

Supplementary Information

Identification of OTB-*N*-acetyl-L-cysteine as a new ochratoxin A metabolite and potential biomarker in human urine

Franziska Sueck, Jonas Specht, Benedikt Cramer and Hans-Ulrich Humpf*

Institute of Food Chemistry, Westfälische Wilhelms-Universität Münster, Corrensstr. 45,
48149 Münster, Germany

(*corresponding author: humpf@wwu.de; +49 251 83 33391)

HRMS data

The exact mass and the most intensive fragments of each analyte is given in Table S1:

Table S1: Molecular formula, exact masses and the most intensive fragments of the synthesised analytes from HRMS using HCD 35% relative energy.

analyte	mode	molecular formula	exact mass (<i>m/z</i>)	fragments (<i>m/z</i>)
OTB-GSH	positive	[C ₃₀ H ₃₄ N ₄ O ₁₂ S+H] ⁺	found 675.1967 (calcd. 675.1967)	600.1639, 447.0851, 353.0799, 296.0585
OTB-NAC	positive	[C ₂₅ H ₂₆ N ₂ O ₉ S+H] ⁺	found 531.1422 (calcd. 531.1432)	384.0746, 366.0640, 255.013, 237.0213
d ₅ -OTB-GSH	positive	[C ₃₀ H ₂₉ D ₅ N ₄ O ₁₂ S+H] ⁺	found 680.2279 (calcd. 680.2281)	605.1958, 353.0801, 296.0587, 278.0481
d ₅ -OTB-NAC	positive	[C ₂₅ H ₂₁ D ₅ N ₂ O ₉ S+H] ⁺	found 536.1735 (calcd. 536.1746)	384.0742, 366.0636, 255.0318, 237.0213
OTHQ-GSH	negative	[C ₃₀ H ₃₄ N ₄ O ₁₃ S-H] ⁻	found 689.1762 (calcd. 689.1770)	-
OTHQ-NAC	negative	[C ₂₅ H ₂₆ N ₂ O ₁₀ S-H] ⁻	found 545.1238 (calcd. 545.1235)	-

UV data

The UV absorption maxima of all four analytes, (d₅-)OTB-GSH and (d₅-)OTB-NAC is 331 nm. This observation agrees with the results from Tozlovanu et al. 2012.

NMR data

OTB-NAC

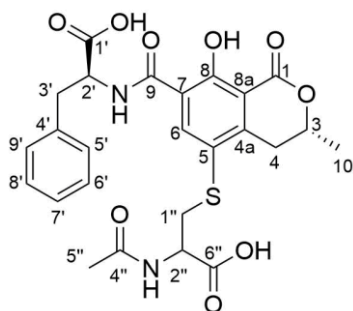


Figure S1: Chemical structure of OTB-NAC with atom labelling.

¹H NMR (600 MHz, CD₃OD/D₂O/DCOOD (75/25/0.1; v/v/v)) δ 8.40 (s, 1H, H-6), 7.31 – 7.22 (m, 5H, H-5'-9'), 4.93 (dd, *J* = 7.0, 5.3 Hz, 1H, H-2'), 4.39 (dd, *J* = 8.5, 4.3 Hz, 1H, H-2''), 3.52 (dd, *J* = 17.2, 3.2 Hz, 1H, H-4.2), 3.38 (dd, *J* = 14.1, 4.4 Hz, 1H, H-1''.2), 3.34 (m, 1H, H-3'.2), 3.20 (dd, *J* = 13.8, 7.2 Hz, 1H, H-3'.1), 3.14 (dd, *J* = 14.1, 8.5 Hz, 1H, H-1''.1), 2.92 (dd, *J* = 17.2, 11.7 Hz, 1H, H-4.1), 1.96 (s, 3H H-5''), 1.55 (d, *J* = 6.3 Hz, 3H, H-10).
δ 4.80 (H-3) overlapping with water signal, identified by HMBC.

¹³C NMR (151 MHz, CD₃OD/D₂O/DCOOD (75/25/0.1; v/v/v)) δ 174.7 (C-1'), 173.6 (C-4''), 171.6 (C-1), 165.1 (C-9), 164.9 (C-6''), 161.2 (C-8), 149.0 (C-4a), 144.3 (C-6), 137.5 (C-4'), 130.4 (C-5'/9' or C-6'/8'), 129.5 (C-5'/9' or C-6'/8'), 128.1 (C-7'), 124.1 (C-5), 120.5 (C-7), 111.2 (C-8a), 77.8 (C-3), 55.6 (C-2'), 53.5 (C-2''), 38.2 (C-3'), 37.8 (C-1''), 34.0 (C-4), 22.5 (C-28), 20.8 (C-10).

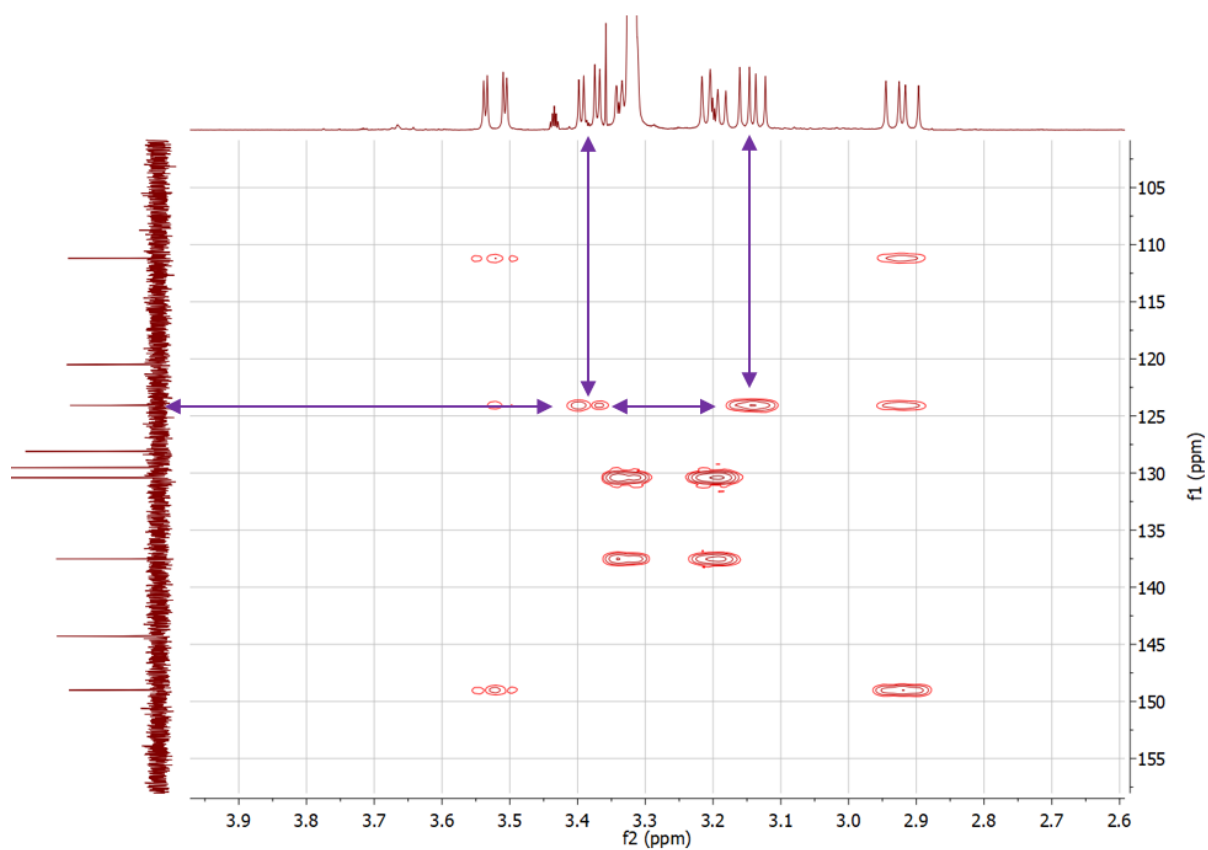


Figure S2: ^1H , ^{13}C -HMBC-spectra of OTB-NAC. The $^3J_{\text{C,H}}$ coupling of H-1''.1 (δ 3.14) and H-1''.2 (δ 3.38) with the C-5 (δ 124.1) are marked with arrows.

OTB-GSH

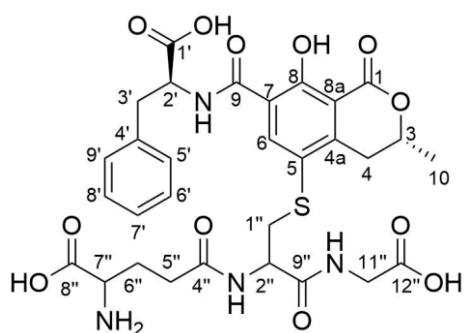


Figure S3: Chemical structure of OTB-GSH with atom labelling.

^1H NMR (600 MHz, $\text{CD}_3\text{OD}/\text{D}_2\text{O}/\text{DCOOD}$ (75/25/0.1; v/v/v)) δ 8.35 (s, 1H, H-6), 7.31 – 7.18 (m, 5H, H-5'-9'), 4.27 (dd, J = 9.8, 4.5 Hz, 1H, H-2''), 3.81 (d, J = 2.5 Hz, 2H, H-11''), 3.69 (t, J = 6.3 Hz, 1H, H-7''), 3.55 (dd, J = 17.2, 3.2 Hz, 1H, H-4.2), 3.34 (dd, J = 3.1, 1.3 Hz, 1H, H-1''.2), 3.18 (dd, J = 14.0, 7.2 Hz, 1H, H-3'.1), 3.03 (dd, J = 14.1, 9.9 Hz, 1H, H-1''.1), 2.98 (dd, J = 17.2, 11.8 Hz, 1H, H-4.1), 2.55 (t, J = 7.2 Hz, 2H, H-5''), 2.23 – 2.08 (m, 2H, H-6''), 1.56 (d, J = 6.3 Hz, 3H, H-10).

δ 4.85 (H-2') and 4.79 (H-3) overlapping with water signal; δ 3.32 (H-1''.2) and 3.31 (H-3'.2) overlapping with methanol signal but identified by HMBC.

^{13}C NMR (151 MHz, $\text{CD}_3\text{OD}/\text{D}_2\text{O}/\text{DCOOD}$ (75/25/0.1; v/v/v)) δ 175.9* (C-1'), 175.3 (C-4''), 174.1* (C-8''), 173.9* (C-12''), 172.7 (C-9''), 171.6 (C-1), 165.3 (C-9), 161.4* (C-8), 149.4 (C-8a), 144.8 (C-6), 138.0 (C-4'), 130.5 (C-15'/9'), 129.5 (C-6'/8'), 127.9 (C-7'), 123.2* (C-5), 120.5 (C-7), 111.3 (C-4a), 77.8 (C-3), 56.5 (C-2'), 55.3* (C-7''), 53.8 (C-2''), 42.5 (C-11''), 38.4 (C-3'), 37.4 (C-1''), 33.9 (C-4), 32.7 (C-5''), 27.5 (C-6''), 20.9 (C-10). * identified by HMBC

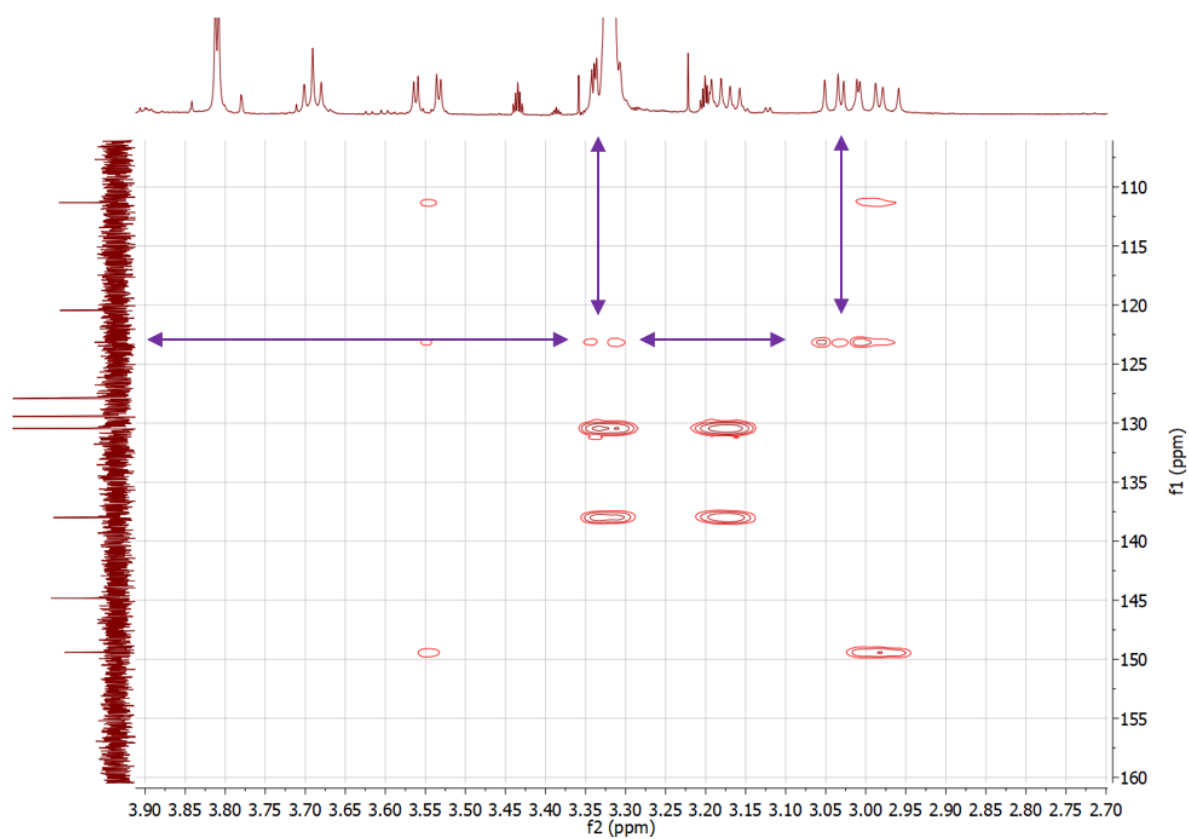


Figure S4: ^1H , ^{13}C -HMBC-spectra of OTB-GSH. The $^3J_{\text{C,H}}$ coupling of H-1''1 (δ 3.03) and H-1''2 (δ 3.32) with the C-5 (δ 123.2) are marked with arrows.