Precision high throughput proton NMR spectroscopy of human urine, serum and plasma for large-scale metabolic phenotyping

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SUPPLEMENTARY MATERIAL

S1 Reagents

KH_2PO_4, Potassium dihydrogen phosphate, 99.99% ACS, anhydrous (Sigma-Aldrich 229806); KOH, Potassium hydroxide, ≥85% KOH (Sigma-Aldrich P1767); Na_2HPO_4, Disodium hydrogen phosphate, 99% anhydrous, (Sigma-Aldrich W239901); D_2O,Deuterium oxide, 99 atom % D (Sigma-Aldrich 435767); NaN_3, Sodium azide, 99.5% (Sigma-Aldrich S2002) [Caution: sodium azide is highly toxic and highly reactive under certain conditions]; TSP, 3-trimethyl-silyl-[2,2,3,3-$^2H_4$]propionic acid, sodium salt 98 atom % D (Sigma-Aldrich 269913). Note: DSS, 4,4-dimethyl-4-silapentane-1-sulfonic acid, can be used as an alternative to TSP and is often considered the ideal standard for aqueous samples. However it gives rise to additional NMR peaks that can obscure part of the complex spectrum.

S2 Equipment

Operation at 600 MHz ^1H observation is recommended but is not mandatory. Typically a 600 MHz Avance III NMR spectrometer (Bruker Biospin Ltd.) or similar. Instruments form other manufacturers are available but all the results shown in this document and the standardisation required for the Phenome Centres have been developed and tested on Bruker spectrometers. Higher field systems are likely to become popular in future work, however comparing spectra at varying field strengths should be approached with caution as dispersion of chemical shifts is different so that peak overlap and the second order nature of some spin systems can be altered. One possible way to circumvent this is to compare quantified metabolites rather than spectral peaks.

The following items of hardware are used - NMR detector - BBI 600 MHz 5-mm Z gradient probe and automated tuning and matching (ATMA unit) (Bruker Biospin Ltd.) or similar; SampleJet (i.e. refrigerated sample storage prior to data acquisition) with a cooling rack and heating station; cooling by BCU of the probe and the SampleJet system. The probe must also be heated. This should produce temperature stability of the order of 0.01 °C; Liquid handler 215 sample preparation robot with Sample Track or similar; Topspin 3.2 software with Icon NMR (Bruker Biospin Ltd.); microplate 96 square well 2mL (Fisher Scientific UK Ltd Part No. FB58005); 96 NMR 5 mm tube SampleJet boxes

(Bruker Biospin Ltd. Part No. Z105684); POM balls to seal sample tube caps (Bruker Biospin Ltd. Part No. Z72497)

S3 Reagent preparation

It should be noted that volumes can be changed but the relative proportions of the reagents must be maintained and reagents must be of analytical grade.

Buffer for urine samples: Prepare 100 mL of 1.5 M KH_2PO_4 buffer by dissolving 20.4 g of the reagent in 80 mL of D_2O. Dissolve 100 mg of TSP and 13 mg of NaN_3 in 6 to 10 mL of D_2O. Mix both solutions very well using sonication. The solution might appear cloudy; this cloudiness should disappear when the pH is adjusted. Adjust the pH to 7.4 by adding KOH pellets. Transfer the solution to a 100 mL volumetric flask and adjust the volume with D_2O. Shake thoroughly to mix completely, and recheck the pH. This volume of buffer is enough to prepare 1500 samples.

Buffer for plasma and serum samples: Prepare 500 mL of 0.075 M NaH_2PO_4 buffer by dissolving 5.32 g of NaH_2PO_4 in 380 mL of water. Adjust masses when using hydrated salts. Add 0.4 g of TSP and shake until the powder is dissolved. Add 5 mL of 4% NaN_3 aqueous solution and shake. Add 100 mL of D_2O. Adjust the pH to 7.4 by adding 1M HCl/NaOH solutions. Transfer to a 500 mL volumetric flask and fill up to 500 mL with water. Shake thoroughly to mix completely, and recheck the pH. This volume of buffer is enough to prepare 1500 samples.

S4 Gilson robot preparative protocols

Gilson robot protocols, like any protocol, should be tested for a specific system. Calibration is required to ensure the volumes aspirated, dispensed, the depth of the needle, and introduction of push buffer are understood and accurately measured. Our protocols were developed to minimize loss of accuracy within the time requirements. The methods followed the following steps for both urine and plasma/serum.

1) Wash needle by purging push buffer (pure water).
2) Aspirate gas gap
3) Aspirate buffer (60uL – urine, 300uL – plasma/serum)

4) Dispense buffer in NMR tube.

5) Aspirate gas gap

6) Aspirate sample (540 uL – urine, 300uL – plasma/serum)

7) Dispense sample in NMR tube.

8) Return to step 1.

Following preparation of an entire rack the tubes are sealed and manually mixed to ensure homogeneity. Samples are then set up for analysis.

S5 NMR experimental set-up

All the automated routines and the parameter sets are provided (§ S9).

***A) Temperature calibration*:** A 99.8% deuterated methanol (MeOD) standard sample in a sealed 5 mm NMR tube is used to calibrate the temperature before each run of samples. The purpose of the temperature calibration is to ensure that urine samples are run at exactly 300 K and plasma/serum samples at exactly 310 K [1]. This is particularly important for plasma/serum as the large protein, lipid and lipoprotein molecular aggregates and their binding properties to small molecules vary significantly with temperature, and so biological conditions are used. The MeOD NMR tube is inserted into the magnet manually and allowed time (~ 5 min) to equilibrate. Following this, the probe is automatically tuned and matched and locked to deuterated methanol and automatically shimmed using a tuning routine after the regular shimming. A standard 90° proton parameter set is used to run an experiment with 2 scans using a pulse length of 1 µs. The short pulse length and highly deuterated methanol ensures the sample is not affected by radiation dampening. When the experiment is processed without any line broadening the methanol peak at 3.33 ppm (from CD_2H-) should have a distinct 1:2:3:2:1 multiplicity due to H-D spin coupling and be symmetrical. Optimization of the magnet shims may be necessary through manual or automatic forms of shimming to achieve these results. Once achieved, the experiment is processed with a line broadening of 3.0 Hz and the real probe temperature calculated by measuring the distance in Hz

between the two methanol peaks, the CH_3 and OH resonances, and by referring to a calibration curve supplied by the instrument manufacturer [2]. If required the target temperature of the probe is adjusted and the procedure repeated until the actual calculated temperature of the sample is 300 ± 0.05 K. The temperature is recorded and later stored in the relevant parameter set for running under automation. The temperature for a urine sample set should be adjusted to 300 K (corresponding to a chemical shift difference in 1.526 ppm) while the temperature for a plasma/serum sample set should be adjusted for a real temperature of 310 K (corresponding to a shift difference of 1.428 ppm).

***B) Water suppression*:** A standard 2 mM sucrose sample (containing 0.5 mM TSP, 2 mM NaN_3 in 90% H_2O:10% D_2O) is loaded into the magnet and is used to check the performance of the water suppression functionality. At this stage it is also worth running a gradient profile of the probe to check its integrity. Firstly, the centre frequency (O1) is optimized by using a 1D NMR experiment with presaturation, a long relaxation delay and 1 scan. Subsequently, the water suppression performance is evaluated by acquiring a full cycle (8 scans) experiment and relatively long delays (~ 10 s). The signal-to-noise value must be higher than 300 (as measured on the anomeric proton of sucrose), the resolution better than 15 % (a measure of the height of the minimum of the anomeric proton as a percentage of the entire peaks signal) and the water hump not bigger than 40/80 Hz (as measured at 100 % and 50 % of the TSP signal intensity respectively).

i) The standard proton presaturation parameter set is read once the true temperature has been set to either 300 K (urine) or 310 K (plasma) and allowed to equilibrate. Tuning and matching will be adjusted in automation, the sample will be locked to 90% H_2O+10% D_2O (*lock*), and shimmed using the automated routine.

ii) The optimal 90° pulse is calculated for the sample using the automated routine. The experiment is run and the data processed with optimization if necessary of the magnetic field homogeneity, done by checking that the line width at half height of the TSP peak is satisfactory (< 0.7 Hz with no line broadening). Furthermore the pulse frequency is optimized by phasing the TSP signal and changing

the pulse frequency value until the rest of the spectrum, including the residual water peak can be phased.

iii) The 90° length is recorded, and this and the saturation frequency and power (presaturation power corresponding to a 25 Hz field) and the rest of the acquisition parameters are read into a new experimental file. The experiment is run and processed using a multiple of 8 scans. At this stage it must be ensured that the water saturation is optimal (the residual water peak should be in phase and provide much less signal than the 2 mM TSP peak) and the line width at half height of the TSP should be less than 0.7 Hz with no line broadening. No first order phase correction should be necessary at this stage.

v) The efficiency of the water suppression is estimated by reprocessing the spectrum using 24.1K points and using the *suppcal* command. This command calculates the water suppression hump which should be smaller than 80 Hz across; the resolution of the spectrum (better than 15%) and the signal to noise value (above 300) as described earlier. If these values are not fulfilled, it is necessary to recalibrate at this stage.

***C) External reference for quantitation*:** Quantification based on reference to a synthetic signal in NMR data has been available for many years. Namely, the ERETIC method (Electronic Reference To access In vivo Concentrations) provides a reference signal, synthesized by an electronic device, which can be used for the determination of absolute concentrations [3]. Lately methodologies have been developing to allow even metabolites in complex biofluid matrices to be quantified by these methods. It is therefore very useful to run a calibration monthly (or before each sample set) on a sample of quantified, resolved signals for quantification reference. A good quantification reference sample consists of 20 standard metabolites commonly found in biofluids which are known to be stable over the long term and as a mixture. Ideally a universal reference is run across all systems enabling the comparison of all instrumentation used. A 1D NOESY experiment using the same parameters used for the samples should be acquired on the reference, and care taken during

deconvolution for quantification of resonances as biofluids are complex and signals often unresolved.

i) The standard presaturation parameter set is read (the same as that used during sucrose set up) and the temperature is allowed to equilibrate (300 K for urine or 310 K for plasma/serum).

ii) The pulse sequence is changed to a standard presaturation sequence, the number of transients to 1, the dummy scans and the line broadening are removed. This set up will be used to optimize the presaturation frequency.

iii) Tuning and matching will be adjusted in automation, the sample will be locked to the solvent for urine or plasma/serum samples (Note: solvent locking parameters can be optimized for particular biofluids), and shimmed using the automated routine.

iv) The 90° pulse length is calculated along with the presaturation frequency and power as described for the sucrose samples (B) steps ii and iii).

v) The data set is updated with the parameters optimized in iv).

vi) The data are acquired using the automation routine intended for use during biofluid analysis. Basically this automation should read in an optimal parameter set, allow the temperature to reach equilibrium, automatically tune, match and shim on the sample and run an experiment with automatically optimized pulses.

vii) The spectrum is using the automation routine intended for use during biofluid analysis. This routine should read in the optimal processing parameters and automatically Fourier transform, phase, calibrate and baseline the data. The spectrum should be checked for quality (as outlined for urine in Figure S1, and for plasma/serum Figure S2).

D) Urine Sample Setup*:* One of the composite QC samples is used to do the set up. The SampleJet mode is changed to the 5 mm shuttle automation mode and the QC sample is introduced. A wait of 5 minutes to equilibrate the temperature (urine samples are run at 300 K) is required.

i) The previously used experimental parameters for urine are then loaded (§ S9).

ii) The spectrometer is tuned and matched by using the automation routine, the spectrum will be locked to the solvent, optimized for urine and shimmed using the automated routine.

iii) The presaturation frequency is optimized by following the steps ii, iv from section C.

iv) The experiment is run by updating the parameter set with the optimized parameters and using the automation routine (vi in Section C).

v) The spectrum is processed using the automation routine and the quality is checked (vii in Section C).

vi) The NOESY parameter file to be used for urine with the optimized acquisition parameters and the calibrated temperature is updated in preparation for the automation.

vii) The run parameter sets for automation are updated and the urine sample set experiments are then set up in automation. In addition to the 1D NOESY-presat experiment, a 2D *J*-resolved experiment will be also run using the parameters given. Both these experiments can be performed with a time of change-over between samples of 15 min per spectrometer.

viii) An experiment quality control exercise is built into the procedure. The width at half height of the TSP signal should be recorded for each sample acquisition. This should be ≤ 1.0 Hz after being processed with a line broadening of 0.3 Hz. It is occasionally impossible to obtain this value if the sample derives from subjects with certain specific conditions that cause high ion content or increased protein concentration in their urine. This may also be the case if, due to drug intake, the urine contains xenobiotics and their metabolites at high concentrations. Furthermore, the residual

water signal should be less intense compared to other metabolite signals (H_2O should in most cases not be the largest signal) and it should not affect the spectral baseline outside of 4.7 – 4.9 ppm. The zero-order phase should not need to be adjusted by more than 0.2°. Finally when a residual is taken in the baseline, there should be no trend in the residual above 10 ppm and below -0.5 ppm. If for any reason a sample spectrum does not fulfil these criteria then the sample must be either rerun or excluded from the data set analysis. Patient pathological conditions may often be connected to the samples which do not pass the analytical criteria. Often factors that make a sample difficult to analyse by NMR (ie. high protein content in urine) can be inherent to a pathological condition; these conditions are often not the best explored by NMR analysis and so researchers should consider another analytical platform.

***E) Plasma Sample Setup*:** One of the QC samples is used to do the set up. The SampleJet mode is changed to the 5mm shuttle automation mode and the QC sample introduced. Enough time should be allowed to equilibrate the temperature (plasma/serum samples are run at 310 K). This process can begin outside the magnet reducing the experimental time.

i) The previously used experiment parameters for plasma/serum are loaded (§ S9).

ii) The spectrometer is tuned and matched by using the automation routine, and the spectrum will be locked to the solvent optimised for plasma and shimmed using the automated routine.

iii) The presaturation frequency is optimized by following the steps ii, iv from section C.

iv) The experiment is run by updating the parameter set with the optimized parameters and using the automation routine (vi in Section C).

v) The spectrum is processed using the automation routine and the quality is checked (see Quality Assurance and Figure S2).

vi) The parameter file used for plasma/serum is updated with the optimized pulse lengths, pulse frequencies and the calibrated temperature in preparation for the automation.

vii) The run parameters in the automation procedures are updated and the plasma/serum samples are set up to run under automation. In addition to the 1D NOESY-presat and the 2D *J*-resolved experiments, the 1D CPMG experiment will be also run in order to attenuate the resonances belonging to macromolecules. A fourth experiment, a 1D diffusion-edited spectrum, can also be run at the discretion of the spectroscopist. If only the 1D NOESY-presat, CPMG and J-resolved experiments are run, the time between sample change-over should be around 19 min for plasma/serum samples

viii) Experiment Quality Control: Unlike urine, the internal standard (TSP) is affected by binding to large molecules such as endogenous proteins. The effect of this is that the signal is broadened. Consequently a signal from a small metabolite such as the H1 proton of α-glucose which is common-place in plasma samples can be used to monitor the quality and consistency of the magnetic field homogeneity (See Quality Assurance). Similarly to urine (apart from the resonance shift due to the temperature difference) the residual water peak should not affect the baseline outside the range 4.6 - 4.8 ppm.

S6 NMR spectroscopic methods

All the parameter sets and sequences given here are in the Bruker format but they can be formatted to be compatible with other types of NMR spectrometers from other instrument manufacturers. In the manual parameter optimization procedure using a urine and a plasma test sample, respectively, power levels for the hard pulse should be selected such that 'hard pulse lengths' P1 are P1 in plasma ~10 µs and P1 in urine ~13 µs. Having specified those pulse lengths, the power levels for hard pulses are in the order of ~11 dB under the experimental conditions and probe specification used in our laboratory. The respective power level needs to be stored in the PROSOL table, and used in the pulse

optimization procedure PULSECAL optimizing the individual pulses for each sample in automation.

***Noesy-presat sequence*:** The ^{1}H NMR spectra are measured using a specified water suppression pulse sequence such as NOESY-presat, which employs the first increment of a NOESY pulse sequence (Bruker terminology - noesygppr1d) with continuous wave irradiation at the water resonance frequency using 25 Hz RF strength during the relaxation delay and also during the mixing time. The sequence has the form –RD – $g_{z,1}$ – 90° – t – 90° – t_m – $g_{z,2}$ – 90° – ACQ, where RD is the relaxation delay (4 s), t is a short delay typically of about 3 µs, 90° represents a 90° RF pulse, t_m is the mixing time (10 ms), $g_{z,1}$ and $g_{z,2}$ are magnetic field z-gradients both applied for 1 ms, and ACQ is the data acquisition period (2.7 s). Application of the gradients ensures that dispersive residual water signals are filtered out and do not contribute to the final spectrum. The spectral window is set to 20 ppm for urine and 30 ppm for plasma/serum. In total 32 transients are acquired with 64k data points for urine or 96k data points for plasma/serum. The receiver gain is set to 90.5 for all of the experiments (§ S9).

***J-resolved sequence*:** The Bruker *J*-resolved pulse sequence (jresgpprqf) takes the form –RD-90°-t_1-180°-t_1-ACQ, where t_1 is an incremented time period, RD is the relaxation delay (2 s), t_1 is the increment delay, 90° represents a 90° RF pulse while 180° is the 180° RF pulse, ACQ is the data acquisition period [4,5]. The spectral window is set to 16.6 ppm for the direct dimension and 78 Hz for the indirect dimension. Two scans are acquired over 40 increments in the indirect dimension. Continuous wave irradiation is applied at the water resonance frequency using 25 Hz RF strength during the relaxation delay RD.

***Carr-Purcell-Meiboom-Gill (CPMG) spin-echo sequence with presaturation*:** A spin-echo spectrum using the CPMG sequence should also be acquired for each plasma/serum sample. CPMG sequences attenuate peaks from molecules with slow rotational correlation times such as the lipids and proteins present in in the biofluids which otherwise give rise to many broad, interfering resonances. The CPMG pulse sequence (cpmgpr1d) [6,7] has the form –RD-90°-(t-180°-t)$_n$-ACQ, where the

definitions are as above, plus t is the spin-echo delay and n represents the number of loops. The acquisition parameters are set up in the same way as the 1D NOESY-pre-sat, the spin-echo delay is set at 0.3 ms and a minimum of 128 loops. Continuous wave irradiation is applied at the water resonance frequency using 25 Hz RF strength during the relaxation delay RD.

***Diffusion-edited sequence*:** A diffusion-edited pulse sequence is often run on plasma or serum samples as, contrasting to a CPMG sequence, it attenuates peaks from the fast diffusing (i.e. generally low molecular weight) molecules and only acquires signals from protons attached to slower moving proteins and larger lipids. Application of this technique has been successful in the past linking lipoprotein sub-fractions at a metabolic level with genetic associations [8,9].

Diffusion-edited spectra are acquired using a pulse sequence (ledbpgppr2s1d) [10] with bipolar gradients and the LED scheme that has the form -RD-90°-G_1-180°-G_1-90°-G_2-T-90°-G_1-180°-G_1-90°-G_2-t-90°-acquire FID, where, in addition to the previous definitions, G_1 is a pulsed-field gradient that is applied to allow editing, and G_2 is a spoil gradient applied to remove unwanted magnetization components. The diffusion delay D is the time during which the molecules are allowed to diffuse (0.12 s); this is the period (90°-G_1-180°-G_1-90°-G_2-T-); and t is a delay to allow the longitudinal eddy currents that arise in the NMR probe metal body by the field gradient switching to decay (5 ms). Continuous wave irradiation is applied at the water resonance frequency using 25 Hz RF strength during the relaxation delay RD.

S7 Spectrum processing methods

***One Dimensional Experiment (NOESY, CPMG and Diffusion-edited)*:** After each spectral acquisition the data is processed automatically. The line broadening is set to 0.3 Hz, a zero-filling by a factor of 2 is used to produce 132K data points for processing, and finally the first-order phase correction is set to 0.0. The data is then automatically phased using only zero-order phase correction, the TSP is calibrated to 0.0 Hz (0.0 ppm) and the spectral reference recorded to be called into the next dataset

acquisition. After processing, the spectrum should fulfil the entire criteria outlined in Quality Assurance (Figure S1 and S2).

***J-resolved experiment*:** Each dataset is processed automatically after data acquisition. Zero-filling by a factor of two is included in the F2 dimension and the digital resolution is increased to 256 points in F1 by zero-filling. The limits for spectral processing are set to ± 20 Hz. Finally the data is Fourier transformed, tilted, symmetrized around the central horizontal (F2) axis and finally automatically baseline corrected in the F2 dimension. Finally the TSP signal is calibrated to 0.0 ppm in the F2 dimension and to 0.0 Hz in the F1 dimension

S8 Quality Assurance

Whilst the automation is running, certain aspects of the run must be monitored to ensure the data are being acquired in a consistently high quality manner. Any spectrum run under automation that does not fit the criteria described below should be either rerun immediately or alternately removed from a dataset before biological biomarker discovery can proceed.

***Line width*:** The homogeneity of the magnetic field during urine spectrum acquisition should be assessed using the TSP resonance. The resulting signal should be symmetrical and at half height the line width should be < 1 Hz after a line broadening of 0.3 Hz has been applied to the data. In plasma/serum, as TSP binds to endogenous proteins, the central peaks from the quadruplet of unbound lactate at 4.13 ppm can be used to assess the field homogeneity. It should be symmetrical (being careful not to confuse overlapping threonine or other signals for asymmetries) and no more than 1.15 Hz at half height. Furthermore the doublet resonance of α-glucose at 5.23 ppm should be resolved to the baseline (or to the overlapping lipid resonance). This resonance of α-glucose at 5.23 ppm is then used to calibrate all peaks for small molecule analysis.

***Baseline*:** The spectral noise (from a line through zero relative intensity) should have no residual trend outside the -0.5 – 10 ppm range for urine and outside the -5 – 15 ppm range for plasma/serum.

***Water Peak Saturation*:** The residual water resonances should have an area consistent with a concentration less than 10 mmol (pure water is 110M in hydrogen). A urine NMR spectrum should not be affected by the residual water resonance outside of the range 4.7 – 4.9 ppm. As plasma/serum is run at 310 K the residual water resonance shifts to a higher ppm value but this should not affect the resulting spectrum outside of 4.6 – 4.8 ppm.

***Phase Error*:** Checking the zero-order phase manually (after automatic processing) around the TSP signal, there should be less than a 0.2° distortion in the value if correcting by eye. A first-order phase correction should not be necessary (set to 0.0°).

***Receiver Gain*:** Baseline artefact problems (giving 'sinc wiggles' in the final spectrum) can occur when the sample concentrations are too large because the analog-digital converter in the detection process is overloaded. For consistency, it is preferred that the receiver gain is not adjusted during a run of samples, but if overload does occur, then the affected spectra should be discarded and the samples should be re-run with a readjusted receiver gain value.

S9 NMR spectrometer pulse sequences and parameter lists

The experimental parameters are optimized on the Bruker Biospin 600 MHz magnets, and outlined here using Bruker terminology. Other spectrometer manufacturers provide equivalent pulse sequences. They however have very different names and syntax and should be dealt with accordingly.

Experimental Parameters Urine

Pulse program	noesygppr1d	
Time domain	65536	
Dummy scans	4	
Scans	32	
Sweep width	20 ppm	
Acquisition time	2.726 s	
Relaxation delay	4 s	
Receiver gain	90.5	
Dwell time	41.6 µs	
Mixing time	0.01 s	
Line broadening	0.3 Hz	

Pulse program	jresgpprqf	
Time domain	8192 in F2	40 in F1
Dummy scans	16	
Scans	2	
Sweep width	16.7 ppm in F2	0.13 in F1
Acquisition time	0.41 s in F2	0.26 s in F1
Relaxation delay	2 s	
Receiver gain	90.5	
Dwell time	50 µs	
Line broadening	0.3 Hz in F2	0.3 Hz in F1

Experimental Parameters Plasma

Pulse program	noesygppr1d
Time domain	98304
Dummy scans	4
Scans	32
Sweep width	30 ppm
Acquisition time	2.726 s
Relaxation delay	4 s
Receiver gain	90.5
Dwell time	27.7 µs
Mixing time	0.01 s
Line broadening	0.3 Hz

Pulse program	cpmgpr1d
Time domain	73728
Dummy scans	4
Scans	32
Sweep width	20 ppm
Acquisition time	3.067 s
Relaxation delay	4 s
Receiver gain	90.5
Dwell time	41.6 µs
Mixing time	0.01 s
Line broadening	0.3 Hz
Number of loops	128

Pulse program	jresgpprqf	
Time domain	8192 in F2	40 in F1
Dummy scans	16	
Scans	2	
Sweep width	16.7 ppm in F2	0.13 in F1
Acquisition time	0.41 s in F2	0.26 s in F1
Relaxation delay	2 s	
Receiver gain	90.5	
Dwell time	50 µs	
Line broadening	1.0 Hz in F2	0.3 Hz in F1

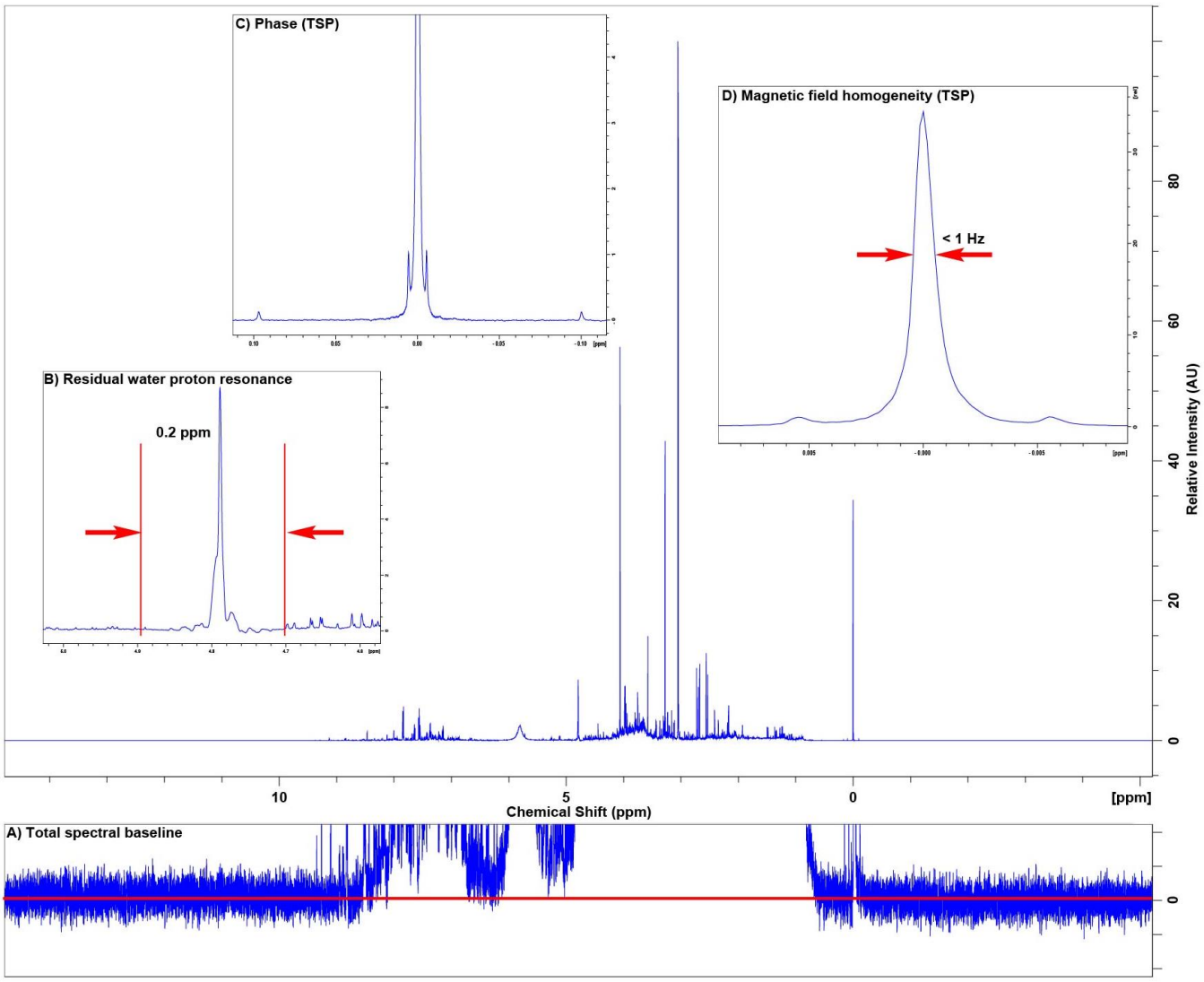

Figure S1. Representative ^{1}H NMR spectra of human urine (using a noesygppr1d pulse sequence as described in the text); quality assurance of the analytical quality of spectra is determined by A) zero trend in the baseline residuals (in the region < -0.5 ppm and > 10 ppm, B) the residual water resonance only affects the region between 4.7 ppm and 4.9 ppm, C) no first order phase correction is necessary to produce a symmetrical TSP resonance, D) the line width at half height of the TSP peak set at 0.0 ppm is < 0.7 Hz , resulting in a final line width of < 1.0 Hz after application of the recommended 0.3 Hz line broadening function to the time domain free induction decay.

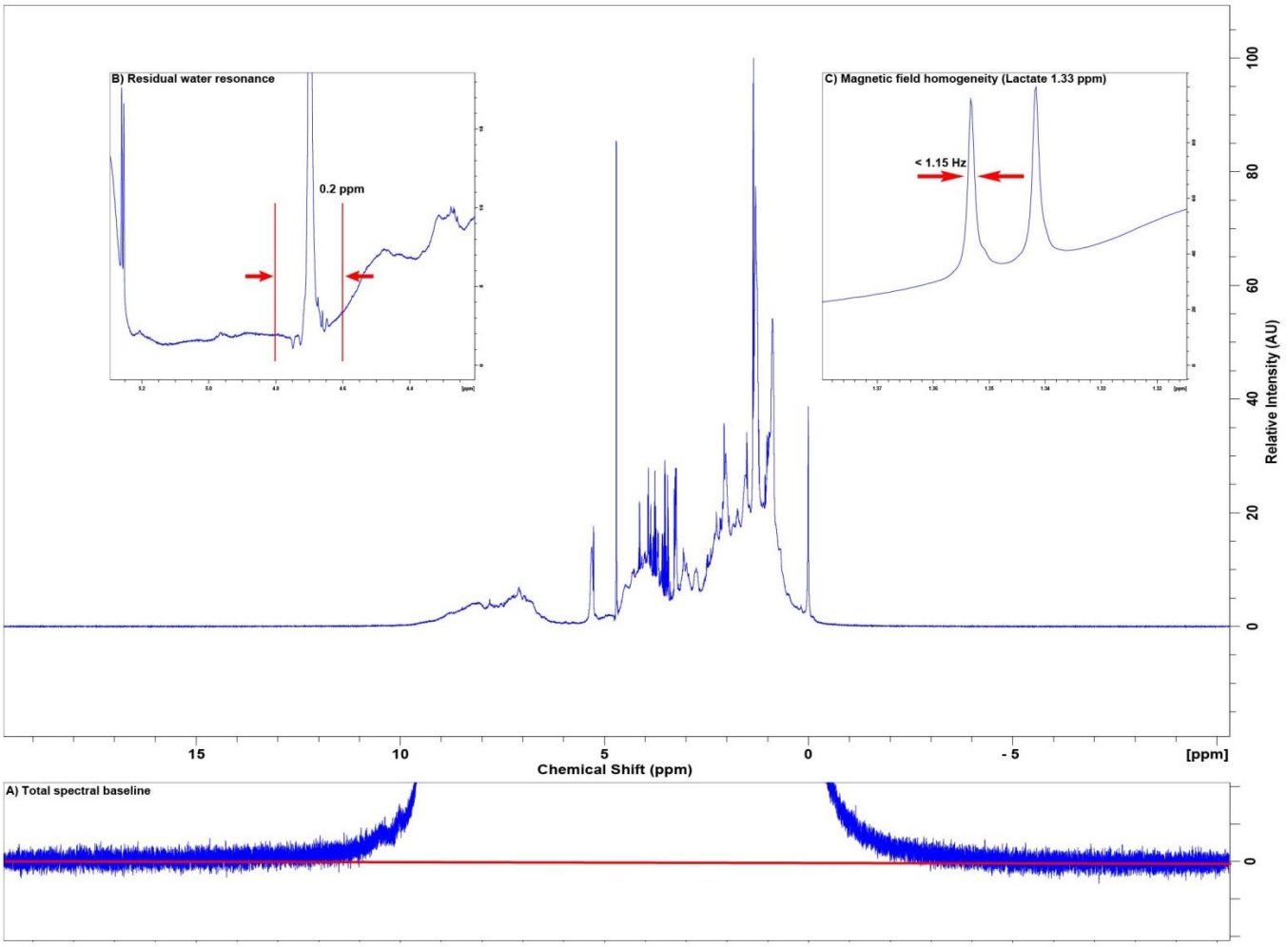

Figure S2. 1D ^{1}H NMR representative spectra of human plasma (using a noesygppr1d pulse sequence and the parameters described above); quality assurance of the analytical quality is during automation is determined by A) zero trend in the baseline residuals (in the region < -5 ppm and > 15 ppm, B) the residual water resonance only effects the region between 4.6 ppm and 4.8 ppm, C) the line width at half height is < 1.15 Hz on one of the central lactate resonances at 1.35 ppm.

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