Ethanol Metabolites by Paper Spray Ionization: Method Development in Negative Ion Mode

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Introduction:

Paper spray is a direct ionization technique that simplifies the mass spectrometric analysis of compounds from biological fluids without time consuming sample preparation and chromatography. Paper spray technology is therefore attractive for compound screening and quantitation in forensic toxicology. The sample collection and storage in a simple paper cassette is attractive for the shipment of samples to the forensic toxicology laboratory.

In this work, we develop protocols in negative ion mode for the screening of ethanol metabolites (ethyl sulfate (EtS) and ethyl glucuronide (EtG)) in urine by coupling paper spray technology to a new generation triple stage quadrupole (TSQ) mass spectrometer (MS).

Methods:

EtS, EtG and their corresponding deuterated standards (IS) were spiked in solvent, synthetic urine or donor urine and analyzed with the Velox 360™ system (Prosolia, Inc., IN) coupled to a Thermo Scientific™ TSQ Endura™ MS. Eight microliter samples were loaded to the paper spray cartridge and allowed to dry for 10 mins. Each method contained SRM transitions for both drugs and labeled internal standards. EtS concentrations ranged from 25 to 1000 ng/mL and EtG ranged from 100-1000 ng/mL. Ions are generated directly from paper when an applied high voltage induces electrospray from the sharp tip of the paper. Thermo Scientific™ TraceFinder™ software was used for data acquisition triggered by contact closure from the paper spray source and for data processing.

Preliminary Data:

Electrospray voltage and extraction solvents were optimized for negative ionization by paper spray. Negative ionization by paper spray is more sensitive to small variations in spray voltage.

We found highest sensitivity with values around 3000 Volts for this paper spray/TSQ system. The two parts of method development in paper spray ionization involves the substrate and the solvent/modifier used for analyte extraction from the paper/biological matrix. We used disposable paper spray cartridges (Velox Sample Cartridges, Prosolia, Inc.) made with Whatman® cellulose chromatography paper, 31ET grade. Therefore, we proceeded to optimize the solvent needed for extraction of EtS. Neat EtS worked well with extraction solvent that was 100% MeOH with 100 ppm acetic acid. The need for a two solvent system became apparent once spiked synthetic urine and urine were analyzed. A typical paper spray solvent used in urine analysis is 90/10 (v/v) acetonitrile/H_2O with 100 ppm acetic acid. Optimization of a two solvent system required varying the volume added to each of the sample and cartridge reservoirs. Linear quantitation was demonstrated with coefficients of determination R^2, of ~0.99 and precision as %RSD ≤20%.

EtG proved more challenging to optimize due to its hydrophilicity, given that the paper spray substrate was kept the same for both analytes. While typical paper spray extraction solvents worked, the limits of detection and quantitation were favored by a novel cartridge design where a solid phase extraction step is followed by paper spray ionization. The next step in method development for EtS and EtG is finding conditions where both analytes can be simultaneously detected in urine.

Novel Aspect: Quantitation of ethanol metabolites in urine by MS in about one sample/minute with very little sample preparation.