbelliferyl sulfate
4-Hydroxybenzoic acid sulfate (15)	4-Ethylphenyl sulfate
4-Hydroxyquinoline sulfate (16)	Methylurolithin sulfate
4-Acetamidophenol sulfate (17)	3-Methylindole-5-O-sulfate
N-Methyltyramine sulfate (18)	Dopamine-3-sulfate
4-Hydroxyphenylacetic acid sulfate (19)	Dopamine-4-sulfate
Mandelic acid sulfate (20)	2-Methoxyphenol-4-vinylphenyl sulfate
3-Hydroxyphenylacetic acid sulfate (21)	Estrone-3-sulfate
Vanillin sulfate (22)	Dehydroisoandrosterone-3-sulfate
5-Aminosalicylic acid sulfate (23)	Cholesterol sulfate
4-Hydroxycoumarin sulfate (24)	4-Nitrocatechol sulfate
Umbelliferone sulfate (25)	
trans-3-Hydroxycinnamic acid sulfate (26)	
trans-4-Hydroxycinnamic acid sulfate (27)	
Raspberry ketone sulfate (28)	
D-3-Phenyllactic acid sulfate (29)	
3-(3-Hydroxyphenyl)propionic acid sulfate (30)	
5-Methoxysalicylic acid sulfate (31)	
Serotonin sulfate (32)	
4-Hydroxy-3-methoxyphenylacetic acid sulfate (33)	
3-Hydroxyhippuric acid sulfate (34)	
4-Hydroxyhippuric acid sulfate (35)	
Syringic acid sulfate (36)	
Sinapic acid sulfate (37)	
3-Hydroxyflavone sulfate (38)	

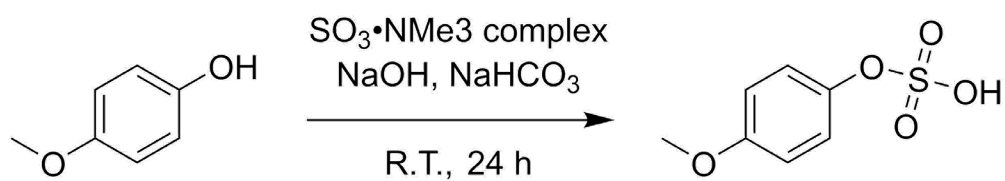
Supplementary schemes

Scheme S1 – Chemical synthesis reaction of 3-methoxyphenol-*O*-sulfate (1) and 4-methoxyphenol-*O*-sulfate

Synthesis of 3-Methoxyphenol-*O*-sulfate (1)

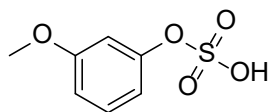


Synthesis of 4-Methoxyphenol-*O*-sulfate (2)



Chemical synthesis

Synthesis of 3-Methoxyphenol-*O*-sulfate



3-methoxyphenol-*O*-sulfate

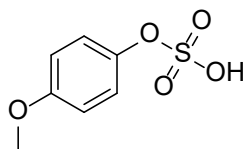
To a solution of 3-Methoxyphenol (44.2 μ L, 0.403 mmol, 1.0 eq) and NaOH (1.2 mL, 1.2 mmol, 3.0 eq), NaHCO₃ (135.5 mg, 1.6 mmol, 4.0 eq) and SO₃•NMe₃ complex (140 mg, 1.0 mmol, 2.5 eq) were added, as illustrated in Scheme 1. The reaction mixture was stirred (800 rpm) at room temperature for 24 hours and concentrated in a freeze drier overnight (-51 °C, 0.05 mbar). The crude product was subjected to HPLC purification [r.t. = 22.5 min, 0-5 min (0% B), 5-20 min (0-100 %B), 20-30 min (0% B) at a flow of 2.5 mL/min; buffer A = ammonium acetate 5 mM (water) and buffer B = ammonium acetate 5 mM (MeOH)] to afford 3-Methoxyphenol-*O*-sulfate.

¹H NMR (400 MHz, D₂O) δ (ppm) = 3.83 (3H, s), 6.53-6.65 (3H, m) 7.25 (1H, t, J = 8.2 Hz);

¹³C NMR (151 MHz, D₂O) δ (ppm) = 55.28, 101.42, 106.12, 108.24, 130.62, 156.75, 160.18;

HRMS (ESI-) calculated for C₇H₇O₅S⁻ (M-H)⁻: 203.0014, found: 203.0057.

Synthesis of 4-Methoxyphenol-*O*-sulfate



4-methoxyphenol-*O*-sulfate

To a solution of 4-Methoxyphenol (50 mg, 0.403 mmol, 1.0 eq) and NaOH (1.2 mL, 1.2 mmol, 3.0 eq), NaHCO₃ (135.5 mg, 1.6 mmol, 4.0 eq) and SO₃•NMe₃ complex (140 mg, 1.0 mmol, 2.5 eq) were added, as illustrated in Scheme 1. The reaction mixture was stirred (800 rpm) at room temperature for 24 hours and concentrated in a freeze drier overnight (-51 °C, 0.05 mbar). The crude product was subjected to HPLC purification [r.t. = 22.0 min, 0-5 min (0% B), 5-20 min (0-100 %B), 20-30 min (0% B) at a flow of 2.5 mL/min; buffer A = ammonium acetate 5 mM (water) and buffer B = ammonium acetate 5 mM (MeOH)] to afford 4-Methoxyphenol-*O*-sulfate.

¹H NMR (400 MHz, D₂O) δ (ppm) = 3.70 (3H, s), 6.80 (2H, d, J = 9.0 Hz), 7.14 (2H, d, J = 9.0 Hz);

¹³C NMR (151 MHz, D₂O) δ (ppm) = 54.57, 113.66, 122.38, 146.05, 156.98;

HRMS (ESI-) calculated for C₇H₇O₅S⁻ (M-H)⁻: 203.0014, found: 203.0047.

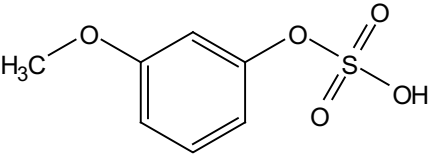
NMR spectra

3-Methoxyphenol sulfate

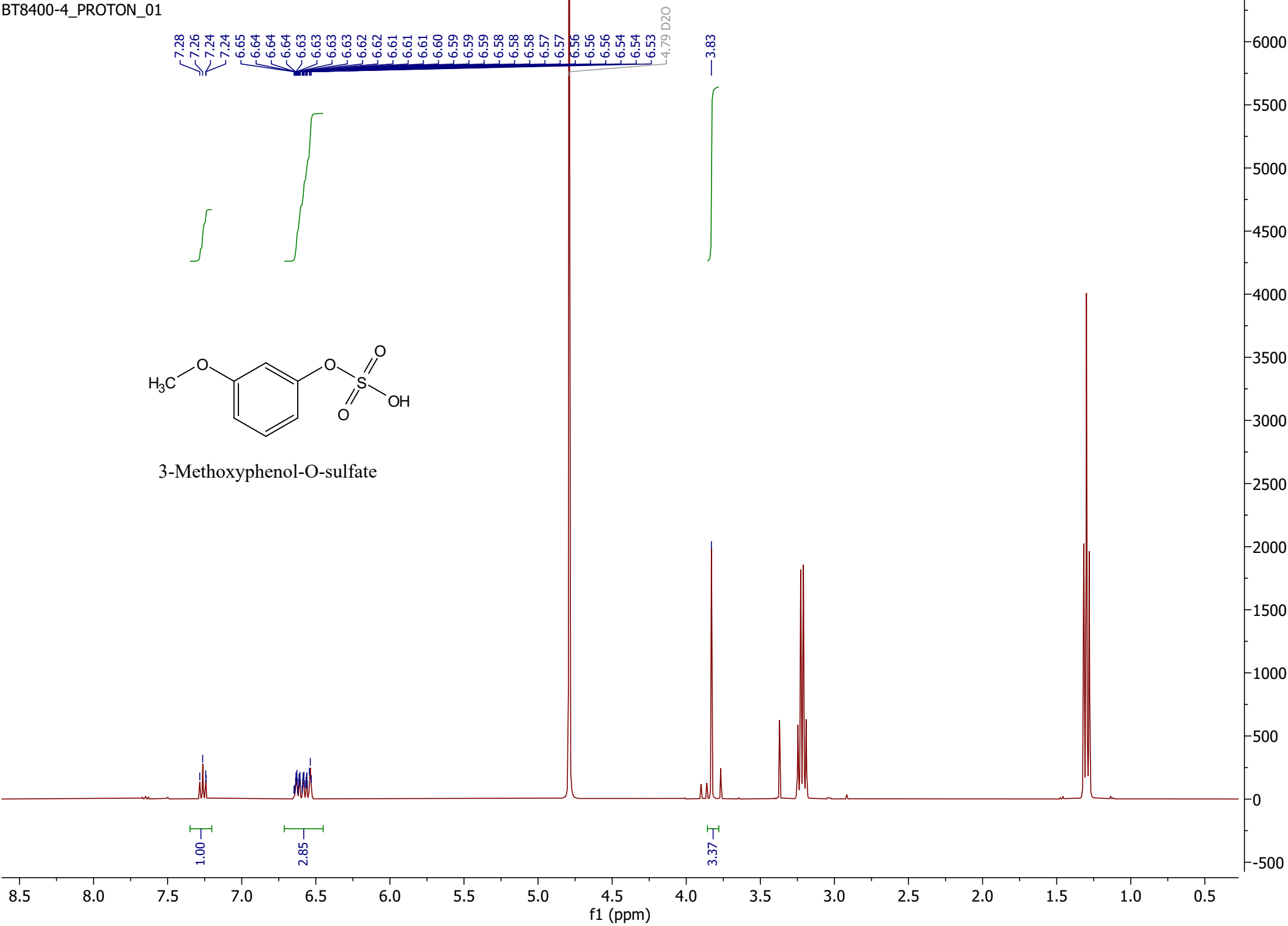
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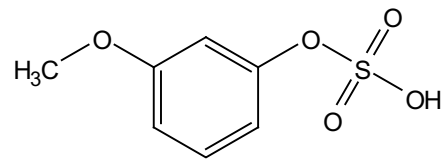
4-Methoxyphenol sulfate

^1H NMR, ^{13}C NMR, COSY, HSQC, HSBC

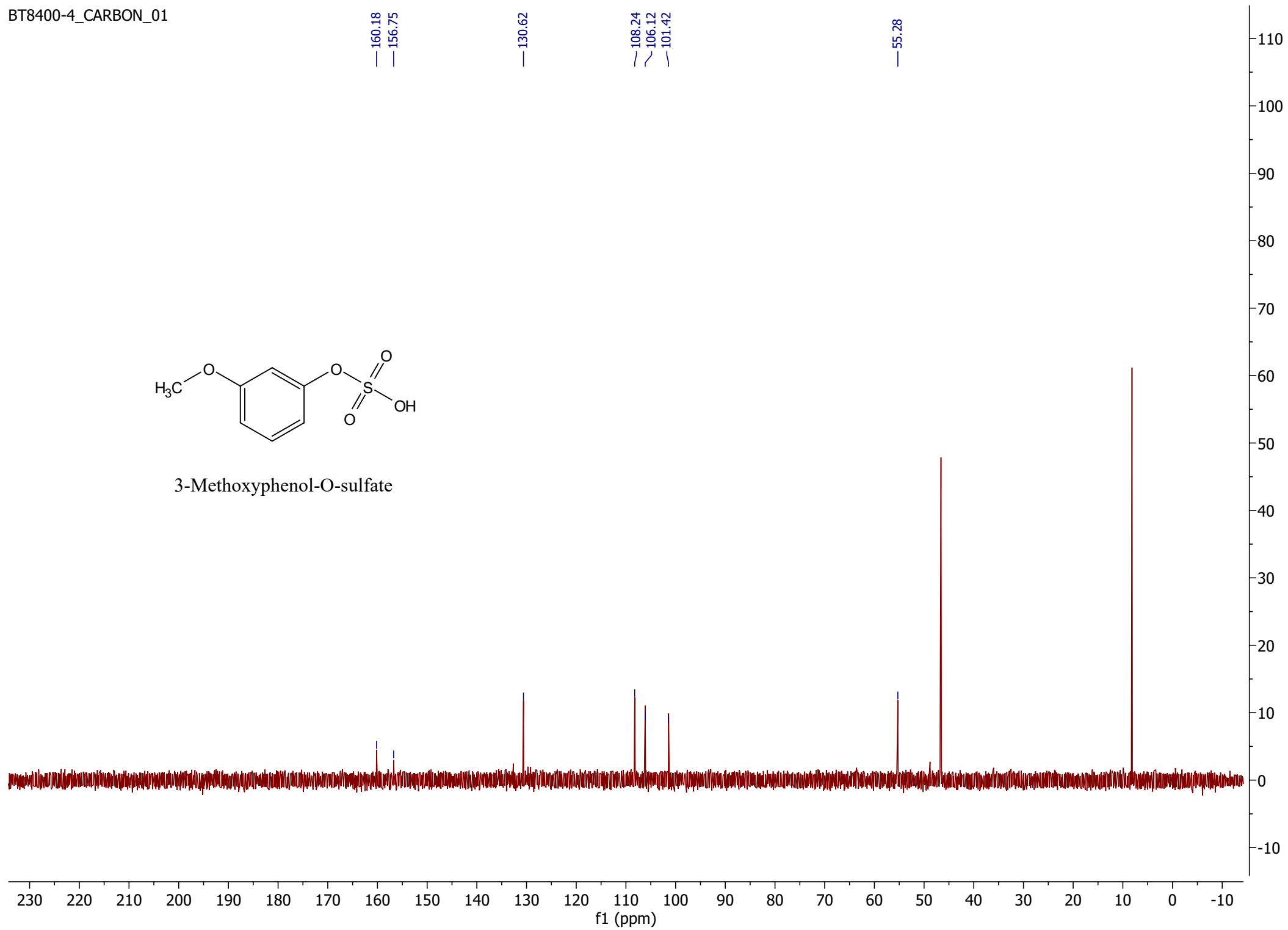


3-Methoxyphenol-O-sulfate

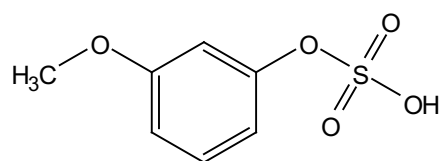




3-Methoxyphenol-O-sulfate



BT8400-4_gCOSY_01



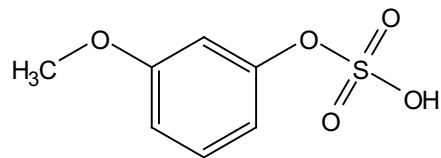
3-Methoxyphenol-O-sulfate

{4.79,4.79}D2O

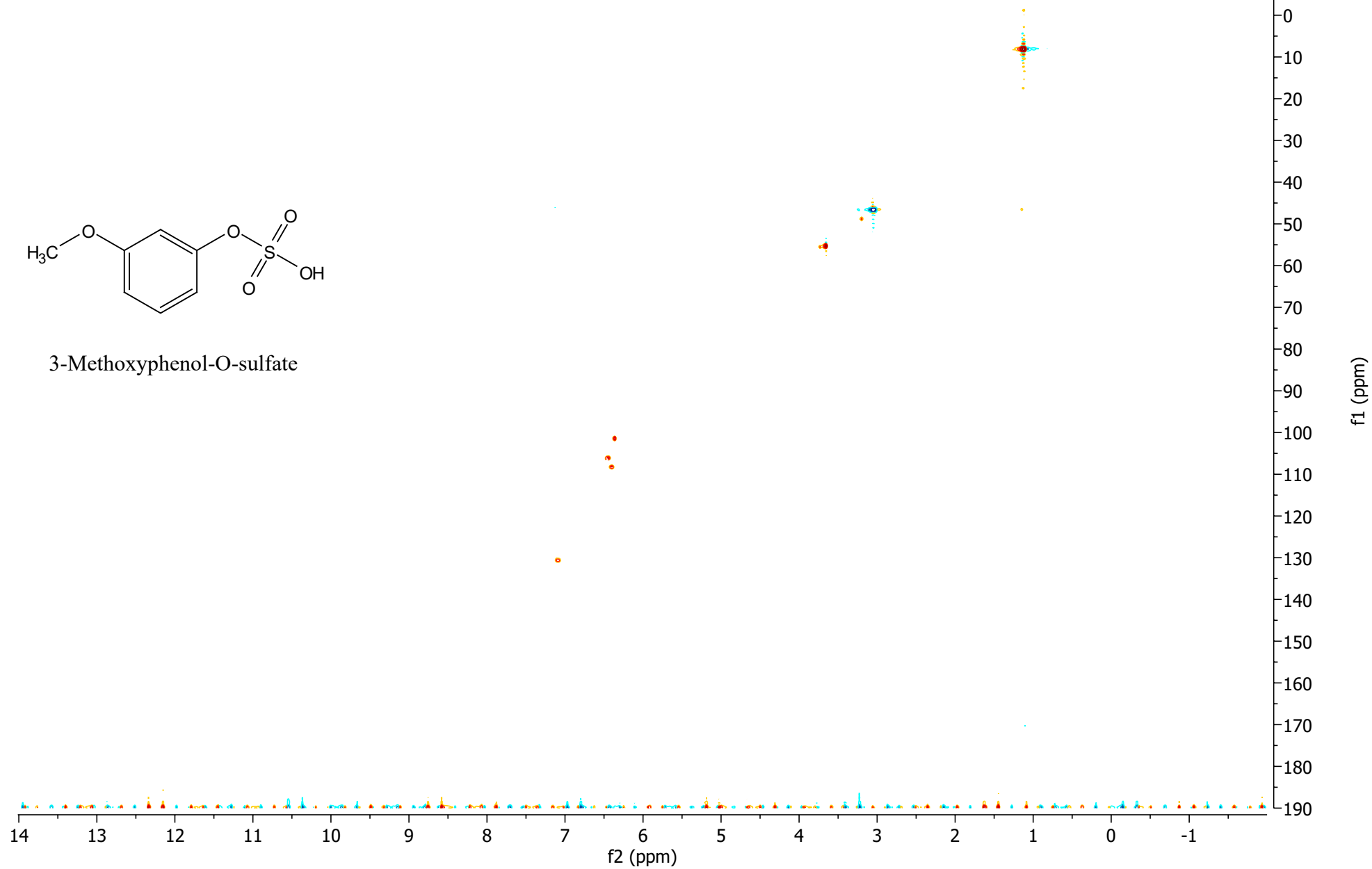
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f2 (ppm)

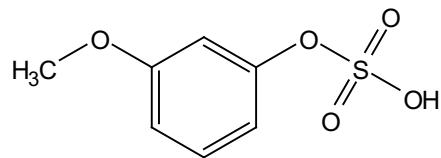
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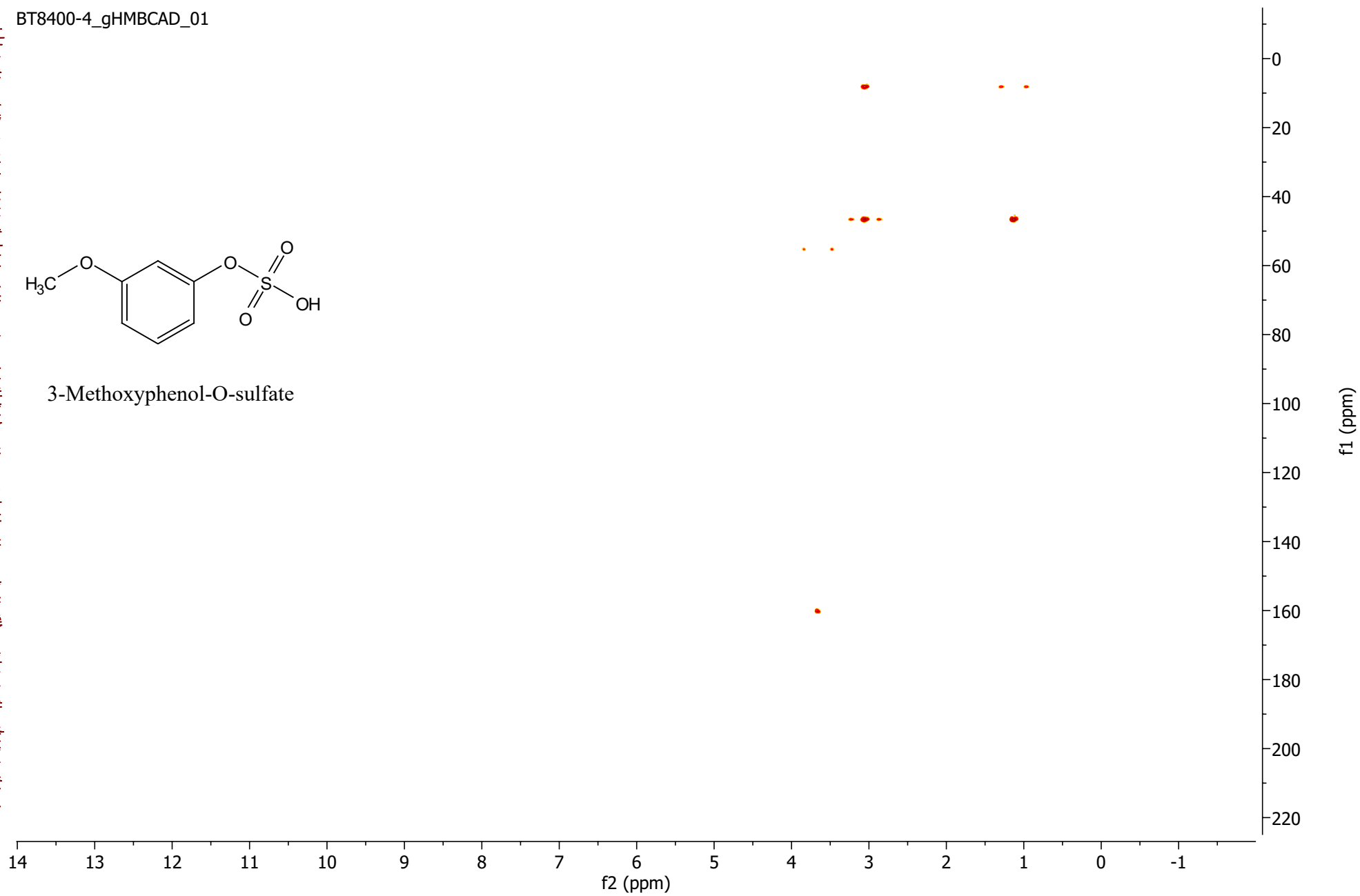
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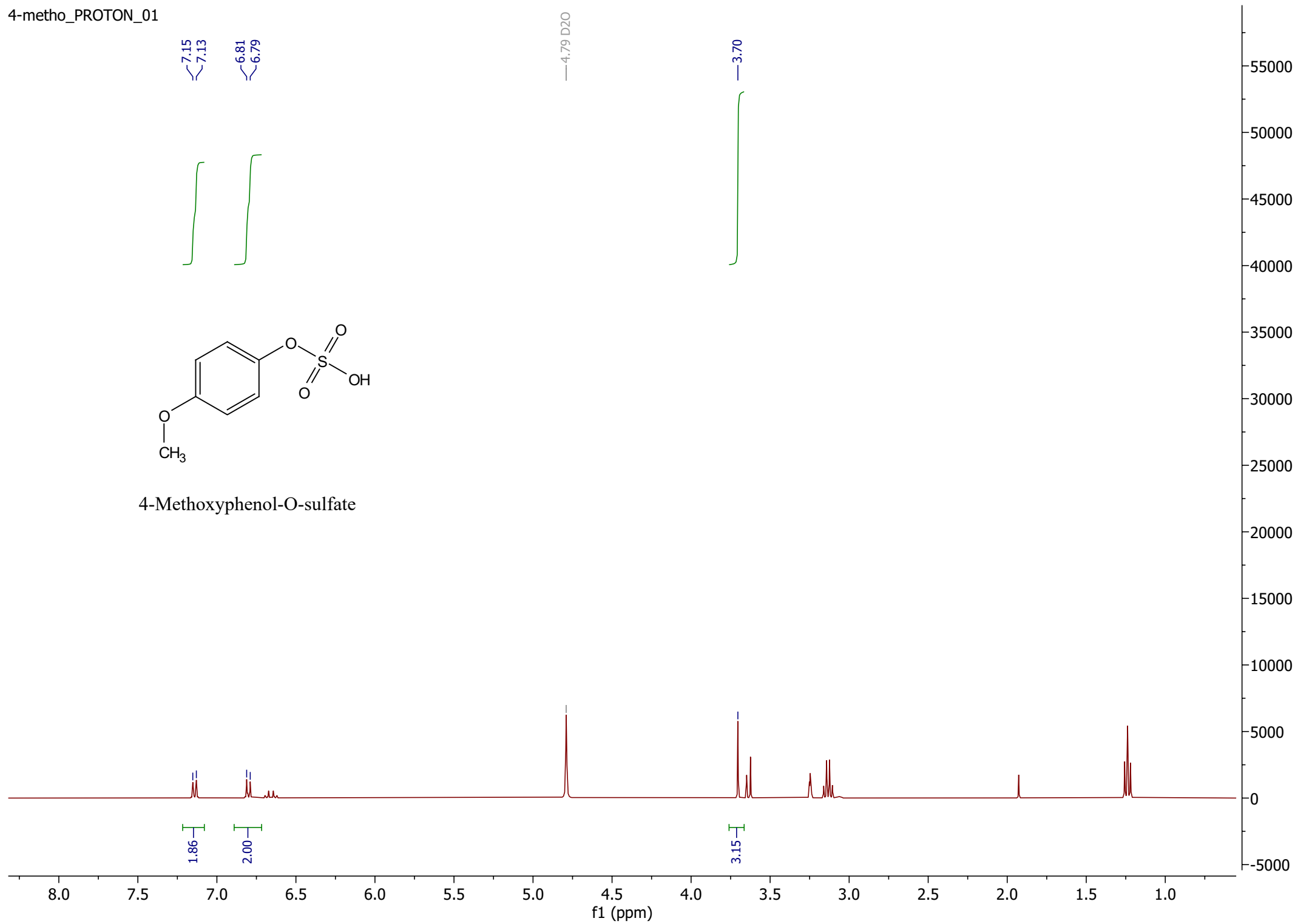


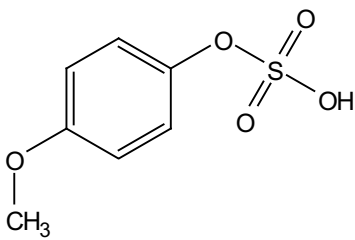
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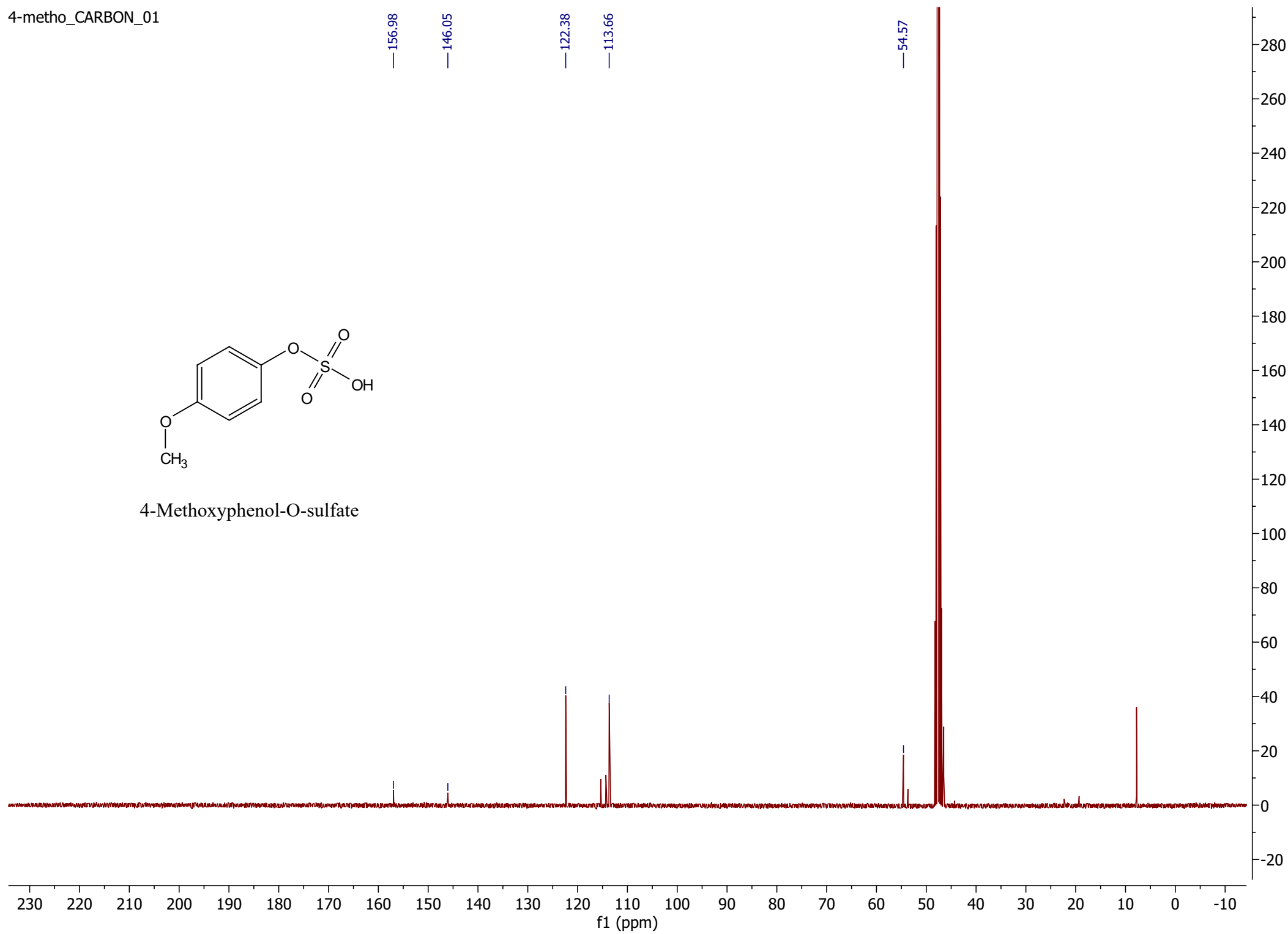
3-Methoxyphenol-O-sulfate



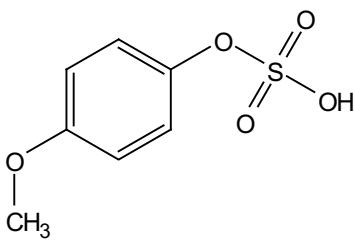




4-Methoxyphenol-O-sulfate



4-metho_gCOSY_01



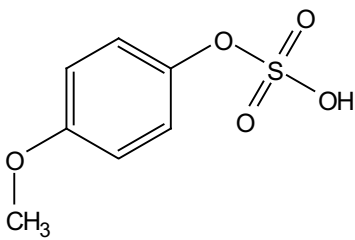
4-Methoxyphenol-O-sulfate

{4.79,4.79}D2O

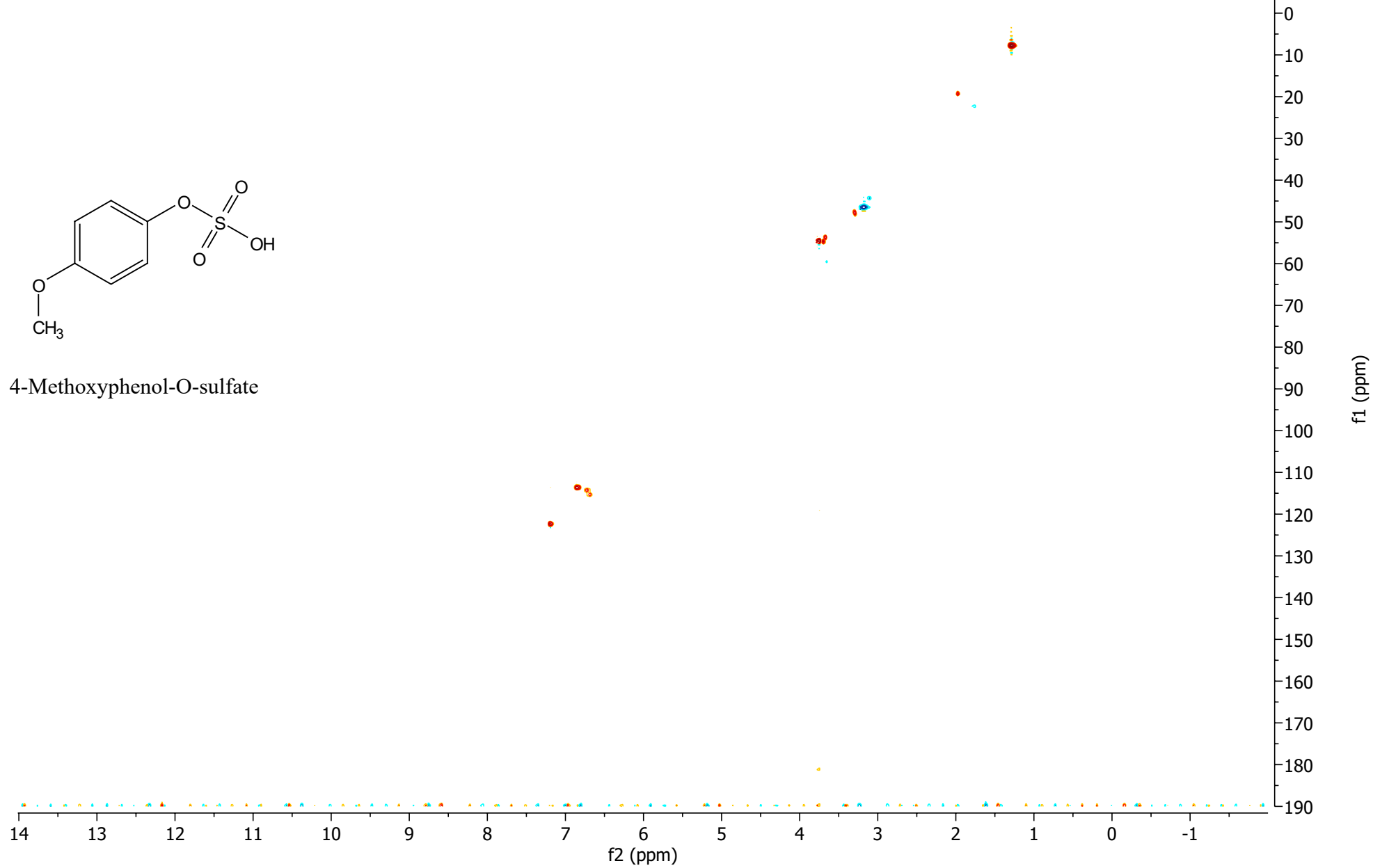
f1 (ppm)

f2 (ppm)

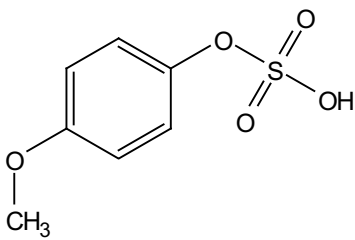
4-metho_HSQCAD_01



4-Methoxyphenol-O-sulfate



4-metho_gHMBCAD_01



4-Methoxyphenol-O-sulfate

