Chemoenzymatic Preparation and Biophysical Properties of Sulfated Quercetin Metabolites

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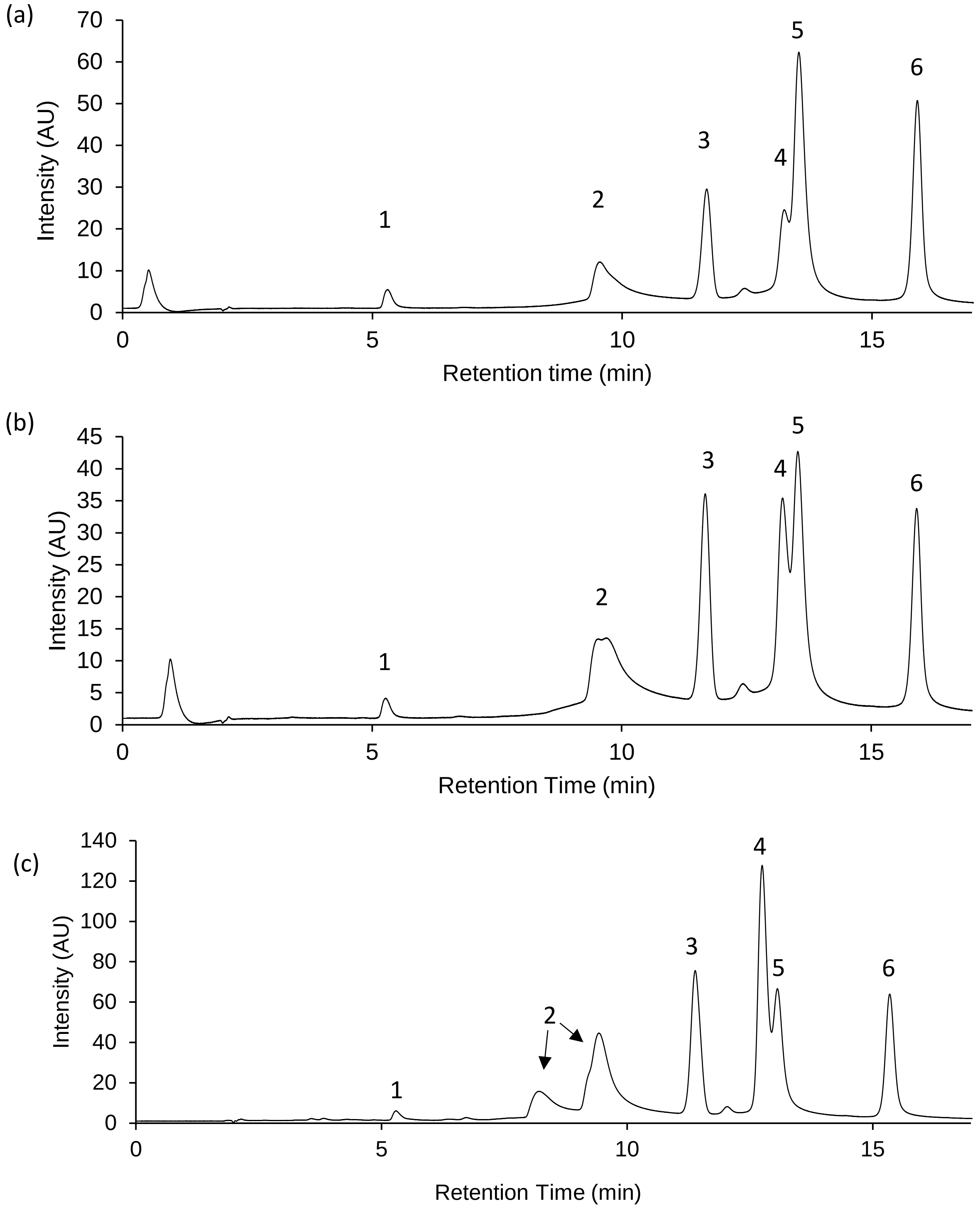

Figure S1. HPLC chromatograms of quercetin sulfation reaction mixture at (a) 1 h, (b) 5 h, (c) 24 h. Peak assignment: 1 – *p*-NP, 2 – quercetin disulfates, 3 – *p*-NPS, 4 – quercetin-3′-*O*-sulfate, 5 - quercetin-4′-*O*-sulfate, 6 – quercetin; detection at 370 nm.

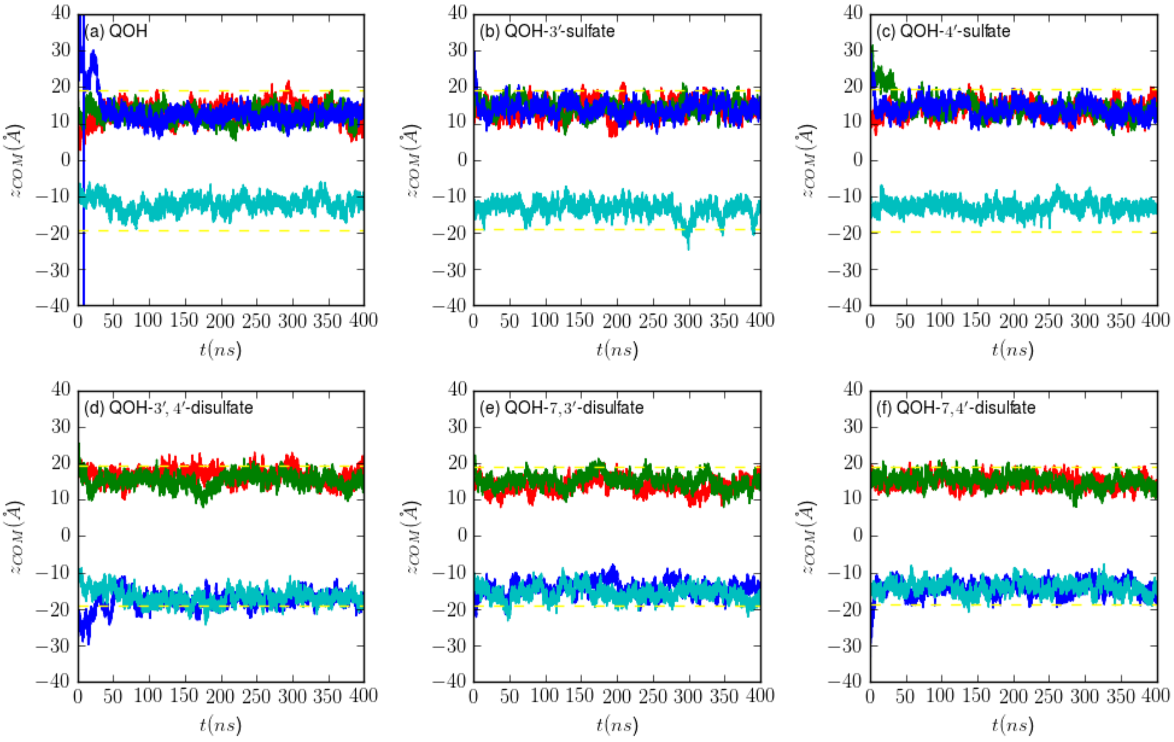

Figure S2. Distance of quercetin derivatives to lipid bilayer center over MD simulations.

Each quercetin replica is depicted in red, green blue and cyan. Average phosphate distances to lipid center is plotted in yellow.

Table S1. Average non-covalent interactions between solute and (a) lipids or (b) lipid tails.

(a)

	E_{int}	E_{elec}	E_{vdw}
quercetin	-78.0±16.2	-47.7±16.4	-30.3±7.7
quercetin 3´-O-sulfate	-134.7±27.2	-92.6±22.7	-42.2±8.7
quercetin 4´-O-sulfate	-138.6±20.2	-94.1±17.2	-44.5±8.0
quercetin 3',4´-O-disulfate	-288.7±53.8	-245.5±47.4	-43.2±10.4
quercetin-7',3´-O-disulfate	-222.2±31.7	-170.1±28.4	-52.1±7.5
quercetin-7',4´-O-disulfate	-216.1±30.2	-163.1±26.0	-53.0±8.5

(b)

	E_{int}	E_{elec}	E_{vdw}
quercetin	-24.1±7.0	-0.9±0.6	-23.3±6.8
quercetin 3´-O-sulfate	-23.0±6.7	-0.4±1.2	-22.6±6.2
quercetin 4´-O-sulfate	-29.4±7.3	-1.2±	-28.2±6.6
quercetin 3',4´-O-disulfate	-17.3±6.4	0.2±	-17.6±6.1
quercetin-7',3´-O-disulfate	-25.6±5.9	-1.2±	-24.4±4.9
quercetin-7',4´-O-disulfate	-30.5±6.8	-1.4±	-29.1±5.9

total (E_{int}, kcal.mol^{-1}), electrostatic (E_{elec}, kcal.mol^{-1}) and van der Waals energies (E_{vdw}, kcal.mol^{-1}) over the last 200 ns

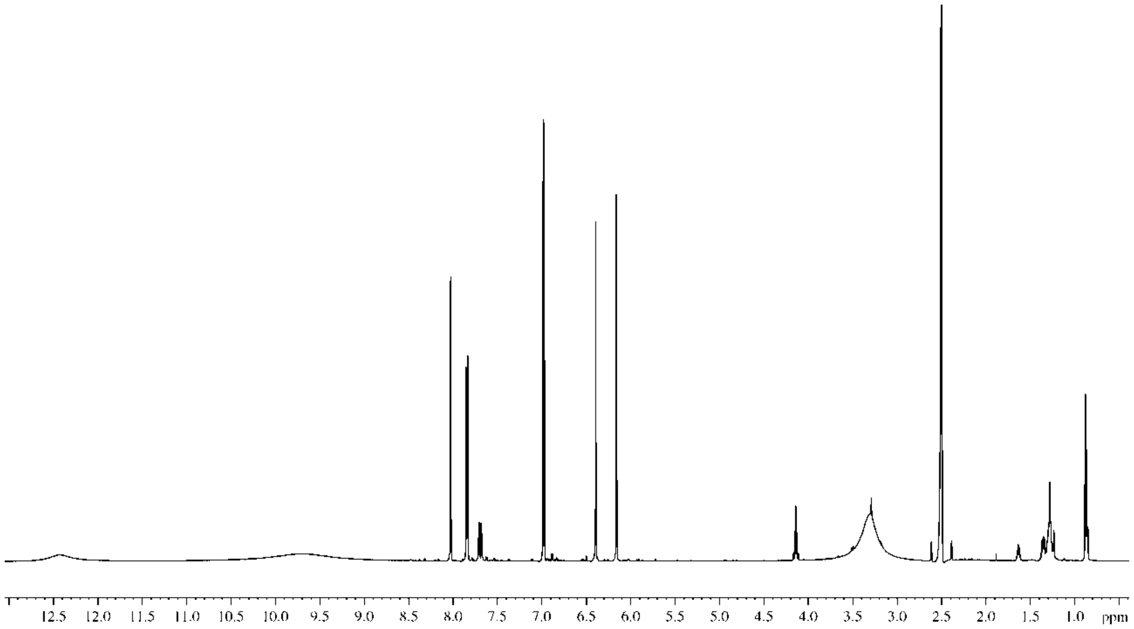

Figure S3. ^{1}H NMR spectrum of quercetin-3′-*O*-sulfate (600.23 MHz, DMSO-*d*$_6$, 30 °C).

Signal assignment: 6.158 (1H, d, *J* = 2.0 Hz, H-6), 6.392 (1H, d, *J* = 2.0 Hz, H-8), 6.977 (1H, d, *J* = 8.6 Hz, H-5′), 7.841 (1H, dd, *J* = 8.6, 2.3 Hz, H-6′), 8.026 (1H, d, *J* = 2.3 Hz, H-2′)

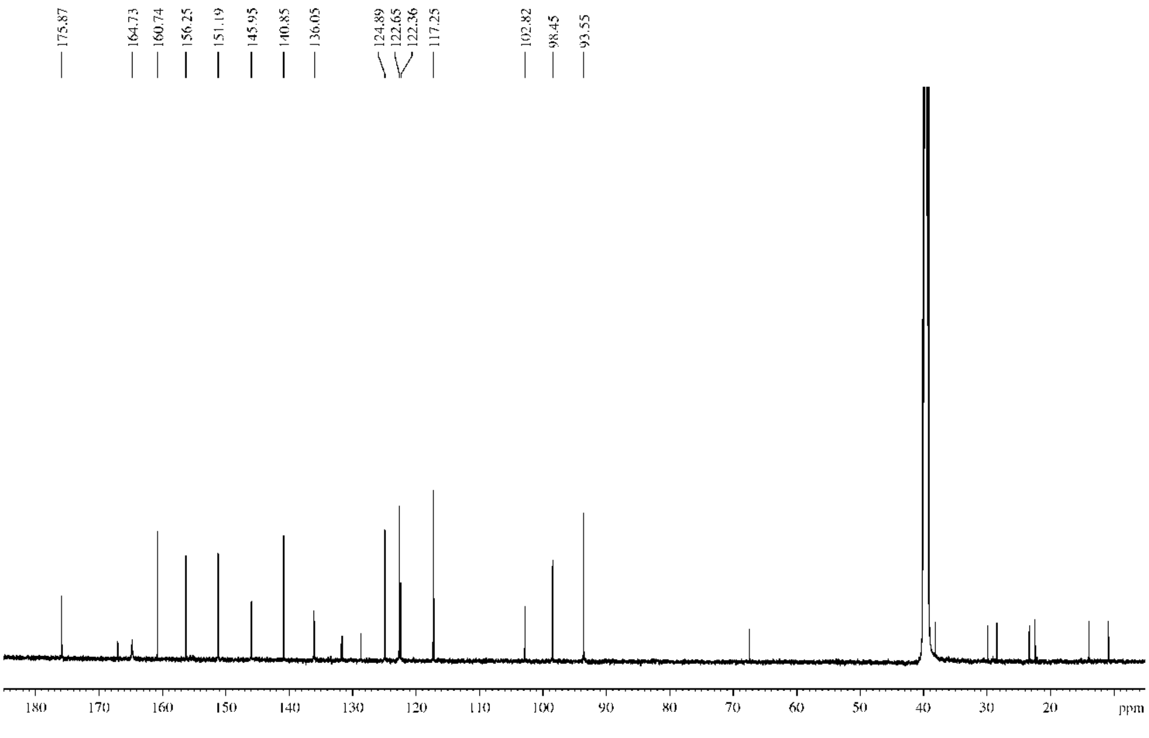

Figure S4. ^{13}C NMR spectrum of quercetin-3′-*O*-sulfate (150.93 MHz, DMSO-*d*$_6$, 30 °C).

Signal assignment: 93.55 (C-8), 98.45 (C-6), 102.82 (C-10), 117.25 (C-5′), 122.36 (C-1′), 122.65 (C-2′), 124.89 (C-6′), 136.05 (C-3), 140.85 (C-3′), 145.95 (C-2), 151.19 (C-4′), 156.25 (C-9), 160.74 (C-5), 164.73 (C-7), 175.87 (C-4)

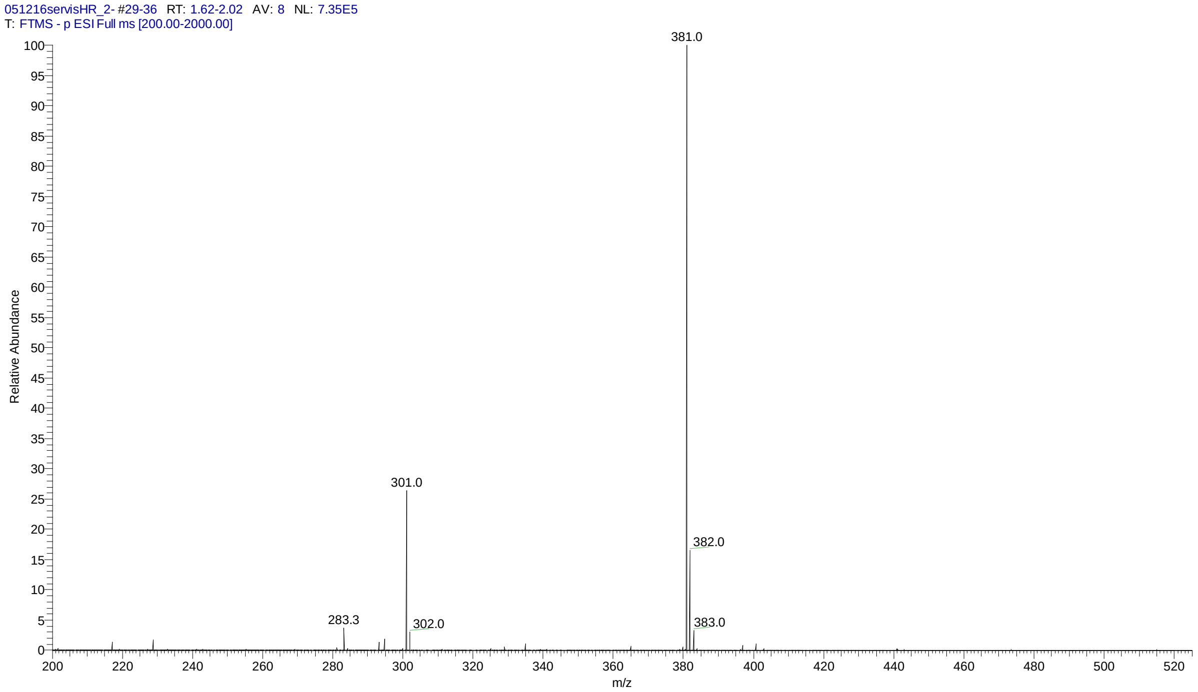

Figure S5. Mass spectrum (ESI−) of quercetin-3′-O-sulfate.

$[M - H]^-$, *m/z* 381.0; $[M - H - SO_3]^-$, *m/z* 301.0.

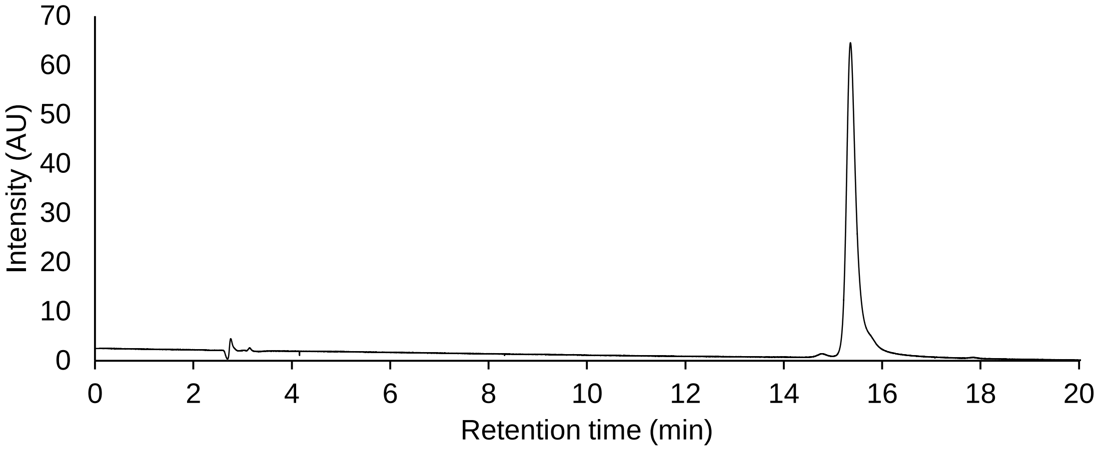

Figure S6. HPLC chromatogram of quercetin-3′-*O*-sulfate.

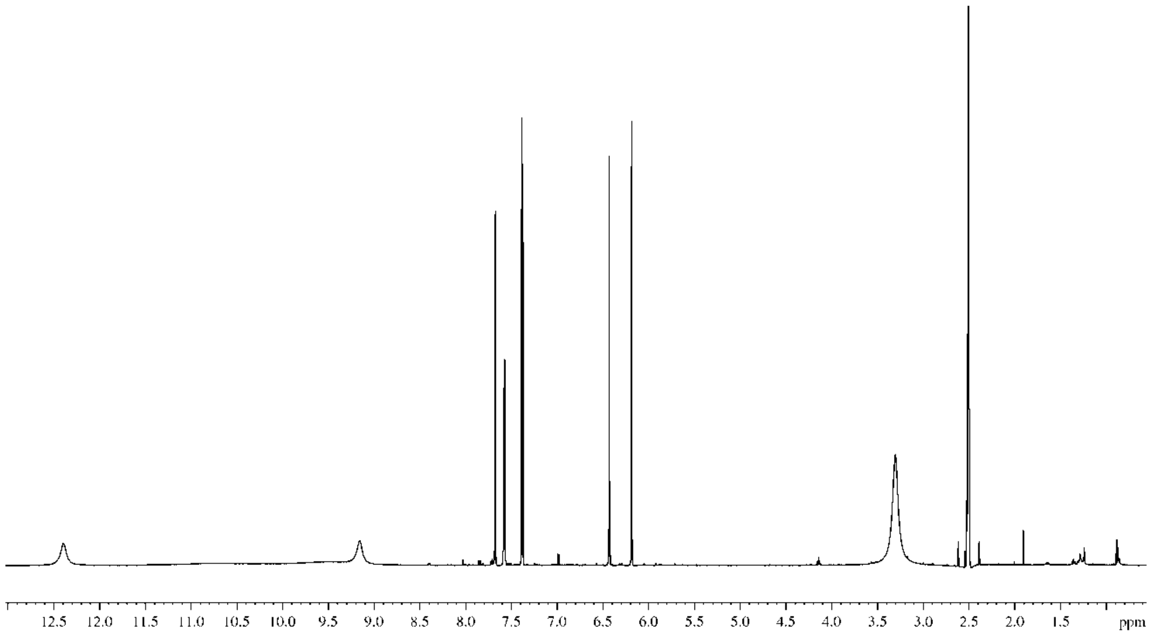

Figure S7. ^{1}H NMR spectrum of quercetin-4′-*O*-sulfate (600.23 MHz, DMSO-d_6, 30 °C).

Signal assignment: 6.181 (1H, d, *J* = 2.1 Hz, H-6), 6.428 (1H, d, *J* = 2.1 Hz, H-8), 7.377 (1H, d, *J* = 8.6 Hz, H-5′), 7.573 (1H, dd, *J* = 8.6, 2.2 Hz, H-6′), 7.672 (1H, d, *J* = 2.2 Hz, H-2′)

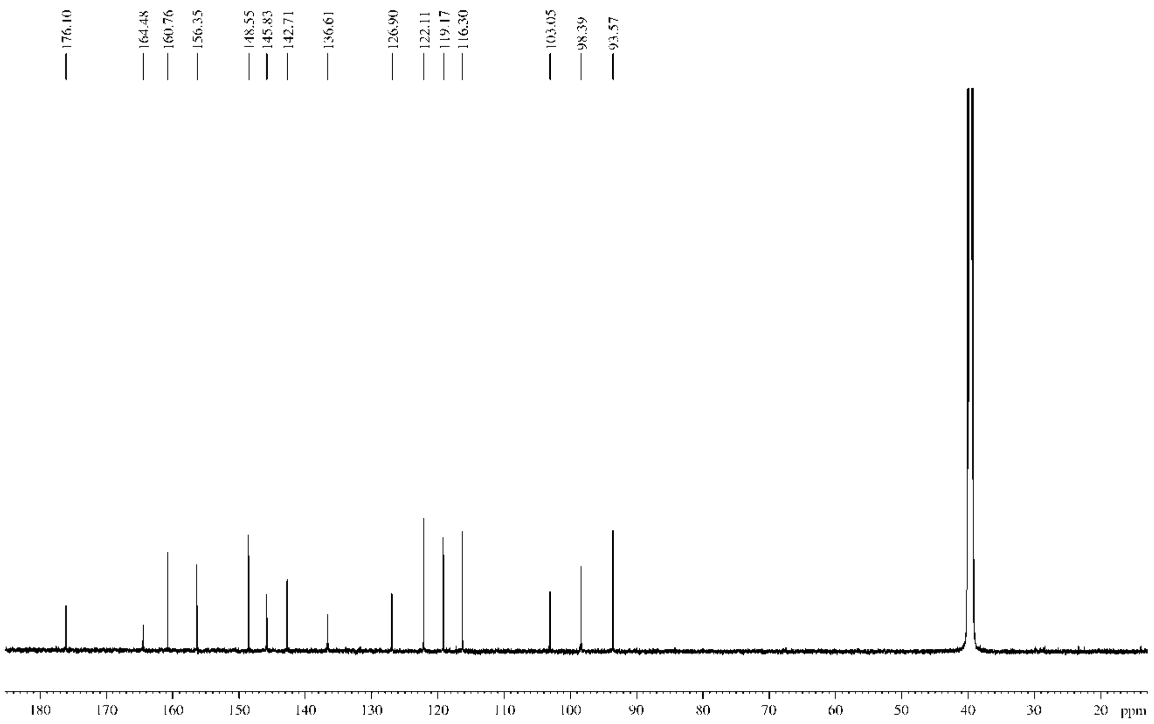

Figure S8. ^{13}C NMR spectrum of quercetin-4′-*O*-sulfate (150.93 MHz, DMSO-d_6, 30 °C).

signal assignment: 93.57 (C-8), 98.39 (C-6), 103.05 (C-10), 116.30 (C-2′), 119.17 (C-6′), 122.11 (C-5′), 126.90 (C-1′), 136.61 (C-3), 142.71 (C-4′), 145.83 (C-2), 148.55 (C-3′), 156.35 (C-9), 160.76 (C-5), 164.48 (C-7), 176.10 (C-4)

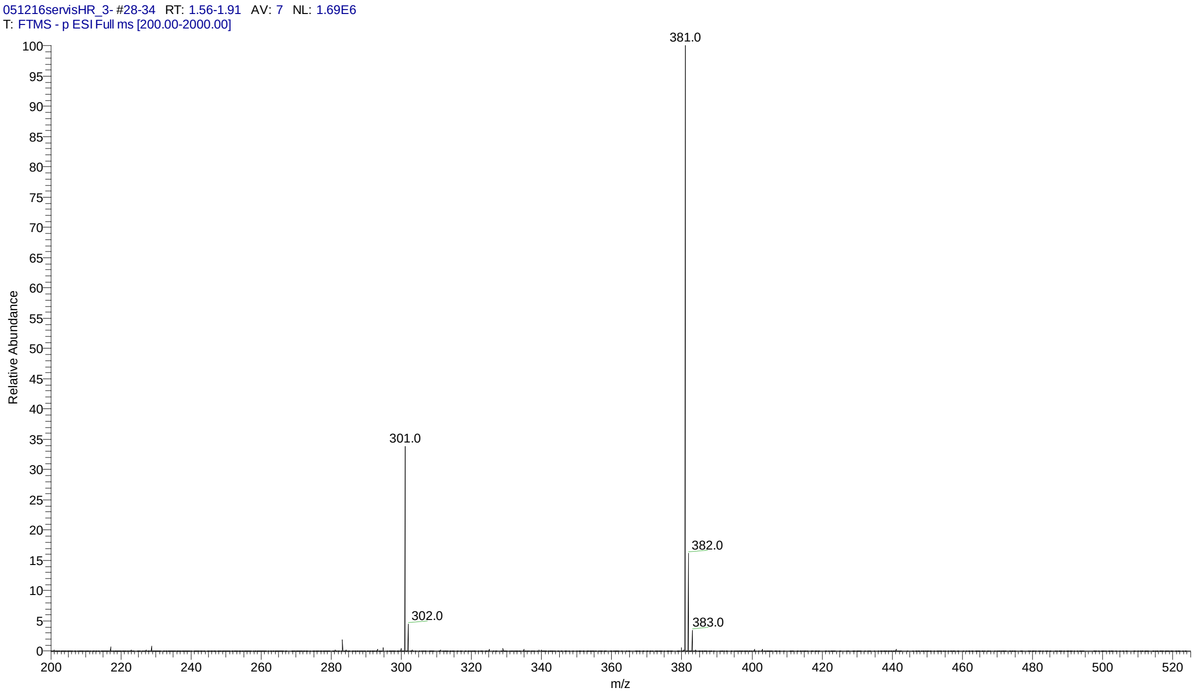

Figure S9. Mass spectrum (ESI−) of quercetin-4′-O-sulfate.

$[M-H]^-$, *m/z* 381.0; $[M-H-SO_3]^-$, *m/z* 301.0.

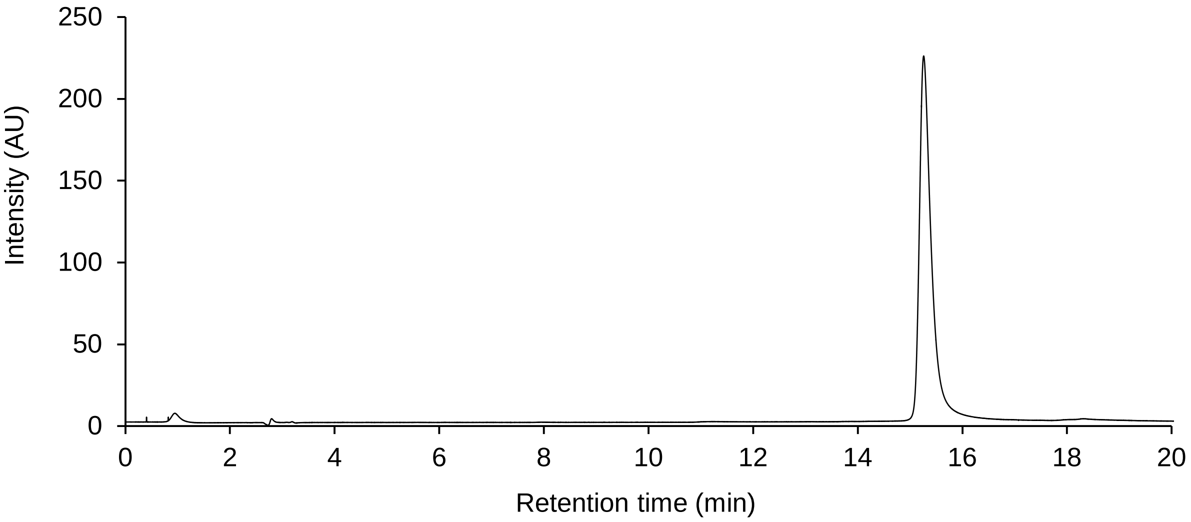

Figure S10. HPLC chromatogram of quercetin-4′-*O*-sulfate.

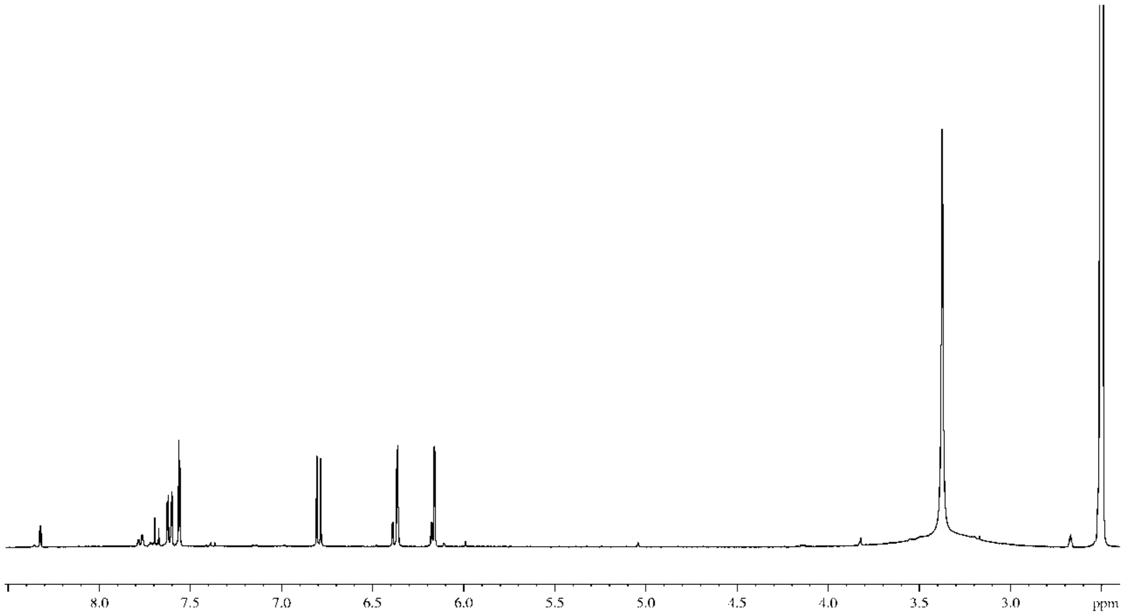

Figure S11. ^{1}H NMR spectrum of quercetin-3-*O*-sulfate (399.87 MHz, DMSO-d_6, 30 °C).

signal assignment: ^{1}H NMR: 6.159 (1H, d, J = 2.1 Hz, H-6), 6.363 (1H, d, J = 2.1 Hz, H-8), 6.795 (1H, d, J = 8.5 Hz, H-5′), 7.560 (1H, d, J = 2.2 Hz, H-2′), 7.613 (1H, dd, J = 8.5, 2.2 Hz, H-6′)

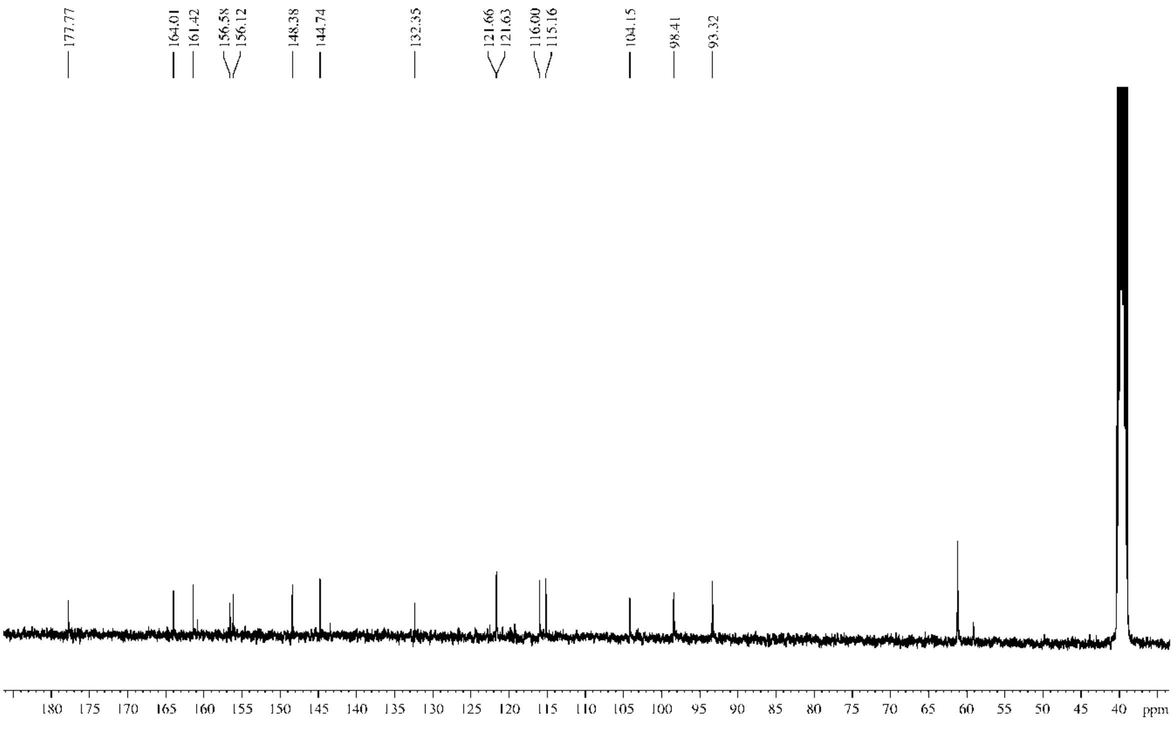

Figure S12. ^{13}C NMR spectrum of quercetin-3-*O*-sulfate (100.55 MHz, DMSO-d_6, 30 °C).

signal assignment: 93.32 (C-8), 98.41 (C-6), 104,15 (C-10), 115.16 (C-5′), 116.00 (C-2′), 121.63[a] (C-1′), 121.66[a] (C-6′), 132.35 (C-3), 144.74 (C-3′), 148.38 (C-4′), 156.12 (C-9), 156.58 (C-2), 161.42 (C-5), 164.01 (C-7), 177.77 (C-4) [a] … might be interchanged

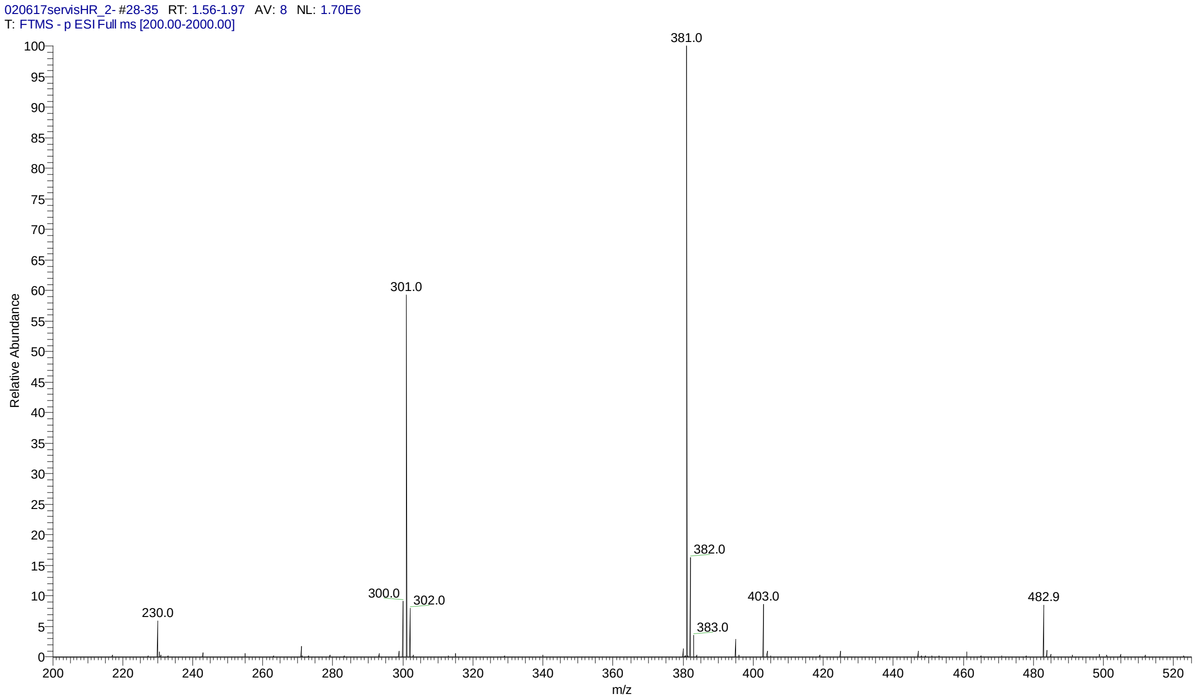

Figure S13. Mass spectrum (ESI−) of quercetin-3-O-sulfate.

$[M-H]^-$, *m/z* 381.0; $[M-H-SO_3]^-$, *m/z* 301.0. Signals of a disulfate were found at *m/z* 482.9 ($[M-2H+Na]^-$), *m/z* 403.0 ($[M-2H+Na-SO_3]^-$), and *m/z* 230.0 ($[M-2H]^{2-}$).

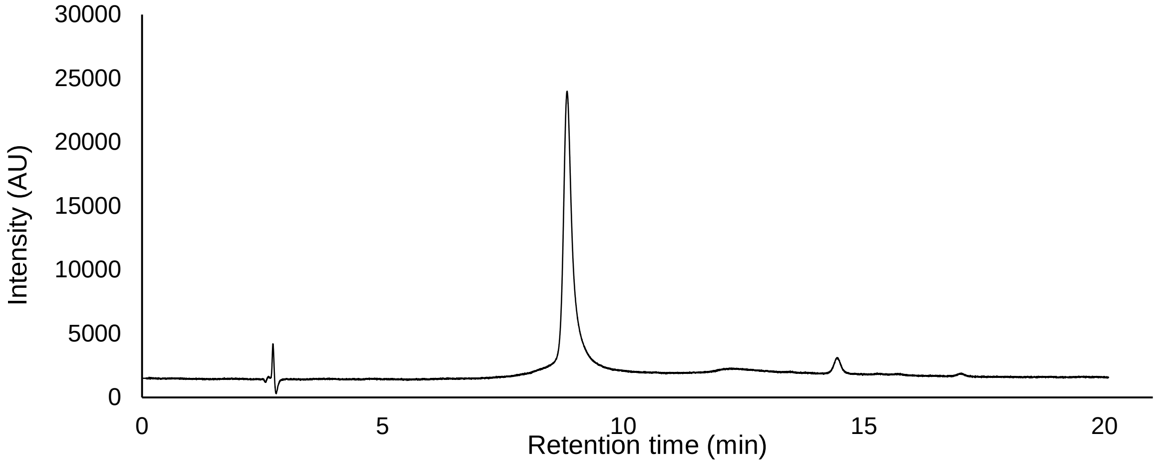

Figure S14. HPLC chromatogram of quercetin-3-*O*-sulfate.

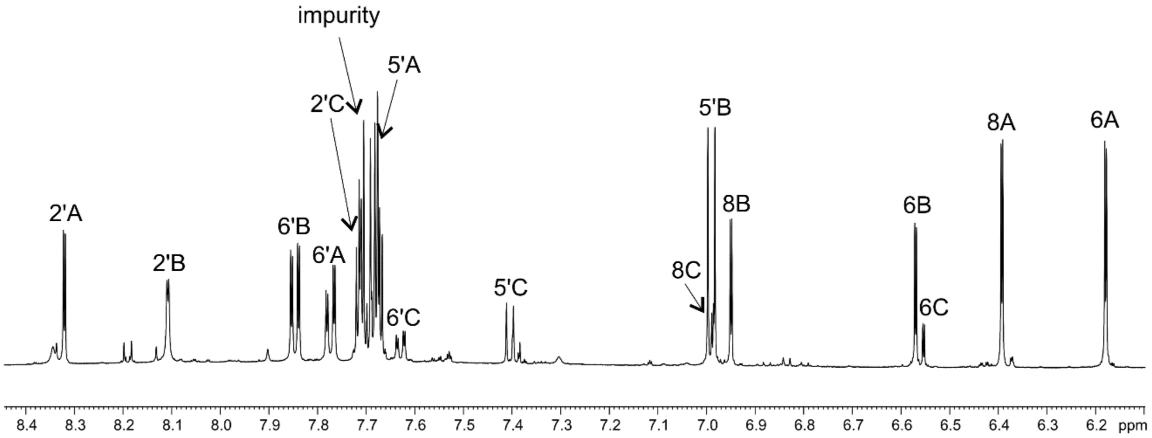

Figure S15. Expanded ^{1}H NMR spectrum of quercetin-di-*O*-sulfates mixture (600.23 MHz, DMSO-d_6, 30 °C). Signals of individual components are marked as **A**: quercetin-3′,4′-di-*O*-sulfate, **B**: quercetin-7,3′-di-*O*-sulfate, and **C**: quercetin-7,4′-di-*O*-sulfate.

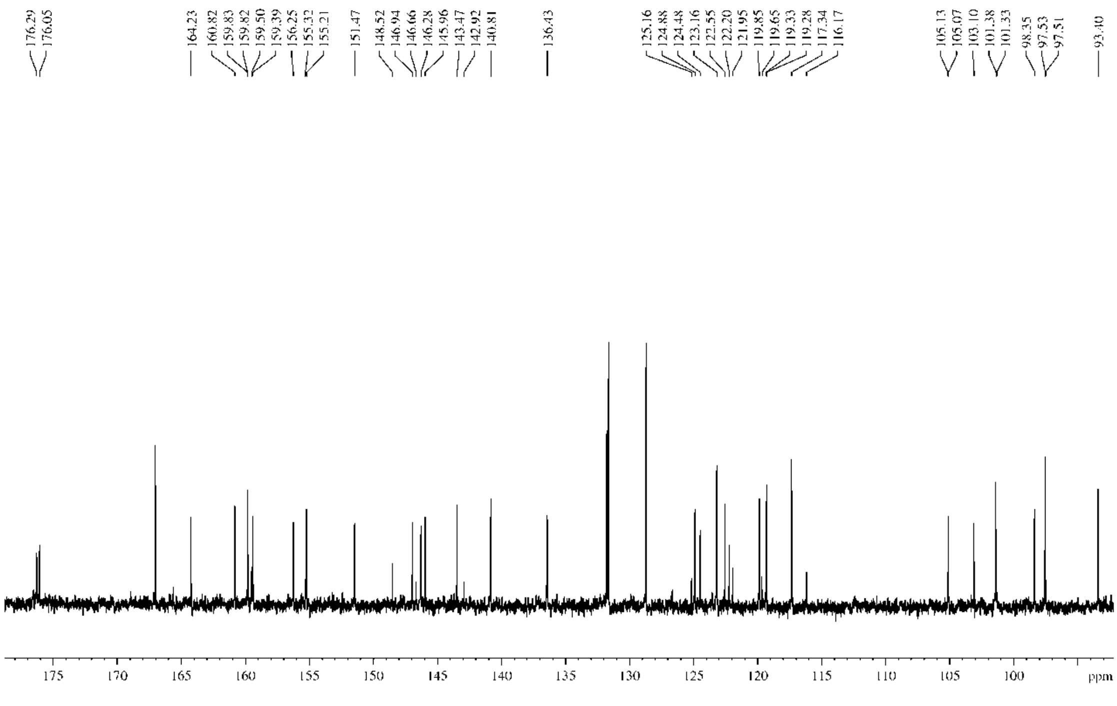

Figure S16. Expanded ^{13}C NMR spectrum of quercetin-di-*O*-sulfate mixture (150.93 MHz, DMSO-d_6, 30 °C).

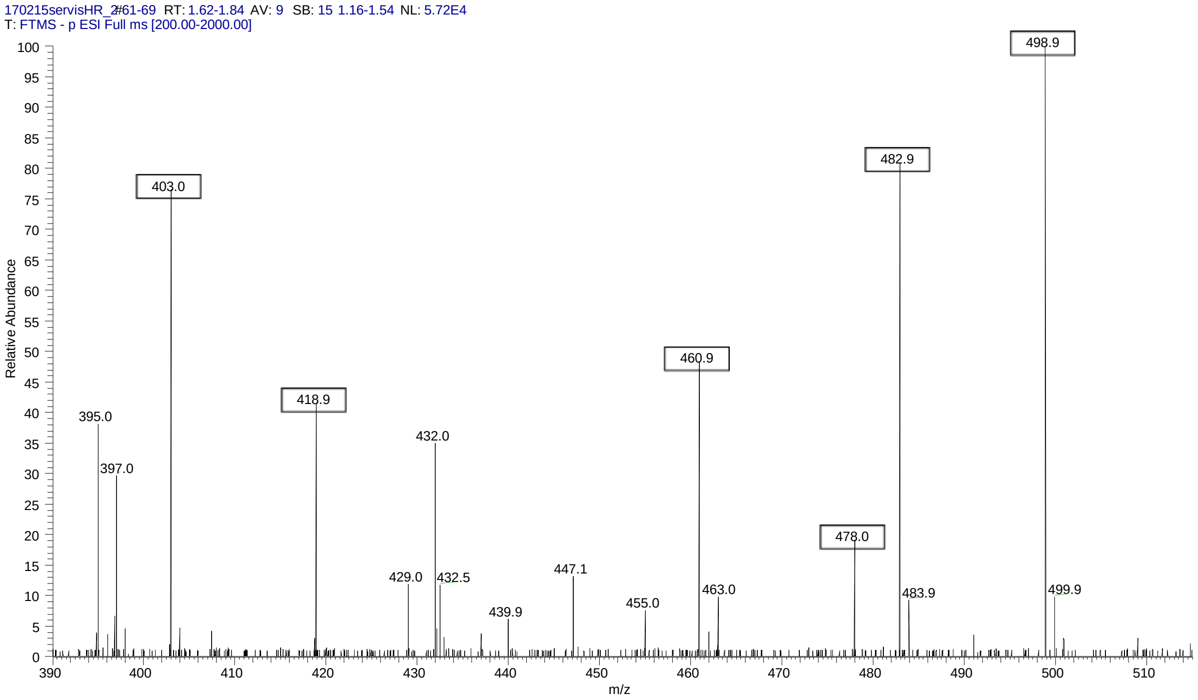

Figure S17. Mass spectrum (ESI−) of quercetin-di-O-sulfates.

$[M-2H+K]^-$, m/z 498.9; $[M-2H+Na]^-$, m/z 482.9; $[M-2H+NH_4]^-$, m/z 478.0; $[M-H]^-$, m/z 460.9; $[M-2H+K-SO_3]^-$, m/z 418.9; $[M-2H+Na-SO_3]^-$, m/z 403.0.

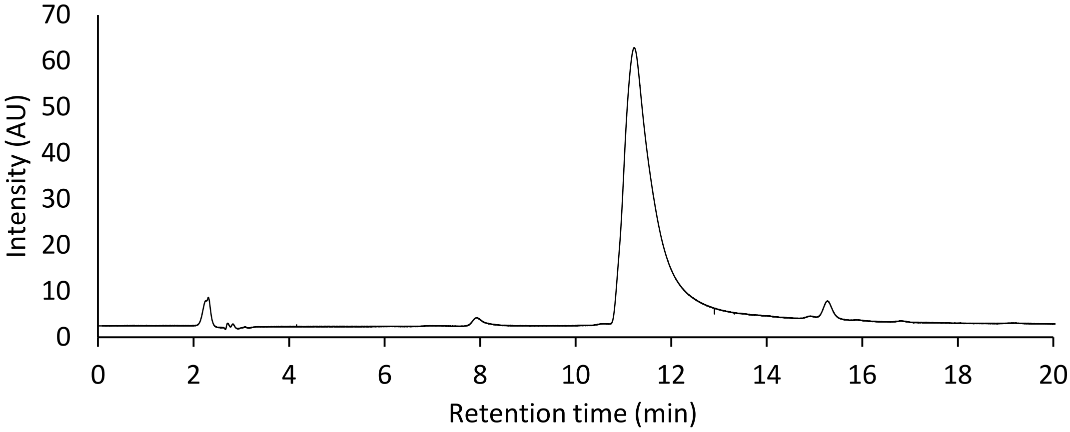

Figure S18. HPLC chromatogram of quercetin-di-*O*-sulfate mixture.