

ELECTRONIC SUPPLEMENTARY MATERIAL

Identification of metabolites in human hepatic bile using 800 MHz ^1H NMR spectroscopy, HPLC-NMR/MS and UPLC-MS

**Iola F. Duarte^a, Cristina Legido-Quigley^b, David A. Parker^b, Jonathan R. Swann^b,
Manfred Spraul^c, Ulrich Braumann^c, Ana M. Gil^a, Elaine Holmes^b, Jeremy K.
Nicholson^b, Gerard M. Murphy^b, Hector Vilca-Melendez^d, Nigel Heaton^d and John C.
Lindon^{b,*}**

^a CICECO, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

^b Department of Biomolecular Medicine, Faculty of Medicine, Imperial College London, Sir
Alexander Fleming Building, South Kensington, London SW7 2AZ UK

^c Bruker Biospin GmbH, Silberstreifen, D76287 Rheinstetten, Germany

^d Liver Transplant Unit, Institute of Liver Studies, King's College Hospital, Denmark Hill,
London SE5 9RS UK

Table S1. The concentration (mean and standard deviation) of selected metabolites in human hepatic bile from 5 donor livers, based on ^1H NMR spectroscopy.

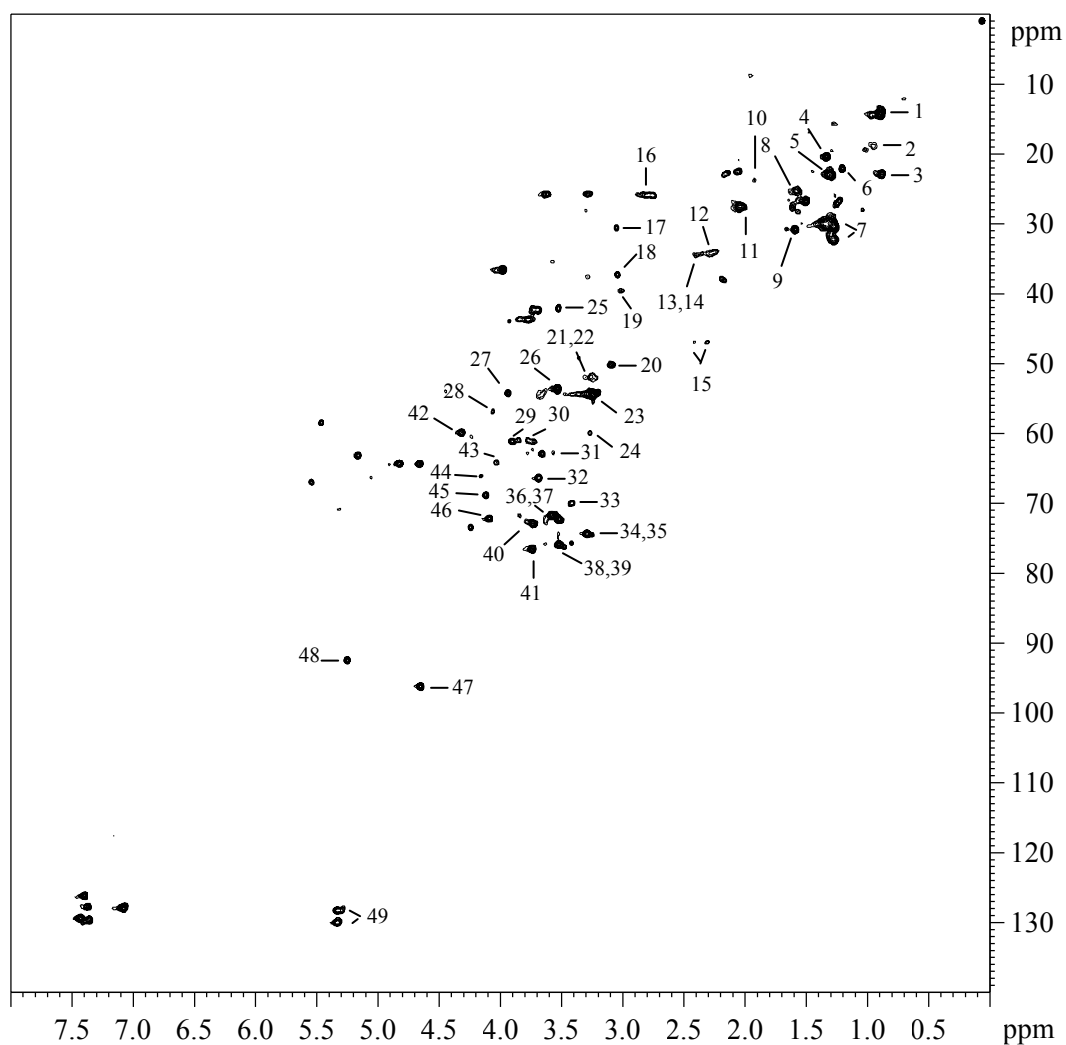
Metabolite	Mean concentration (mM)	Standard deviation
Alanine	1.51	1.02
Citrate	0.55	0.36
Creatine	0.25	0.10
Creatinine	0.07	0.03
Formate	0.04	0.04
Glucose	2.33	2.25
Glucuronate	2.72	0.64
Lactate	3.53	2.56
Succinate	0.20	0.07

Figure S1. Expansions of the 800 MHz ^1H - ^1H TOCSY NMR spectrum of human hepatic bile: (a) region between δ 0.2 – 6.0, (b) an expansion, with some assignments indicated. The cross peaks marked with full rectangles belong to glucuronate, whereas those in dashed rectangles arise from glucose; other compounds identified in this region are: 1- myo-inositol, 2- betaine, 3- choline, 4- phosphocholine, 5- choline in phosphatidylcholine (PTC), 6- glycerol.

Figure S2. 800 MHz ^1H - ^{13}C HSQC NMR spectrum of human hepatic bile. Assignment: 1- PTC f.a. CH_3 , 2- valine γCH_3 , 3- leucine δCH_3 + other, 4- lactate βCH_3 , 5- PTC f.a. CH_3 - CH_2 -(CH_2) $_n$, 6- β -hydroxybutyrate γCH_3 , 7- PTC f.a. (CH_2) $_n$, 8- PTC f.a. CH_2 - CH_2 -CO, 9- PTC f.a. CH_2 - CH_2 -CH=CH, 10- acetate βCH_3 , 11- PTC f.a. CH_2 - CH_2 -CH=CH, 12- PTC f.a. CH_2 - CH_2 -CO, 13- succinate $\alpha,\beta\text{CH}_2$, 14- α -ketoglutarate βCH_2 , 15- β -hydroxybutyrate α -CH, 16- PTC f.a. CH=CH- CH_2 -CH=CH, 17- creatinine N- CH_3 , 18- creatine N- CH_3 , 19- α -ketoglutarate γCH_2 + lysine ϵCH_2 , 20- conjugated taurine S- CH_2 , 21- choline N(CH_3) $_3$ + carnitine N(CH_3) $_3$, 22- betaine N(CH_3) $_3$, 23- choline N(CH_3) $_3$ in PTC, 24- trimethylamine oxide (TMAO) N(CH_3) $_3$, 25- glycine αCH , 26- choline βCH_2 , 27- creatine N- CH_2 , 28- creatinine N- CH_2 , 29- α -glucose C6H, 30- β -glucose C6H, 31- glycerol CH_2 , 32- choline βCH_2 in PTC, 33- α -glucose C4H + β -glucose C4H, 34- β -glucose C2H, 35- β -glucuronate C2H, 36- α -glucose C2H, 37- α -glucuronate C2H, 38- β -glucose C3H, C5H, 39- α -glucuronate C4H + β -glucuronate C3H, C4H, 40- α -glucose C3H + α -glucuronate C3H, 41- β -glucuronate C5H, 42- choline αCH_2 in PTC, 43- choline αCH_2 , 44- β -hydroxybutyrate βCH , 45- lactate αCH , 46- α -glucuronate C5H, 47- β -glucose C1H + β -glucuronate C1H, 48- α -glucose C1H + α -glucuronate C1H, 49- PTC f.a. CH=CH. f.a. = fatty acyl.

Figure S3. (a) Expansion of the 800 MHz ^1H - ^1H TOCSY NMR spectrum of the bile extract showing signals arising from correlations to protons H3 (δ 3.49 and δ 3.62), H7 (δ 3.88 and δ 3.89) and H12 (δ 4.05) of bile acids; (b) Expansion of the ^1H - ^{13}C HSQC spectrum (^1H at 800 MHz) of the bile extract. Assignment: 1- C18,H18 (B), 2- C18,H18 (A,C), 3-C21,H21 (A;C), 4- C21,H21 (B), 5- C19,H19 (A,B,C), 6- C15,H15 β (B,C), 7- C15,H15 β (A), 8- C1,H1 β (A,B,C), 9- C11,H11 β (B), 10- C7,H7 β (C), 11- C16,H16 β (A,B,C) + C6,H6 α (C), 12- C12,H12 α (B), 13- C17,H17 (B), 14- C11,H11 β (B), 15- C7,H7 α (C), 16- C2,H2 α (A, B, C), 17- C22,H22 (A, B, C), 18- C20,H20 (A,B,C) + C8,H8 (C), 19- C4,H4 β (C), 20- C5,H5 (A,B), 21- C5,H5 (C), 22- C14,H14 (B), 23- C15,H15 α (B,C), 24- C15,H15 α (A), 25- C11,H11 (A), 26- C11,H11 (C), 27- C2,H2 β (A,B,C), 28- C6,H6 α (A,B), 29- C8,H8 (B), 30- C8,H8 (A), 31- C4,H4 β (A,B), 32- C14,H14 (C), 33- C6,H6 β (C), 34- C16,H16 α (A,B,C), 35- C9,H9 (A), 36- C9,H9 (B,C), 37- C6,H6 β (A,B), 38- C1,H1 α (A,B,C) + C4,H4 α (C), 39- C4,H4 α (A,B), 40- C12,H12 β (B), 41- C14,H14 (A), 42- C17,H17 (A,C), 43- C23,H23 (A,B,C), 44- N-CH₂ conj. taurine, 45- CH₂ conj. glycine, 46- CH₃ methanol, 47- S-CH₂ conj. taurine, 48- N(CH₃)₃ choline, 49- N(CH₃)₃ choline in PTC, 50- C7,H7 (A,B), 51- C3,H3 (C), 52- C3,H3 (A,B), 53- C12,H12 (A,C); A- cholic acid (CA) conjugates, B- chenodeoxycholic acid (CDCA) conjugates, C- deoxycholic acid (DCA) conjugates; Carbon and proton numbers are as shown in Figure 5.

Supplementary Figure S2



Supplementary Figure S3

