Impact of Storage and Handling Conditions on Metabolites in a Human Plasma Standard Reference Material

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Novel aspect
Comprehensive approach to quantify the effects of storage and handling conditions on different classes of metabolites in frozen human plasma.

Introduction
The National Institute of Standards and Technology (NIST) has developed a Standard Reference Material, Metabolites in Human Plasma (SRM 1950), to serve as a common material to help researchers evaluate qualitative and quantitative analytical methods. The intended use of SRM 1950 should significantly contribute in the current steps towards standardized metabolomic approaches.
The study presented here has systematically evaluated the influence of SRM 1950 handling and storage conditions on the plasma metabolome by monitoring 69 representative metabolites with two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC $\times$ GC-TOFMS).

Methods
SRM 1950 is a human plasma pool obtained from a representative mixture of healthy male and female donors and packaged in 1 ml vials stored at -80°C. 15 vials were randomly selected, thawed and pooled to prepare the batch for the study. 200 µl aliquots were spiked with several internal standard solutions: valine $^{13}C_5$, lysine $^{13}C_6$, glutamic acid, $^{13}C_5$, myristic acid d_{27}, stearic acid d_{35}, malic acid d_3, citric acid d_4, caffeine $^{13}C_3$, and cholesterol $^{13}C_3$. Extraction and protein precipitation were performed with a mixture of methanol/ethanol. After centrifugation, the extract was then evaporated to dryness prior to oximation and derivatization. Rtx-5/Rxi-17columns were connected to a Pegasus TOFMS. Masses were acquired in full scan from 40 to 800 m/z.

Preliminary results
The 69 target compounds encompass several classes of endogenous metabolites such as 25 amino acids (including secondary amino acids), 19 organic acids, 15 fatty acids, urea, cholesterol and glycerol. In addition, 7 exogenous metabolites (acetaminophen, ibuprofen, naproxen, benzoylecgonine, salicylic acid, caffeine and valproic acid) were also monitored. The first freeze-thaw cycle consisted in thawing 15 vials of SRM 1950 from -80°C to room temperature and pooling them together to prepare the batch for this study. The batch was immediately returned to the freezer at -20°C for 7 days after having withdrawn nine aliquots of 200 µl for analysis. The 9 samples were divided into 3 groups. The first group was sat at room temperature in a hood for 1h prior to protein precipitation. The second and the third groups were left 4 h and 24 h prior to protein precipitation, respectively. A procedural blank and a quality control (SRM 1950 stored at -80°C) were added to each series of triplicate samples. The baseline variability in the measurements was calculated from the 1 h triplicates. The relative standard deviations

(RSD, %) were between 0.5 and 32% with an average value of 8%. The overall RSDs from triplicate measurements at 4 and 24 h were similar to those obtained after 1 h. The experimental design consisted in repeating the same series of trials every 7 days over 4 weeks (second to fifth freeze-thaw cycles and 1, 4 and 24 h). The preliminary results did not show dramatic increase or decrease (e.g. 10-fold) of the metabolite levels over the freeze-thaw cycles and over the time within each cycle. Additional details of this work will be presented including characterization of the effect of these common laboratory sample handling practices on SRM 1950 plasma metabolome with the objective to highlight potential analytical bias.