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Conclusions: In our hands, the automated MA provides adequate throughput for large studies with minimal sample handling time. The DBS-HF results correlate reasonably well with WB-HF and DBS samples appear to be very stable for up to 4 freeze/thaw cycles.

E-53

Rapid Quantification of Caffeine and Theobromine in Multiple Human Biofluids via UPLC-MS/MS

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High through-put targeted analyses of clinically relevant low abundance metabolites in human biofluids often require extensive sample preparation prior to quantitation. In this report, a novel protocol employing a centrifugation based sample pretreatment and ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) has been developed and validated for quantification of 1,3,7-trimethylxanthine (caffeine) and 3,7-dimethylxanthine (theobromine) in human saliva, plasma and urine. This single method circumvents the conventional pre-analytical protocols (e.g., desalting, protein removal and/or preconcentration) typically required for physiological measurements of these methylxanthines. All saliva, plasma and urine samples were respectively processed as outlined below. Initially the samples were centrifuged at 12000 g for 5 min to remove any particulate matter from the sample. 500 µL of the supernatant was then transferred to a 10 kDa molecular weight cut-off filter and centrifuged for 10 min at 12000 g. The low MW filtrate was then diluted 20-fold in distilled water prior to injection onto a C18 BEH (1.7 µm particles x 2.1 cm x 50 mm) column. The metabolites were separated using a gradient elution with mobile phases of 0.1 % formic acid in distilled water (phase A) and acetonitrile (phase B) respectively. A constant flow rate of 0.6 mL/min was employed throughout with phase B initially maintained at 2 % from 0 to 0.5 min. The sample was eluted with increasing the % of solvent B to 10, 13, 14 and 50 % at 0.5, 0.7, 1.25 and 1.5 min respectively. Total analysis time per sample was 3.0 min. Positive-mode electrospray ionization was used with a triple quadruple MS/MS instrument operated in multiple reaction mode with 13C caffeine included as an internal standard. The deleterious effects of sample matrix on UPLC-MS/MS quantitation were avoided by the described assay, with detection limits of 32 and 27 nmol/L for caffeine and theobromine respectively (S/N ~ 3) being achieved. 63 nmol/L represents the LOQ of the method, for all matrices, as the analyte peak area variation (CV) was ~ 15 - 20 % at this level. The assay was linear over a 160-fold concentration range with the average correlation coefficients (R2) for both caffeine and theobromine being 0.9997 and 0.9968 respectively. The intraday (N = 10) and interday (N = 25) peak area variation for 125 nmol/L caffeine and theobromine in saliva, plasma and urine were 5 and 10 % (intra-day) and 9 and 13 % (inter-day) respectively. The intra- and inter-day precision of caffeine and theobromine elution times were < 1 and 3 % respectively for all biofluids and concentrations tested. Recoveries for caffeine and theobromine ranged from 114 to 118 % and 99 to 105 % at the 500 nmol/L and 15 µmol/L concentration levels respectively. The developed sample pretreatment and UPLC-MS/MS method provides a single rapid and sensitive protocol applicable to the selective quantification of nmol/L levels of caffeine and theobromine in multiple human biofluids.

E-54

Trace elements and biochemical analytes concentrations from a healthy elderly population living in São Paulo city, Brazil.

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Despite the growing of elderly population around the world there are few data about reference values to be used in laboratory tests for this population. This fact encouraged us to study the issue and we evaluate some trace elements and biochemical analytes concentrations in blood samples from elderly people. This study was submitted and approved by our Internal Review Board (IRB). An elderly population, without clinical evidence of serious chronic diseases, attended at the ambulatory of Clinical Geriatric from Clinical Hospital of São Paulo University Medical School was evaluated. The selection of these individuals was based on the SENIEUR protocol (*SENIor EUROpean Protocol*). The blood samples of 94 elderly people, 26 men aging 72±6 years and 68 women aging 72±9 years, were analyzed. The blood, after 12 hours fast, was collected by venipuncture using sterile standard metallic needles. It was collected in two types of evacuated tubes (Vacutainer Systems - Becton Dickinson, EUA): SST II *Advance* gel and clot activator tube and a specific tube for trace elements analysis, without heparin. An aliquot of serum (3.0 mL) was transferred to a flask (Nalgene) and freeze-dried for trace element determinations. Neutron activation analysis (NAA) was applied for trace elements determination. About 150 mg of freeze-dried serum were irradiated at the IEA-R1 research nuclear reactor together with elemental standards. Short and long irradiations were carried out under a thermal neutron flux of about 4 x 10^{12} n cm^{-2} s^{-1} for Br, Ca, Cl, Fe, Na, Rb, Se and Zn determinations. After adequate decay times, the irradiated samples and standards were measured using a Hyperpure Ge detector Model GX2020 coupled a gamma-ray spectrometer. The radioisotopes measured were identified according to their half-lives and gamma-ray energies and the element concentrations were calculated by comparative method. The certified reference material, NIST 1566b Oyster Tissue was analyzed to evaluate the accuracy and precision of the results. The mean concentration values obtained by NAA were: Br: 3.46± 0.82 mg/L, Ca: 9.59 ± 0.93 mg/dL, Cl: 88.60 ± 8.49 Meq/L, Fe: 134.73 ± 113.12 µg/L, Na: 133.15±12.65 Meq/L, Rb: 321.83± 58.68 µg/L, Se: 76.12 ± 24.82 µg/L and Zn 96.82 ± 14.43 µg/L. Biochemical analyses were carried out on Roche/Hitachi MODULAR *ANALYTICS* PP (Roche Diagnostics GmbH, Mannheim, Germany), using specific kits from Roche Diagnostics, too. The biochemical mean values obtained were: uric acid: 5.0±1.5 mg/dL, total bilirrubin:0.72±0.28 mg/dL, Na:141±3 mEq/L, K:4.5±0.4 mEq/L, Ca:9.4±1.2 mg/dL, ionized Ca:5.1±0.6 mg/dL, P: 3.5±0.5 mg/dL, Mg:2.02±0.28 mg/dL, glucose:93±11 mg/dL, urea:37±13 mg/dL, creatinine:0.86±0.20 mg/dL, Fe:102±33 µg/dL, ferritin:173±155 ηg/mL, total protein:7.3±0.5 g/dL, albumin:4.4±0.4 g/dL, total cholesterol:213±37 mg/dL, HDL-cholesterol 60±15 mg/dL, LDL-cholesterol:130±33 mg/dL, triglycerides:122±64 mg/dL, AST:21±6 U/L, ALT:18±9 U/L, alkaline phosphatase:79±25 U/L and GGT:26±33 U/L. This elderly group did not present deficiency or excess of trace elements. The biochemical parameters results indicate the need to establish specific reference values to this type of population.

E-55

Validation of a method for serum methyl malonic acid using UPLC/MS/MS

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Methyl malonic acid (MMA) is considered to be a sensitive and specific marker for cobalamin status. Current methods have used derivatisation or extensive sample preparation, which hinder their application in routine laboratories. We report a simple method that uses UPLC to separate MMA from the isobaric compound succinic acid. The sample (serum 100 µL) and d_3-methyl malonic acid (d_3-MMA) internal standard (10 µL) were extracted with methanol (0.6 mL). The supernatant was evaporated and reconstituted with 200 µL of 0.2% (v/v) formic acid. Mobile phase A was water containing 0.2% (v/v) formic acid and mobile phase B was acetonitrile. The extract (40 µL) was injected onto an ACQUITY® BEH 2.1 x 100mm, 1.8 µm column. Isocratic elution of MMA was performed using 5% B for 1.0 min. The column was then washed with 100% B for 1.5 min and re-equilibrated with starting conditions. The flow rate was maintained at 0.45 mL/min throughout and the column was kept at 45° C. Typical operating pressure on the column was 520 bar. Total run time was 5.0 min injection to injection. The analysis was performed in negative ion mode using a Waters® Quattro Premier™. The tuning conditions for MMA and d_3-MMA were as follows: electrospray capillary voltage 1.0 kV, sample cone voltage 17 V, and collision energy 10 eV. Desolvation gas flow and temperature were maintained at 600 L/h and 400°C respectively; source temperature was 140°C. MMA and d_3 -MMA were monitored in multiple reaction monitoring (MRM) mode with dwell times of 0.2 seconds, the transitions were *m/z* 116.8>72.8 and *m/z* 119.8>75.8 respectively. MMA eluted at 0.92 mins with minimal ion suppression. The assay was linear to 1000 nmol/L with an LOD of 10 nmol/L and LLOQ of 50 nmol/L. Within batch CV and between batch CV was 3.1% and 8.5% respectively across the range 160-800 nmol/L. Average recovery was 94.4% (range 86-102%). We report an LC-MS/MS assay that shows advantages over some existing methods; it requires a small sample volume and has a simpler, less expensive extraction procedure.