patient sample, standard or control, was pipetted into an Eppendorf vial containing 180 µL of methanol. The sample was vortexed for 30 seconds, and spun for 2 minutes at 13400 x g to remove proteins. 10 µL of the supernatant was injected onto a C8 column (BDS Hypersil, 3 µm, 50 cm x 2.1 mm, Thermo Electron Corp.) under an oven temperature of 55°C. The injected sample was loaded on the LC column for 0.9 minutes at a flow rate of 0.6 mL /min with 100% Eluant A (0.1% formic acid in water) and 0% Eluant B (Acetonitrile), then ramped to 80:20 (A:B) and maintained for 1.8 minutes for the compounds elution. The column was then washed with 100% Eluant B for 0.8 minutes, followed by 0.5 minutes of 100% Eluant A. The column was equilibrated for 1.0 minute with Eluant A prior to the next injection. Total time required between injections was 5 minutes. The HPLC was connected to an API-5000 tandem mass spectrometer (Applied Biosystems, CA) with Turbo electrospray for molecular ion detection, positive mode. Results: The LC-MSMS method was able to separate MTX (m/z^+ = 455.1/ 308.2) and DAMPA (m/z^+ = 326.1/175.0). In a clinical sample with known DAMPA, the method was able to measure MTX without interference. The assay lowest detection limit was 0.01 µmol/L and sample values above the highest standard 1.0 µmol/L were quantified further from the extract supernatant by serial dilution without re-extraction. Within run precision (CV) was < 11% (n=10) and between day precision (CV) was < 11% (n=16). The linear regression was y = 0.9863x + 0.0087 µmol/L, R^2 = 0.9824, and standard error = 0.1079 for 34 samples with an MTX below 5 µmol/L. Conclusion: The presented method allows the accurate, precise, and rapid determination of MTX free from interference by DAMPA and provides clinicians the data necessary to correctly monitor leucovorin therapy.

A-30

Carboblatin Monitoring by Flameless Atomic Absorption Spectrophotometry and Metallothionein Levels in Pediatric Patients with Solid Tumors

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BACKGROUND: Carboplatin (CPt) is often used for the treatment of solid pediatric malignancies. Pharmacokinetic studies in children have shown a large degree of interpatient variability of AUC achieved on surface area-based doses. Carboplatin pharmacokinetic variability in children may lead to life-threatening toxicity (if overdosed) or inadequate treatment (if underdosed). Hence, it is recommended that carboplatin dosing is based on renal function, with the intention to achieve a specific, protocol-dependent area under the carboplatin plasma concentration-versus-time curve (AUC). Increase of platinum concentration in organism leads to increasing metallothionein (MT) gene expression. Subsequently therapeutic effect might be markedly reduced.
OBJECTIVES: The aim of the study was to to determinate plasma CPt concentration to calculate AUC values after body surface area-based dosing using spare sampling strategy for therapeutic drug monitoring and to analyze changes of serum metallothionein levels during therapy.
PATIENTS AND METHODS: Nine patients (5 retinoblastoma, 2 rhabdomyosarcoma, 1 neuroblastoma, 1 desmoblastoma), age range 4 months-18 years, were included into the study. Sixteen analyses of 20 were valid. Carboplatin was administered as 1 hour intravenous infusion in absolute dose ranging from 74 to 1445 mg according to body surface area-based dosing scheme in one or more cycles. Blood samples were obtained at 0, 1, 4 a 24 hours post dose. The carboplatin concentration in the samples was analyzed by flameless atomic absorption spectrophotometry (Varian 220 Z, Australia). The pharmacokinetics of free plasma platinum was assumed to represent those of intact carboplatin for the first 24 hours, where AUC of carboplatin was also calculated using a trapezoidal rule method assisted by the pharmacokinetic program MW/PHARM 3.30 MEDIWARE NL. Differential pulse voltammetry (Brdicka reaction) was used for MT detection in plasma (EcoChemie, Netherland).
RESULTS: The mean plasma concentration (±SD) of CPt was 42.44±21.41 mg/L immediately after infusion, 18.59±8.93 mg/L at 1 hour, 4.95±2.44 mg/L at 4 hours, and 1.89±1 mg/L at 24 hours after dose. AUC was calculated for CPt and platinum (Pt) revealing AUC_{0-24} for CPt mean 8.86±4.83 SD and 4.65±2.54 SD mg/mL.min for Pt, respectively. We found no significant correlation between the CPt dose and AUC for CPt (R=0.64). The mean concentration (±SD) of MT was higher (upper reference range is 0.6 µmol/L) at all measured hours post dose (1.45±0.49 µmol/L immediately after infusion, 1.41±0.61 µmol/L at 1 hour, 1.41±0.56 µmol/L at 4 hours, and 1.47±0.47 µmol/L at 24 hours post dose).
CONCLUSIONS: The simple carboplatin-dosing equation developed is not being used yet in pediatric patients. The use of TDM in BSA-based carboplatin dosing may avoid both underdosing and overdosing. MT can bind significant amounts of carboplatin and may efficiently contribute to carboplatin resistence of the tumors. This study suggests use of TDM in surface area-based carboplatin dosing in children.
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A-31

A Rapid, General Unknown Screen in Blood by Ultra Performance Liquid Chromatography - Time of Flight Mass Spectrometry (UPLC-TOF)

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Objective: Our objective was to develop a novel general unknown screening method for the simple and rapid analysis of drugs of abuse and therapeutic drugs in whole blood, serum and plasma. Relevance: Commonly used methods for general unknown screening in clinical and forensic laboratories include immunoassays, GC/MS, and HPLC-Diode Array Detector. Limitations of these techniques include long analytical run times, inadequate sensitivity, high specimen volume requirements and high reagent costs. Our method provides a sensitive, specific, rapid and cost effective solution for screening over 280 drugs using a simple protein precipitation extraction followed by UPLC-TOF analysis. Methodology & Validation: A 250 µL aliquot of specimen was precipitated with 1.0 mL of acetonitrile containing Proadifen as an internal standard. Specimens were vortexed, centrifuged, and the supernatant was transferred into a 96-well plate. Chromatographic separation was performed at 30°C on a Waters ACQUITY UPLC system, followed by mass spectrometric analysis on a Waters LCT Premier XE Time of Flight mass spectrometer. A Waters ACQUITY UPLC HSS T3, 2.1 x 100 mm, 1.8 µm particle size column was used. Mobile phases consisted of Solvent A:0.05% Formic Acid in DI water and Solvent B: 0.05% Formic Acid in Optima grade Methanol. Initial mobile phase composition was 90%A:10%B. Following a short hold, a linear gradient was employed with a final mobile phase composition of 5%A:95%B. The total run time was 8 minutes per specimen. Scans were completed in both positive and negative electrospray ionization modes to identify both acidic and basic compounds. A low voltage scan was used for parent mass identification and a high voltage scan was used to identify mass fragments by in-source collision induced dissociation (CID). To extend the linear range, dynamic range enhancement (DRE) was used. Real time accurate mass data was acquired by reference to an independently sampled reference material (Leucine Enkephalin, [M+H] = 556.2771 amu). Validation experiments included an extensive comparison of results from our historical screening methods (ELISA & GC/MS) with the UPLC-TOF method. Results & Conclusions: A simple protein precipitation method was developed to screen for drugs of abuse and therapeutic drugs using UPLC-TOF mass spectrometry. The analytical run time was reduced from 26 minutes using GC/MS to 8 minutes using UPLC-TOF. A larger library could be developed on the TOF allowing us to expand the panel of drugs in our comprehensive general unknown screen from 120 analytes (GC/MS) to 280 analytes (UPLC-TOF). The new method also allowed us to eliminate 11 of 13 ELISA assays. A comparison study (GC/MS + ELISA vs. UPLC-TOF) demonstrated superior results using the TOF method. Detection limits varied for all major analytes; however the limits are appropriate for clinical and forensic use. The UPLC-TOF method has an expanded library of analytes, exceptional sensitivity and specificity, a much simpler specimen preparation step, and a faster analytical run time allowing us to cut our analysis time for a batch of 30 samples from 13 hours to 4 hours.

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Development of a No-pretreatment Immunoassay for Sirolimus on the Siemens Dimension Vista® Clinical Chemistry System

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Monitoring sirolimus (Rapamune®, Wyeth Laboratories) in whole blood is a standard practice during immunosuppressive therapy for transplant patients. We describe the development and initial analytical performance of a fully automated immunoassay for the measurement of sirolimus* in whole blood on the Siemens Dimension Vista system. An EDTA whole blood sample (8.5 µL) is automatically lysed on board, incubated first with antibody β-galactosidase conjugate and later with chromium dioxide particles coated with a sirolimus analog. Sirolimus molecules in the sample form immuno-complexes with the antibody conjugate, and the excess molecules of antibody conjugate are bound by the chrome particles. The chrome-conjugate