Determination of Nicotine and Metabolites in Urine, Serum, Plasma and Meconium by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

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Introduction

Laboratory testing to detect tobacco use may benefit smoking cessation programs, qualify patients for pulmonary therapy, orthopedic procedures (such as spinal fusion), organ transplant, and may detect infants exposed to nicotine in utero. The detection of anabasine, a native tobacco plant alkaloid, can distinguish nicotine replacement therapy from active tobacco use.

In 2001, 17.5% of teenage mothers smoked during pregnancy. Only 18% to 25% of all women quit smoking once they become pregnant (1). Smoking during pregnancy is associated with several adverse outcomes for fetuses, including increased risk for stillbirth, infant mortality, Sudden Infant Death Syndrome, preterm birth, and respiratory problems. Carbon monoxide and nicotine from tobacco smoke may interfere with fetal oxygen supply—and because nicotine readily crosses the placenta, it can reach concentrations in the fetus that are much higher than maternal levels. Nicotine concentrates in fetal blood, amniotic fluid, and breast milk, exposing both fetuses and infants to toxic effects. Withdrawal during the first days of life can also occur from in utero exposure.

The adverse effects of smoking during pregnancy can include slowed fetal growth and decreased birth weights, the latter reflecting a dose-dependent relationship—the more a woman smokes during pregnancy, the more infant birth weight is reduced. Smoking during pregnancy can affect cognition and is associated with behavioral problems. In addition, smoking more than a pack a day during pregnancy nearly doubles the risk of the child becoming addicted to tobacco if he or she starts smoking. Even second-hand exposure to cigarette smoke can cause problems. For example, strong associations have been found between second-hand smoke and low birth weight and premature birth. Exposure during the postnatal period has been associated with a number of physical health outcomes, including Sudden Infant Death Syndrome, respiratory illnesses (asthma, respiratory infections, and bronchitis), ear infections and cavities, and increased medical visits and hospitalizations (2).

Meconium is currently the preferred specimen over fetal urine or blood to detect prenatal drug exposure because specimen collection is non-invasive and meconium provides a longer detection window. Drugs and their metabolites collect in meconium beginning at about 5 months gestation. Thus, meconium can identify exposure to drugs during the last trimester of a full-term pregnancy.

Methods

Determination of nicotine and metabolites in urine, serum, plasma, and meconium by liquid chromatography tandem mass spectrometry (LC-MS/MS) was performed at ARUP Laboratories, Inc., Salt Lake City, UT.

A single method for the quantitation of nicotine and metabolites in three specimen types was validated.

3-OH-cotinine was the most predominant analyte in urine; cotinine was the most predominant analyte in serum and plasma. Cotinine and nicotine are detected in urine, but nicotine is rarely seen in serum/plasma. Cotinine, and 3-OH-cotinine are all readily detected in meconium, with 3-OH-cotinine sometimes exceeding the nicotine concentration. Nornicotine and anabasine were not detected in meconium at significant levels.

Validation

Quantitation was achieved using a four-point calibration curve (2, 5, 20 and 200 ng/mL or ng/g). Calibration standards were matrix-matched separately from the calibrators were included for all three specimen types. Deuterated analogs for all analytes were included as internal standards. Two transitions were monitored for each analyte and internal standard. The method was validated for linearity, accuracy, precision and specificity by analyzing spiked samples and residual patient samples. The analytical measurement range was 2.5-2,000 ng/mL for all analytes in urine and serum/plasma, 4-2,000 ng/g for nicotine and trans-3-hydroxycotinine and 2-2,000 ng/g for cotinine, nornicotine and anabasine in meconium. Accuracy (% recovery) and precision (%CV) were within ±15 % of established values.

Cortizos

Cortinone: 2.9 5.4
Nicotine: 2.8 7.8
3-OH-cotinine: 2.8 7.8
Anabasine: 4.6 2.6

table 1.

Total Ion Chromatogram

Patient Specimens

Urine, serum and plasma patient specimens were analyzed and compared to another LC-MS/MS method. Good correlation was achieved between the two methods, with results agreeing within ±15% and a linear regression analysis for all analytes that had a slope between 0.85 and 1.15, y-intercepts less than the LOD, and an r² value ≥ 0.97.

Meconium results

12 meconium specimens that screened positive for cotinine by ELISA and 15 meconium specimens obtained from mothers who admitted to cigarette use during pregnancy were extracted and analyzed. Results and discussion follow.

Discussion

A single method for the quantitation of nicotine and metabolites in three specimen types was validated.

The distribution of nicotine and metabolites differs among the three specimen types.

Conclusions

1. “Prenatal Exposure to Drugs of Abuse, A Research Update from the National Institute on Drug Abuse” (2009)

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