

Title: Preparation of human plasma for high-resolution metabolomics

SOP: HRM_SP_082016_01 Revision: 1

Date effective: 30 July 2016

Summary statement:

Samples are prepared for metabolomics analysis using established methods (Johnson et al. (2010). *Analyst*; Go et al. (2015). *Tox Sci*). Prior to analysis, plasma aliquots were removed from storage at -80°C and thawed on ice. Each cryotube is then vortexed briefly to ensure homogeneity, and $50\ \mu\text{L}$ transferred to a clean microfuge tube. Immediately after, the plasma is treated with $100\ \mu\text{L}$ of ice-cold LC-MS grade acetonitrile (Sigma Aldrich) containing $2.5\ \mu\text{L}$ of internal standard solution with eight stable isotopic chemicals selected to cover a range of chemical properties. Following addition of acetonitrile, plasma is then equilibrated for 30 min on ice, upon which precipitated proteins are removed by centrifuge ($16.1 \times g$ at 4°C for 10 min). The resulting supernatant ($100\ \mu\text{L}$) is removed, added to a low volume autosampler vial and maintained at 4°C until analysis ($<22\ \text{h}$).

Chemicals Needed:

- $5000\ \mu\text{L}$ LC-MS grade acetonitrile
- $125\ \mu\text{L}$ stable isotope internal standard solution containing: $[\text{}^{13}\text{C}_6]$ -D-glucose, $[\text{}^{15}\text{N}, \text{}^{13}\text{C}_5]$ -L-methionine, $[\text{}^{13}\text{C}_5]$ -L-glutamic acid, $[\text{}^{15}\text{N}]$ -L-tyrosine, $[3,3\text{-}^{13}\text{C}_2]$ -cystine, $[\text{trimethyl-}^{13}\text{C}_3]$ -caffeine, $[\text{U-}^{13}\text{C}_5, \text{U-}^{15}\text{N}_2]$ -L-glutamine, $[\text{}^{15}\text{N}]$ -indole

Materials Needed

- $250\ \mu\text{L}$ q3June2014
- $100\ \mu\text{L}$ NIST SRM 1950
- $150\ \mu\text{L}$ conditioning plasma
- 40 Study samples ($\geq 50\ \mu\text{L}$ of sample required)
- Labeled 1.5mL microfuge tubes
- Calibrated P200 and P1000 Micropipettes with $200\ \mu\text{L}$ and $1000\ \mu\text{L}$ tips
- Refrigerated centrifuge at 4°C with speed $\geq 16,100 \times g$
- Vortexer
- Labeled, low-volume LC vials with snap caps

Instrumentation

- Centrifuge, Eppendorf 5430R, Room 225: Prior to starting sample preparation set speed to $16,100 \times g$ and temperature to 4°C . Cool using “fast cool” option. When loading samples, makes sure samples are evenly distributed around the wheel.

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Sample preparation

Samples are to be prepared daily, and placed in the autosampler for analysis immediately upon completing sample preparation

1. Remove conditioning, QC and study samples from storage at -80°C and thaw on ice
2. Remove internal standard solution from storage at -80°C and thaw.
3. Label clean, microfuge tubes.
4. Add 125 μL of internal standard solution to 5000 μL acetonitrile, vortex and store on ice.
5. Carefully pipette 50 μL of thawed sample to appropriate microfuge tube. Ensure no air bubbles or clogs occur in pipette tip. Use a fresh tip for each sample
6. Carefully pipette 100 μL of acetonitrile/internal standard solution into each tube and close snap top.
7. Vortex each tube for 10 sec.
8. Place tube on ice and allow to equilibrate for 30 min.

9. Return remaining sample volume to storage at -80°C .
10. Following equilibration period, centrifuge tubes at 4°C for 10 min at $16.1 \times g$.
11. Label clean, LC vials.
12. Carefully pipette 100 μL of supernatant into corresponding LC vial.
13. Cap.
14. Load into autosampler racks based on predetermined run order.

References

1. Johnson JM, Yu T, Strobel FH, Jones DP. A practical approach to detect unique metabolic patterns for personalized medicine. *The Analyst*. 2010;135:2864-2870.
2. Soltow QA, Strobel FH, Mansfield KG, Wachtman L, Park Y, Jones DP. High-performance metabolic profiling with dual chromatography-Fourier-transform mass spectrometry (DC-FTMS) for study of the exposome. *Metabolomics*. 2013;9:S132-S143.
3. Go YM, Walker DI, Liang Y, Uppal K, Soltow QA, Tran V, et al. Reference Standardization for Mass Spectrometry and High-resolution Metabolomics Applications to Exposome Research. *Tox. Sci*. 2015;148:531-543.



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01	Douglas I. Walker	Creation of SOP	30 July 2016