Supplemental Information

Quantitating Metabolites in Protein Precipitated Serum Using NMR Spectroscopy

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This file contains 8 pages S1 to S8. The first page is the title page; page S2 contains a detailed protocol for the synthesis of the smart isotope tag - ^{15}N-Cholamine and pages S3 to S8 contain six Supplemental Figures (Figure S1 to Figure S6).

Synthesis of the smart isotope tag - ^{15}N-Cholamine. The smart isotope tag, ^{15}N-Cholamine, was synthesized using the two-step reaction following the protocol published recently from our laboratory (Tayyari, 2013). Briefly, in the first step, ^{15}N substituted phthalimide was synthesized by mixing (2-bromoethyl)trimethylammonium bromide with ^{15}N-phthalimide potassium in a 250 mL round bottom flask and dry DMF (100 mL) was added under nitrogen gas. The mixture was then refluxed at 100 °C with stirring for 12 h. The supernatant from the reaction mixture was separated, dried using a rotary evaporator and the resulting residue was washed using acetonitrile and acetone solvents to obtain the pure ^{15}N-substituted phthalimide. In the second step, ^{15}N-substituted phthalimide was converted to ^{15}N-cholamine by the alkali and acid hydrolysis, respectively. For the alkali hydrolysis, 1 N NaOH was added to the aqueous solution of ^{15}N-substituted phthalimide and the mixture was kept stirring for 30 min. For acid hydrolysis, the resulting mixture was mixed with 12 N HCl, the temperature was raised to 70°C and kept stirring for 12 h. Finally, the mixture was dried using a rotary evaporator and the resulting residue was washed with acetonitrile and a 25:75 mixture of water/acetone to yield the pure product, ^{15}N-cholamine.

Reference

Tayyari F, Gowda GA, Gu H, Raftery D. ^{15}N-Cholamine-A Smart Isotope Tag for Combining NMR- and MS-Based Metabolite Profiling. Anal Chem. 85(18):8715-21 (2013).

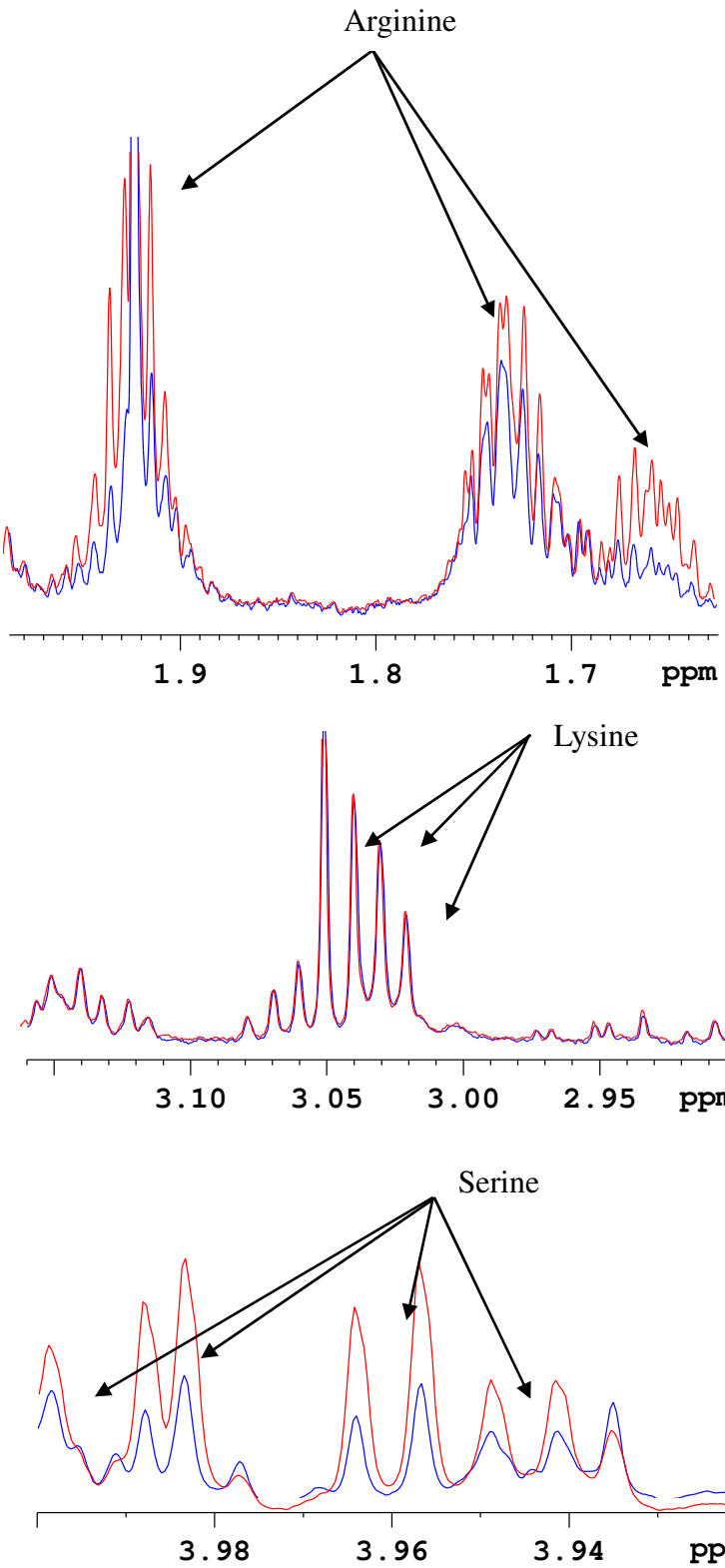

Figure S1: Portions of the protein precipitated serum spectra before (blue) and after (red, higher intensity) spiking with authentic compounds to distinguish metabolites in the overlapping regions for quantitation using Chenomx software: (a) and (b) spectra spiked with arginine; spectra in (b) indicate that lysine peaks, which do not alter by spiking with arginine, do not overlap with arginine, which helped distinguishing lysine from arginine; (c) spectra spiked with serine.

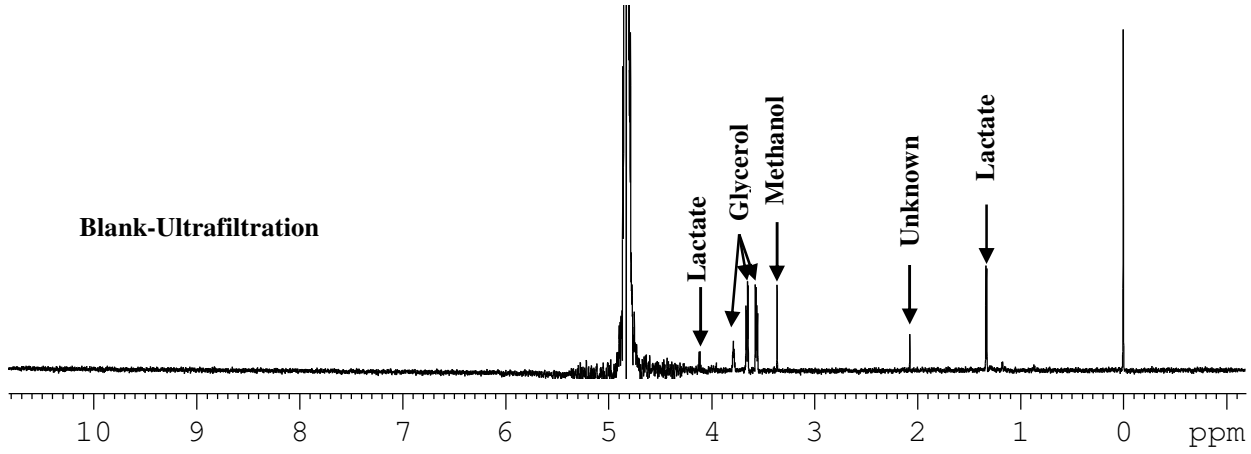

Figure S2: A typical ^{1}H 1D NMR spectrum of a blank (deionized water) sample obtained by ultrafiltration under identical conditions as for the serum samples. Lactate, glycerol, methanol and an unidentified peak are seen as the predominant contaminants from the filter membrane.

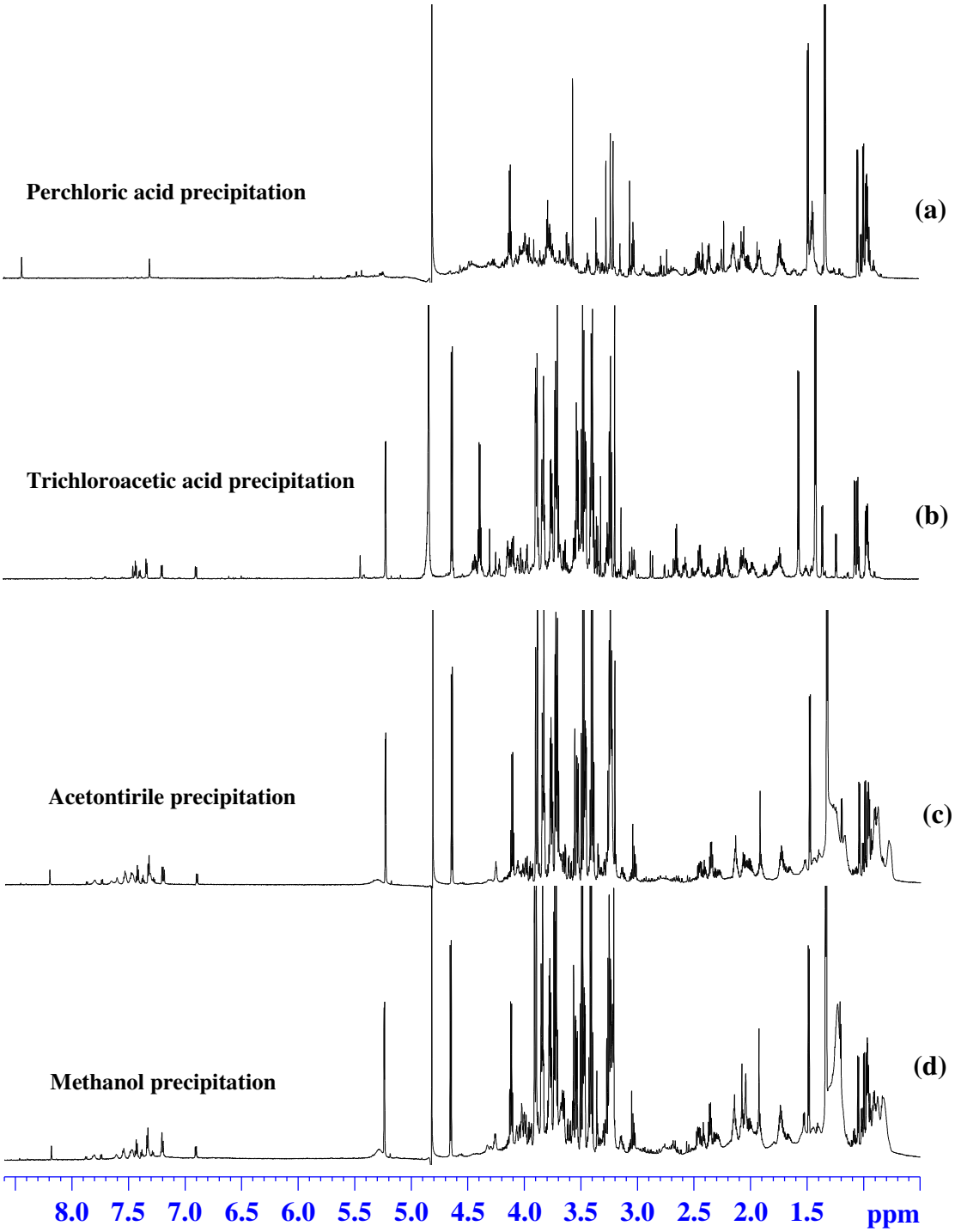

Figure S3: ^{1}H 1D NMR spectra of the same serum sample obtained using ZGPR sequence after protein precipitation using (a) perchloric acid (PCA); (b) trichloroacetic acid (TCA); (c) acetonitrile and (d) methanol. While both methanol and acetonitrile show residual macromolecular signals, which hinder metabolite quantitation using ZGPR or 1D NOESY sequence, TCA and PCA deleteriously affect the integrity of serum metabolite profile.

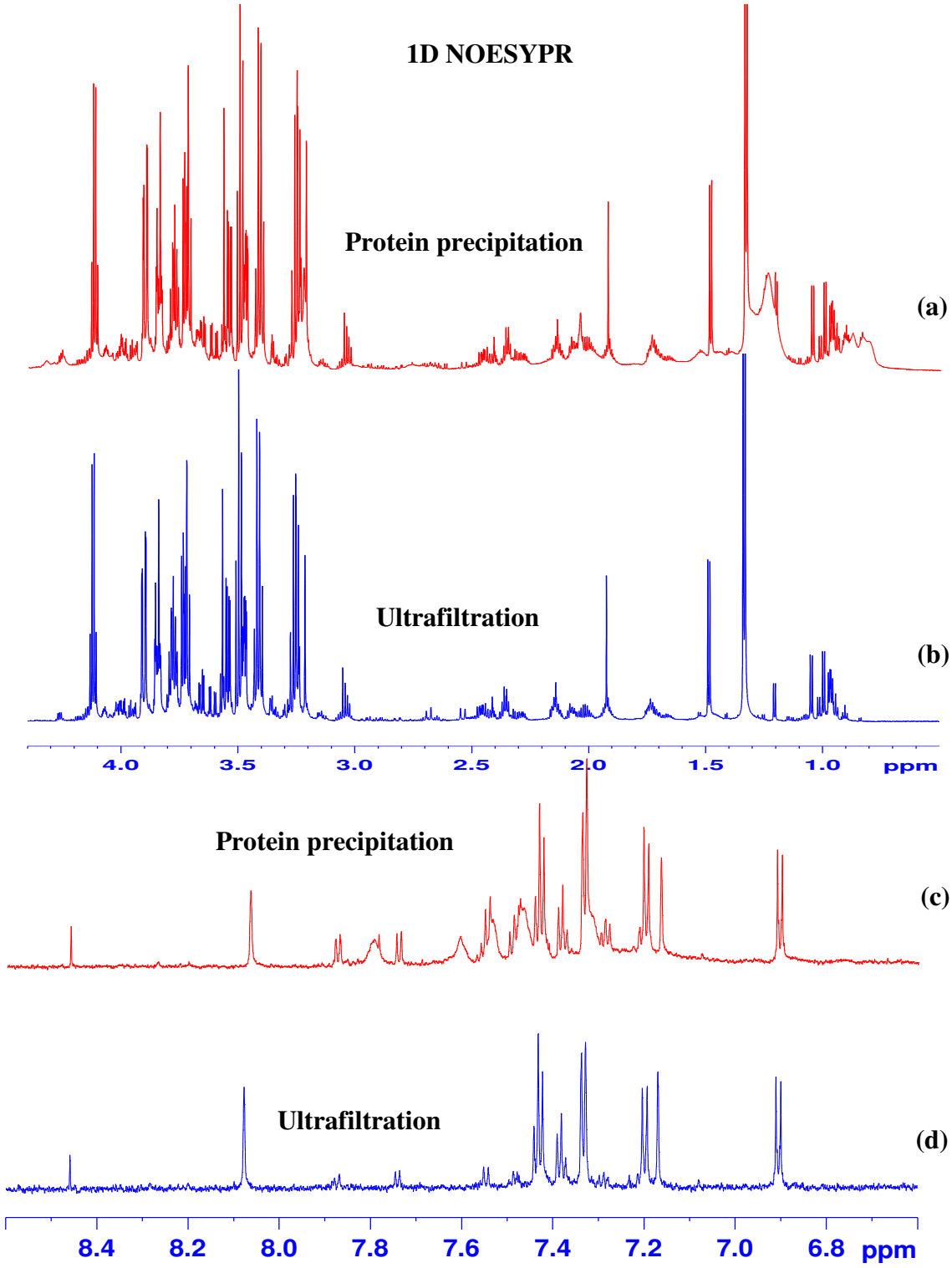

Figure S4: Typical 1D NMR spectra obtained using 1D NOESYPR sequence highlighting that residual macromolecule signals are present in the protein precipitated serum using methanol (a and c) and are absent in ultrafiltered serum (b and d).

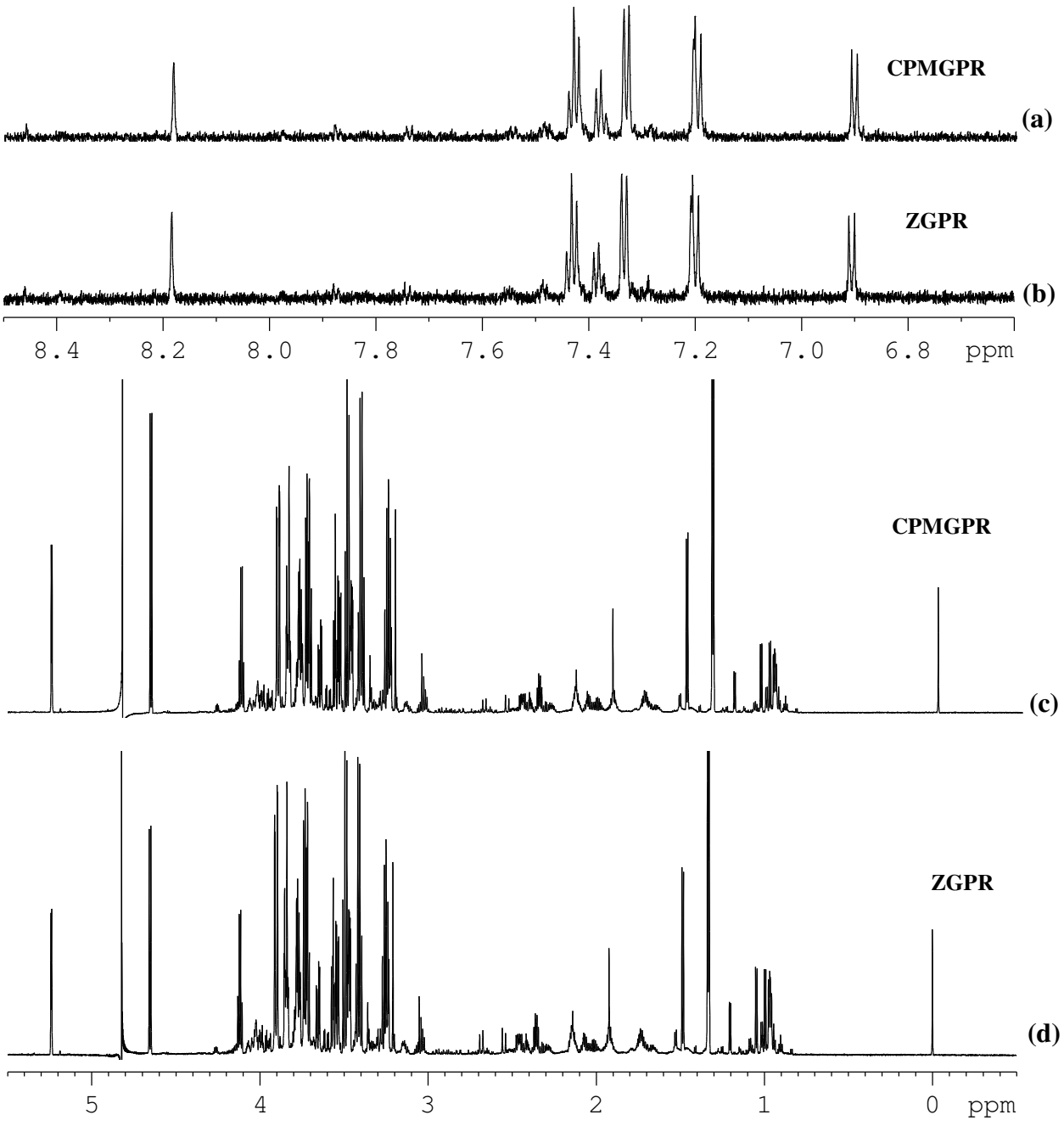

Figure S5: Comparison of ^{1}H 1D NMR spectra of the same serum sample obtained after protein removal by ultrafiltration using CPMGPR sequence (a and c) and ZGPR sequence (b and d). Qualitatively, virtually identical spectra are obtained from both the sequence. A comparison of metabolite concentrations in the two experiments showed an underestimation of concentrations in CPMG experiment by an average of 4.6%.

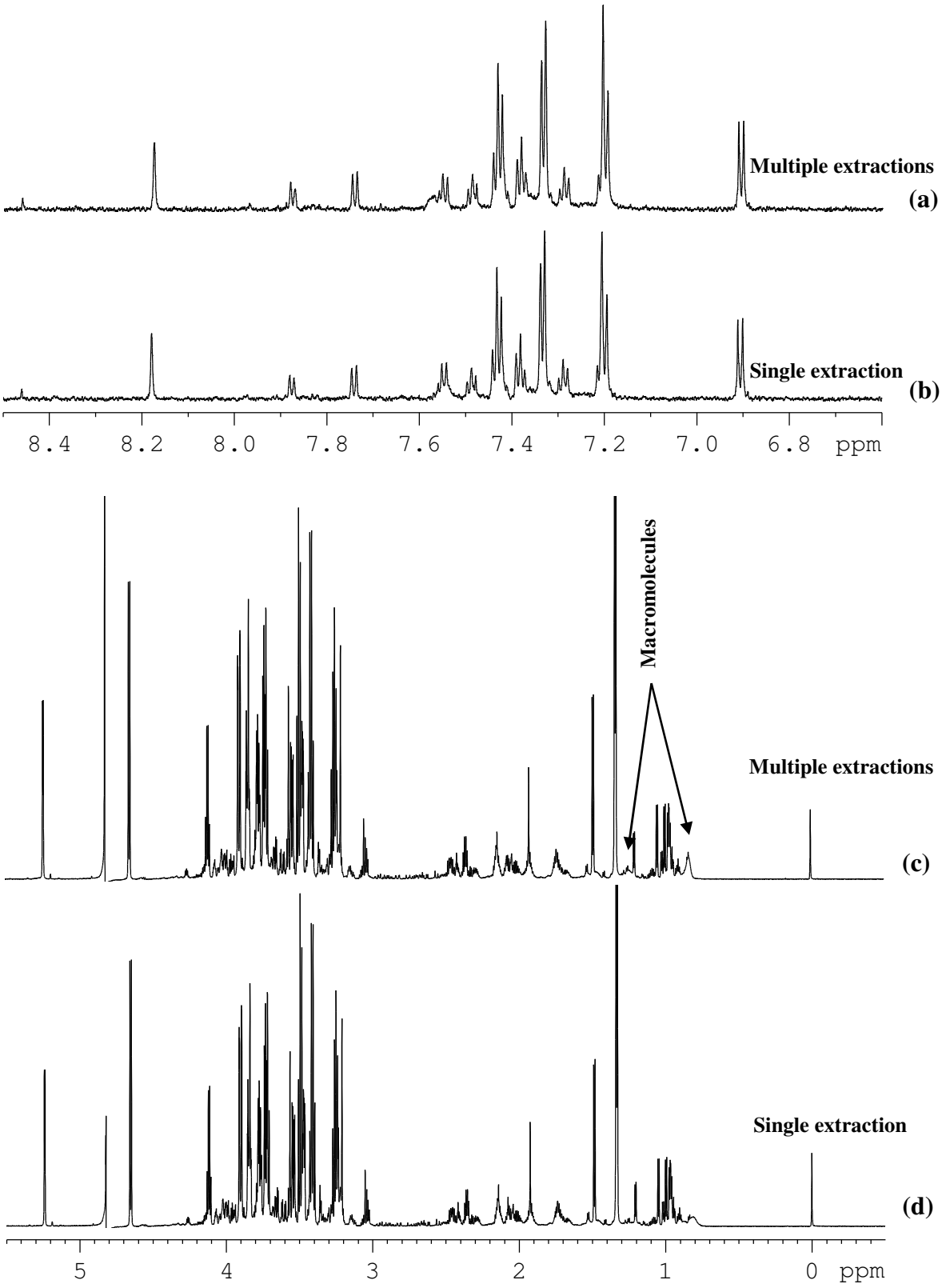

Figure S6: Comparison of serum spectra obtained by multiple (3-fold) extraction (a and c) and single extraction (b and d) using 2:1 methanol. Note that multiple extractions, while improves metabolite recovery marginally (by an average 3.9%), it increases the residual macromolecular contributions.