Poster Note 64490

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

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Overview

Purpose: Develop an ionization method in negative mode for screening of ethanol metabolites in urine using paper spray technology and mass spectrometry for use in forensic toxicology.

Methods: Urine spiked with ethyl sulfate (EtS) and ethyl glucuronide (EtG) were analyzed as dried urine samples by paper spray ionization coupled to Thermo Scientific™ Orbitrap™ mass spectrometers (MS). Positive control donor samples are analyzed after method development.

Results: Demonstrated the potential of paper spray ionization in negative mode MS for the screening of ethanol metabolites with minimum sample preparation and no chromatography. Paper spray is easy to use and provides answers in seconds. The potential of this technique for quantitation of EtG and EtS is also explored.

Introduction

Paper spray is a direct ionization technique that simplifies the mass spectrometric analysis of compounds from biological fluids. Both qualitative and quantitative analysis of small molecules from complex matrices such as blood and urine are possible without time-consuming sample preparation and chromatography. Samples are collected and stored in a simple paper cassette for easy shipment of samples to the forensic toxicology lab. Paper spray technology is therefore attractive for forensic toxicology compound screening.

EtG and EtS have been identified as specific and direct metabolites of recent alcohol consumption. Small amounts of ethanol (0.1%) are converted into EtG and EtS by conjugation pathways in the liver, and their presence in blood and urine are used as confirmation for ethanol consumption. EtG and EtS are detectable in serum for 10–14 h and in urine for 25–44 h. Therefore, these metabolites close the gap between short-term markers (*e.g.*, ethanol) and long-term markers (*e.g.*, carbohydrate-deficient transferrin in serum)[1].

In this work, we develop a negative ionization mode mass spectrometry method that is compatible with paper spray ionization for the analysis of ethanol metabolites in urine and coupling to an Orbitrap mass spectrometer.

Methods

Sample Preparation

EtS and EtG were spiked in urine with their corresponding deuterated internal standards (IS), EtS-d5 and EtG-d5 (all from Cerilliant). Standards were spiked in three different matrices: urine, synthetic urine or solvent. The concentration of IS was kept at 500 ng/mL in all samples. Three positive donor samples were also analyzed.

EtS-d5 and EtG-d5 were spiked into negative donor and positive donor urine samples and submitted to dilution/precipitation (1:3 urine to methanol) and centrifugation for 10 min at 3,000 g. Same protocol was followed with synthetic urine and neat samples. The IS-containing supernatant was then spiked with EtS and EtG to obtain calibration curves: 190 uL supernatant plus 10 uL EtS and Etg at various concentrations to make a calibration curve in the range of 5 to 1000 ng/mL. Eight microliters were loaded onto paper spray cartridges and allowed to dry at room temperature for 10 minutes. The recommended extraction solvent for negative ionization from paper was used, 100% methanol with 100 ppm acetic acid. Spray stability in negative ion mode benefits from a low surface tension solvent such as methanol.

FIGURE 1. Velox 360 paper spray system showing, clockwise from top left: paper spray ion source, mechanism for dispensing solvent to the sample, paper cassette indicating sample deposition, and spotted paper cassette electrospraying into mass spectrometer inlet.

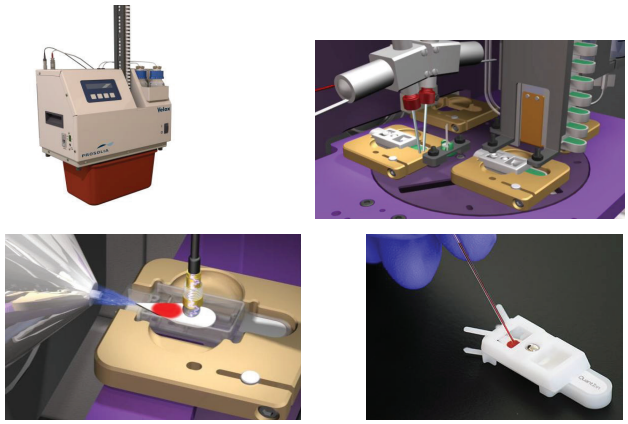
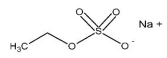
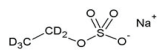
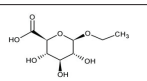
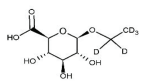


Table 1. Ethanol urine metabolites indicative of ethanol consumption. Deuterium-labeled internal standards were added to all samples.

Ethanol Metabolite	*m/z*	Fragment 1	Fragment 2	Molecular Structure
Ethyl sulfate	124.9914	79.9574	96.9601	
Ethyl sulfate–d5	130.0228	79.9574	97.9664	
Ethyl glucuronide	221.0666	75.0088	85.0296	
Ethyl glucuronide–d5	226.0981	75.0088	85.0296	

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Results

Ethanol metabolites by paper spray ionization

Paper spray ionization has primarily been shown for the analysis of small molecules that ionize in positive mode. Conditions for negative ionization usually require a low surface tension solvent and a spray voltage about 500 V less than that used in positive mode[2]. This is to avoid corona discharge which would increase the spray current and decrease analyte signal. All experiments shown were conducted with 100% methanol/ 100 ppm acetic acid, which fulfills the low surface tension negative ionization requirement.

Preliminary results for the screening of ethanol metabolites by paper spray MS/MS are shown in Figures 2 and 3, with expected fragments for EtG (*m/z* 221.07→75.0088, 85.0296) and EtS (*m/z* 124.99 →79.9574, 96.9601) and outstanding mass accuracies.

Figures 4 and 5 show calibration curves for EtS and EtG, respectively. The ethyl sulfate anion electrosprays well from the Whatman (GE Healthcare Life Sciences, Pittsburgh, PS) cellulose paper used in the VSC, which makes it amenable for quantitative analysis. %RSD (std. dev/mean x100) was found to be between 5 to 20% for the concentration range of 10 to 1000 ng/mL. EtG ionization showed poor reproducibility compared to EtS.

Positive donor samples were analyzed (Fig. 6) by the same methodology. Even though positive results were obtained, the variability obtained is too large to consider the technique robust enough to evaluate the donor signal quantitatively. Please, note some signal detected in the urine from the negative donor. This is potentially attributed to availability due to other ethanol sources (sanitizer gel, for example).

FIGURE 2. MS/MS spectra with resolving power of 35,000 (FWHM at *m/z* 200) confirming the presence of EtS in negative ion mode (Table 1) for spiked urine samples (200 ng/mL). Data collected with a Q Exactive Plus MS. Mass accuracy is < 1ppm for both fragments.

124.99→79.9574, 96.9601

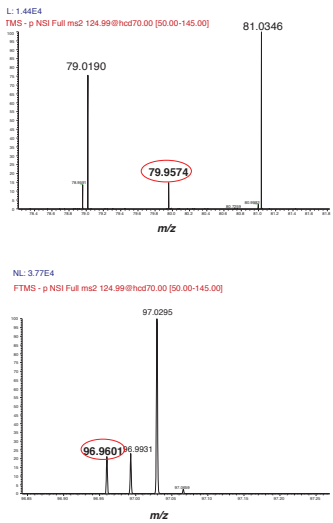


FIGURE 3. MS/MS spectra with resolving power of 35,000 (FWHM at *m/z* 200) confirming the presence of EtG in negative ion mode (Table 1) for spiked urine samples (200 ng/mL). Data collected with a Q Exactive Plus MS. Mass accuracy is < 1ppm for both fragments.

221.07→75.0088, 85.0296

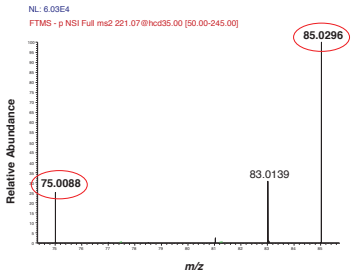


FIGURE 4. Normalized analyte to IS areas vs. concentration plots for spiked EtS in, top to bottom: neat, synthetic urine and urine matrices.

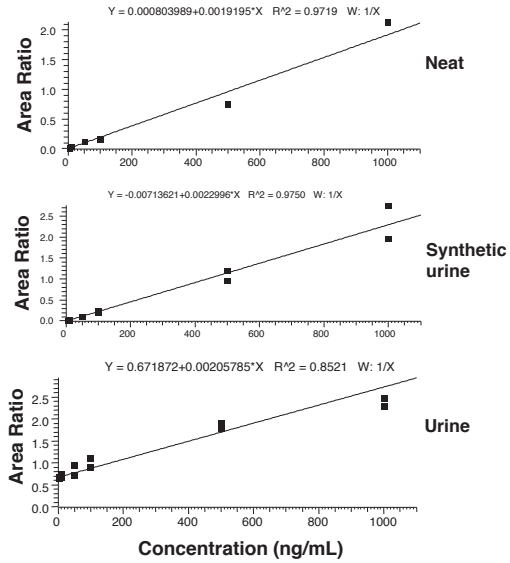


FIGURE 5 Normalized analyte to IS areas vs. concentration plots for spiked EtG in, top to bottom: neat, synthetic urine and urine matrices. EtG does not ionize well under the substrate/solvent conditions used.

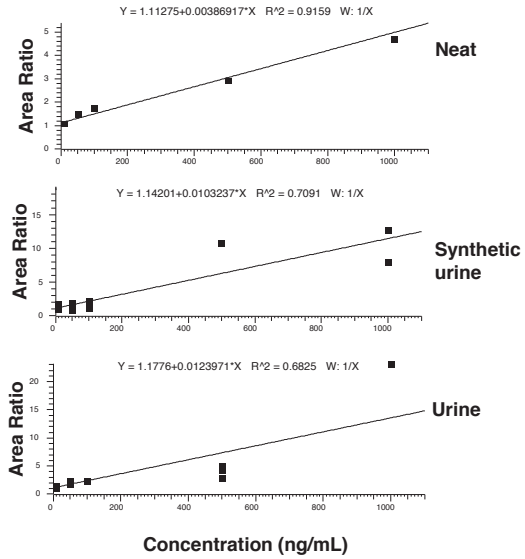
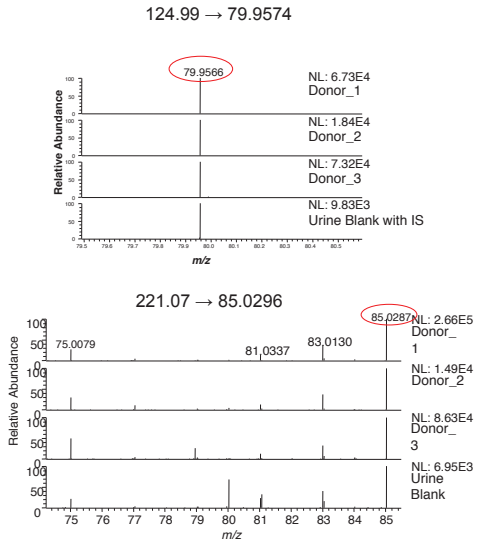


FIGURE 6. Positive donor urine samples, top to bottom, donor 1, donor 2, donor 3 and negative teetotaler urine donor blank. Mass accuracy was 10 ppm.



Conclusion

- We have developed a negative ion mode method for paper spray technology coupled to Thermo Scientific Q Exactive mass spectrometers.
- The Velox 360 system has been demonstrated as a forensic screening technique for ethyl sulfate and ethyl glucuronide from urine samples in a single experiment and from a single urine sample spot, all in much shorter timeframes than it is possible using LC MS techniques.
- As the electrospray signal from paper lasts ~40 s, it can accommodate full scan MS at high resolving power and many MS/MS experiments for identification and confirmation in screening applications.
- The ethyl sulfate anion electrosprays well with an extraction solvent conducive to negative ionization and from cellulose paper which, in the presence of an internal standard, makes it promising for quantitative analysis.
- Ethyl glucuronide ionization showed poor reproducibility compared to EtS. MS/MS data will be recollected at resolving powers of 70,000 (FWHM at *m/z* 200; RP of 35,000 used in this study) to rule out interferences in the measurements.
- Alternative substrates or paper treatments suitable for more efficient ionization of EtG should also be investigated.

References

1.Jalbermann et al, JCS 50 (2012) 51-56.

2.Manicke et al, IJMS 300 (2011) 123-129.

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