Applications of disposable pipette extraction for analysis of drugs of abuse, vitamin D metabolism, testosterone and inborn errors of metabolism by gas and liquid chromatography/mass spectrometry

Yujing Wen

ABSTRACT

This dissertation covers method development for chemical analyses of drugs of abuse in urine, vitamin D metabolites and testosterone in serum, and organic acids in urine for inborn errors of metabolism. It also includes a review of chemical diagnosis profiles of four common organic acidemias.

To find a simple extraction procedure with high extraction efficiency for urinary organic acids with wide pK_a range and polarity, two major ion exchange phases of weak cation and anion were experimented. For weak anion exchange, oxalic acid was found to irreversibly bind to the sorbent and small polar acids, uracil and thymine cannot be effectively extracted. For weak cation exchange, four extraction protocols were designed and results were discussed. Finally, an optimized, fast and simple solid phase extraction method was developed, which can simultaneously extract 33 urinary organic acids. The average recovery is 82.5%.

A fast, simple and accurate liquid chromatography tandem mass spectrometry (LC-MS/MS) method was developed by using an efficient disposable pipette extraction
(DPX) technique, which could simultaneously analyze 51 drugs within 6 minutes. The method has an accuracy of 97 to 107%; a precision of less than 20% including the lowest concentration, a sensitivity with the lower limits of quantification lower than the corresponding cut-off values, and a linearity with a correlation coefficient greater than
0.990. The method was also successfully cross validated by testing and comparing commercially available quality controls.

Early diagnosis is critical for patients with organic acidemias. The diagnosis is based on the complex metabolite profile of each organic acidemia. As more and more definitive biomarkers were identified, the profiles were changing. In this review, the current chemical diagnosis profiles of Isovaleric academia, β-methylcrotonylglycinuria, propionic acidemia and methylmalonic acidemia were summarized for both MS/MS and GC/MS tests.

The accuracy of the analytical methods for vitamin D metabolites has been questioned and the complex sample preparation has deferred the application of LC-MS/MS method in high through-put clinical labs. To address the above issues, a simple, accurate and fast sample clean-up method was developed by using DPX-WAX (weak anion extraction) resin. The method analyzed all major vitamin D metabolites including the frequently-ignored but important and tough structural isomer of 25-hydroxyvitamin D₃. DPX extraction efficiency was high with over 90% recovery. Intra-day and inter-day precisions are less than 5% even at the lowest concentration range tested (1 ppb). The correlation coefficient for the straight line calibration relationship was 0.997, and the average accuracy over five concentration levels was 96.7%; for three independent QC samples at three concentration levels, the average accuracy was 99.4%.

Testosterone analysis in serum by LC-MS/MS is a challenging task due to its low ionization efficiency, especially for samples from women and children with concentration less than 50 ng/dL. Most methods have to add a complex derivatization procedure or use liquid-liquid extraction. We developed a simple and fast DPX extraction procedure with average recovery of 101% and intra-day and inter-day precisions are less than 15% including the lowest
concentration tested (10 ng/dl). The correlation coefficient for the straight line calibration relationship was over 0.999. The average accuracy over five concentration levels was from 97.5 to 105.6% and the accuracy of four independent quality control samples was from 96.9 to 113.6%.