Supplementary Material

In vitro glucuronidation of the antibacterial triclocarban and its oxidative metabolites

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Drug Metabolism and Disposition

Supplementary Table S1
Optimized ESI-MS parameters for detection of TCC and its metabolites in SRM mode.

compound	*m/z* [M-H]	*m/z* fragment	cone voltage [V]	collision energy [V]
DCC (I.S.)	279	126	28	16
TCC	313	160	26	15
2'-OH-TCC	329	168	24	14
6-OH-TCC	329	202	22	14
3'-OH-TCC	329	168	24	14
2'-SO_3-*O*-TCC	409	168	28	25
N-gluc-TCC	489	336	30	19
N'-gluc-TCC	489	302	40	12
2'-*O*-gluc-TCC	505	168	40	12
6-*O*-gluc-TCC	505	343	40	12
3'-*O*-gluc-TCC	505	344	40	12

Supplementary Table S2

Activity of the microsomal preparations for the conjugation of 4-(trifluoromethyl) umbelliferone (TFMU) and trifluoperazine (TFP). Microsomal incubations with 100 µM TFMU and 200 µM were carried out as described for TCC and its metabolites. The glucuronide concentration was determined by HPLC as described previously (Maul et al. 2011; Uchaipichat et al, 2006). The activity and SD (nmol min^{-1} mg $protein^{-1}$) are given as mean of three independent determinations.

microsomes / UGT	*activity* TFMU *nmol min^{-1} mg^{-1}*	TFP *pmol min^{-1} mg^{-1}*
HLM	55.7±3.93	12.7±3.6
MLM	46.4±5.32	<LOD (5)
CLM	107±1.48	127±3.7
RLM	70.8±3.76	<LOD (5)
HIM	10.4±0.82	<LOD (5)
HKM	30.0±1.28	<LOD (5)
UGT1A1	2.30±0.17	
UGT1A3	1.93±0.32	
UGT1A4	<LOD (0.04)	488±20.6
UGT1A6	20.7±0.45	
UGT1A7	6.69±0.75	
UGT1A8	2.44±0.03	
UGT1A9	10.8±0.96	
UGT1A10	2.44±0.03	
UGT2B4	0.67±0.13	
UGT2B7	2.73±0.10	
UGT2B15	3.19±0.05	
UGT2B17 [1]	<LOQ (0.2)	

[1] Activity of 0.37 nmol min^{-1} mg $protein^{-1}$ for the conjugation of the substrate eugenol (According to manufacturer)

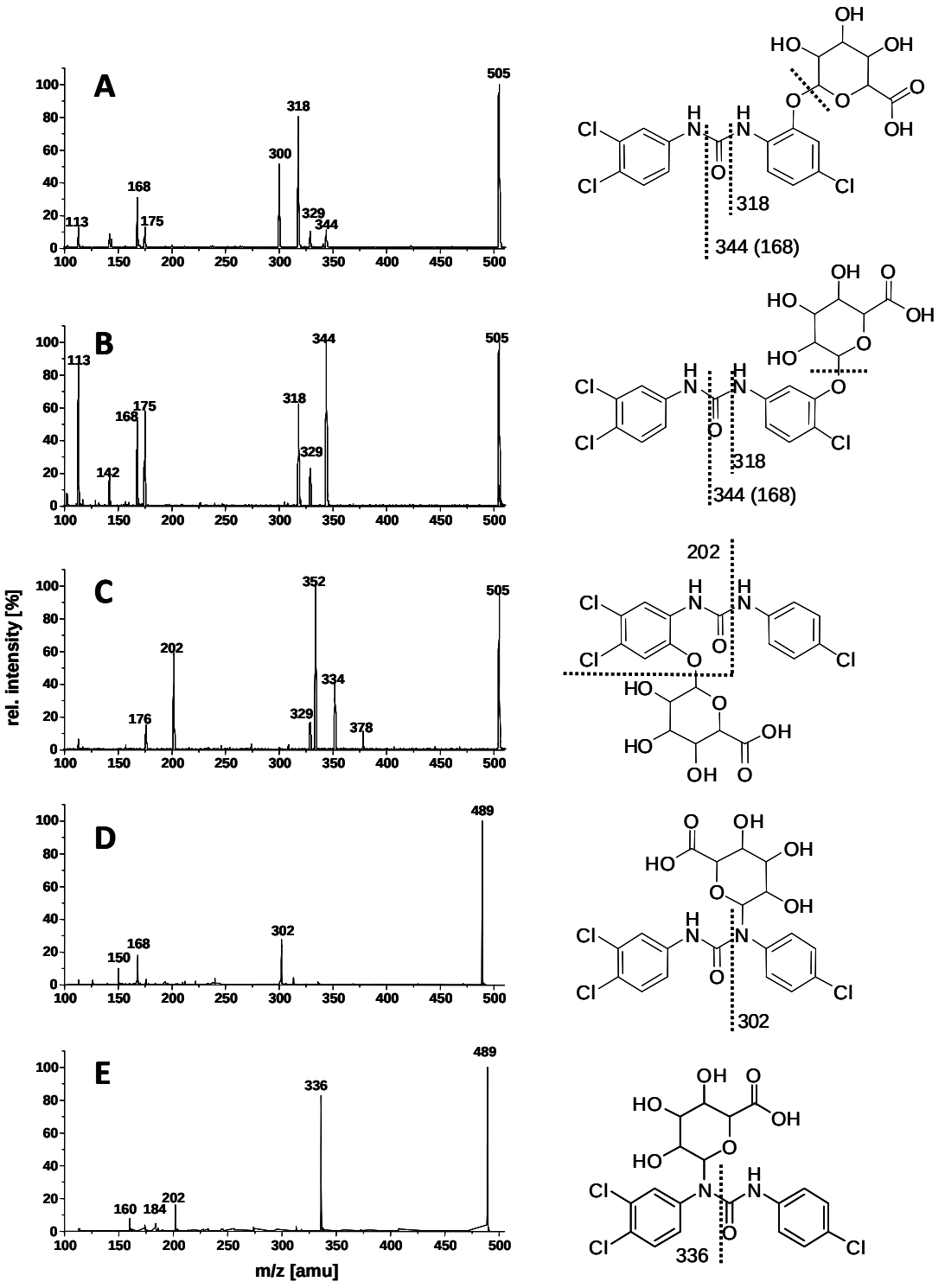

Figure S1:
Left: Negative electrospray ionization product ion spectra of the TCC-glucuronide conjugates. **A**: 2'-*O*-gluc TCC; **B**: 3'-*O*-gluc-TCC; **C**: 6-*O*-gluc-TCC; **D**: *N*'-gluc-TCC, **E**: *N*-gluc-TCC. **Right:** Structures of the glucuronides. The lines depict the fragmenation sites leading to the major fragments. The adjacent numbers show the *m/z* values of the resulting fragments.

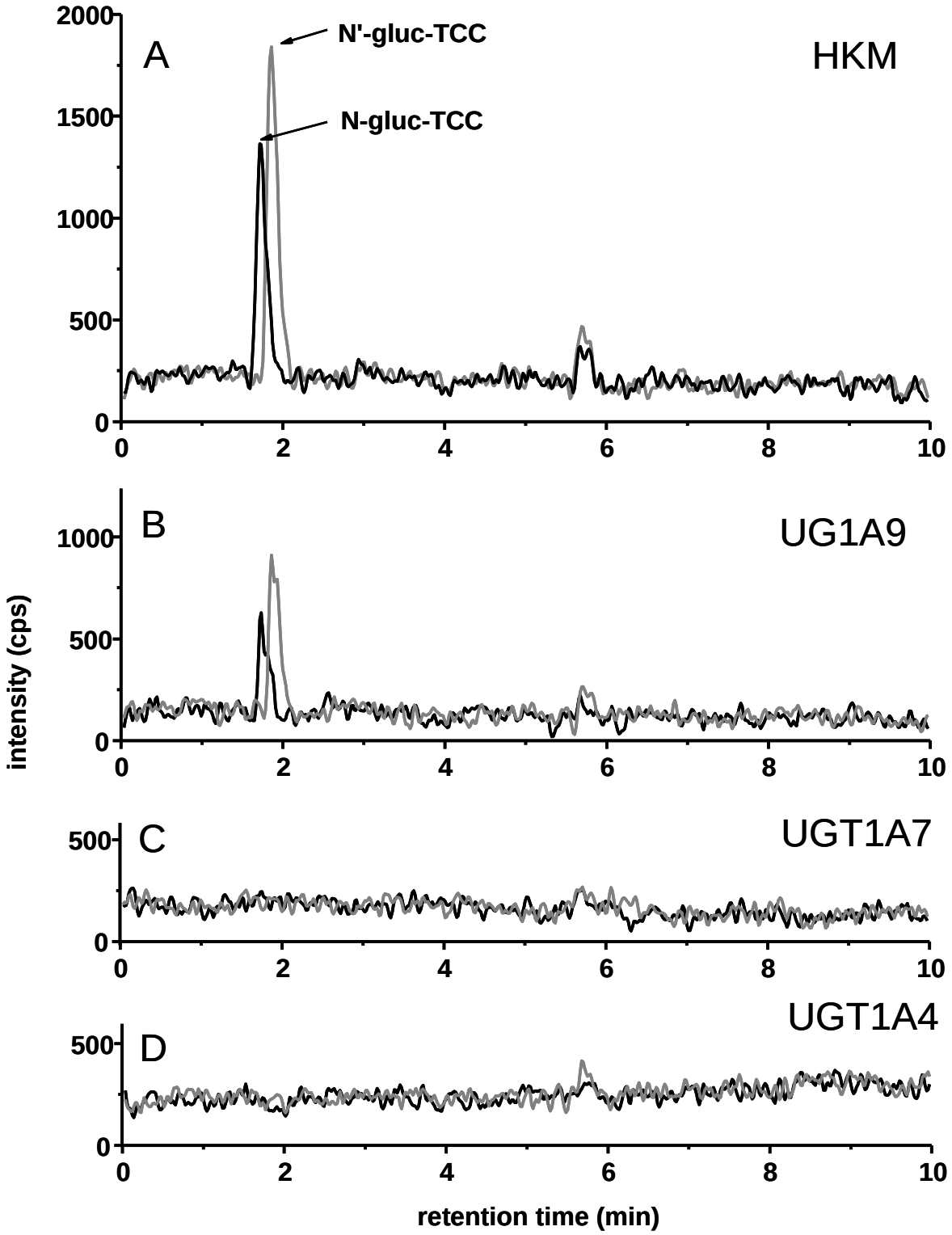

Figure S2:
LC-ESI-MS/MS chromatograms of microsomal incubations of TCC. Shown are the SRM signals of *N*-Gluc-TCC- (black) and *N*'-Gluc-TCC (grey) of 30 minute incubations (25 µg/ml protein) of TCC with (A) HKM and (B) UGT1A9. Analysis after incubation with (C) UGT1A7 and (D) UGT1A4 are shown exemplary for microsomal preparations not converting TCC.

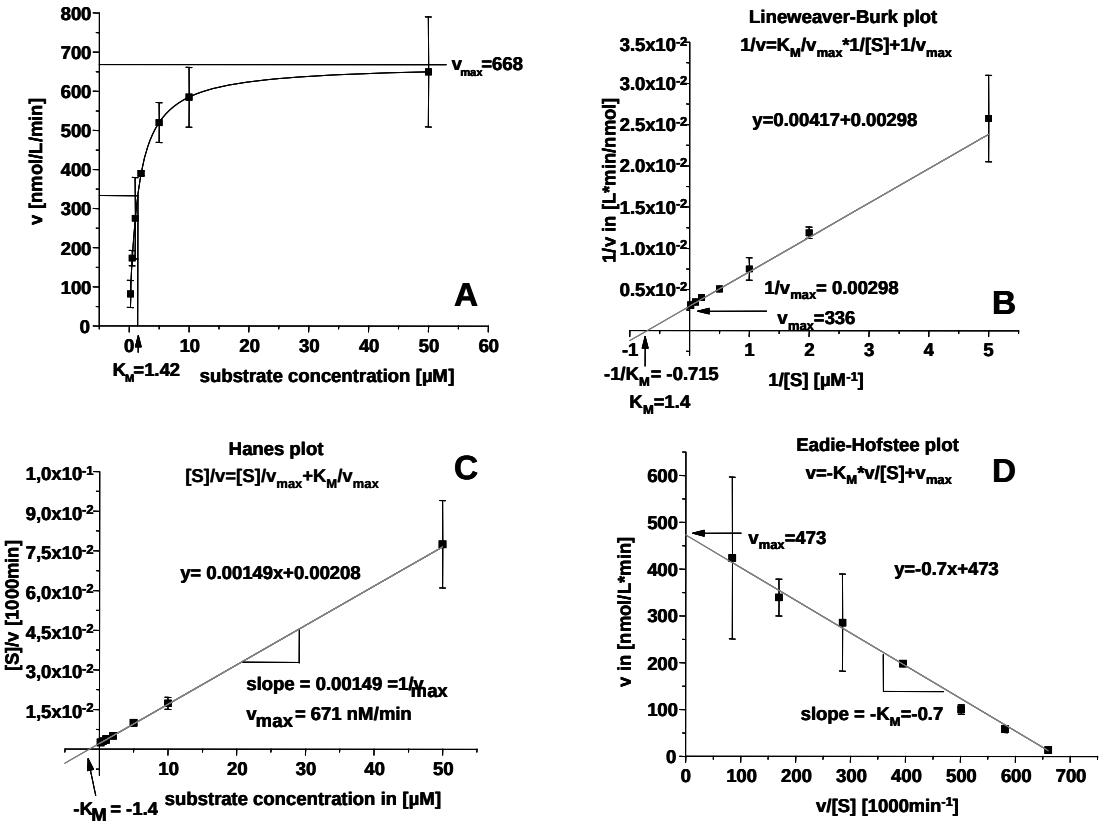

Figure S3
Determination of K_M and v_{max} values by fitting to the Michaelis-Menten function and utilization of various linearization methods. The data derived from a glucuronidation experiment of 2'-OH-TCC by CLM is shown exemplarily. **A:** Mathematical fitting **B:** Lineweaver-Burk plot; **C:** Hanes plot; **D:** Eadie-Hofstee plot.