High-Resolution, Accurate-Mass Screening of Anesthetic Compounds and Their Metabolites in Urine by Paper Spray Q Exactive Mass Spectrometry

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Overview

Purpose: To couple paper spray ionization to a high-performance mass spectrometer and demonstrate its efficacy in the analysis of drugs and metabolites in urine for clinical research.

Methods: Human urine samples collected at various time points after undergoing general anesthesia; urine spotted on paper cartridge directly with no prior sample preparation. MS and MS^2 data was acquired with high resolution and accurate mass for identification of drugs and known metabolites using a paper spray source (Prosolia, IN) coupled to Thermo Scientific™ Orbitrap™ mass spectrometer.

Results: Eight anesthetic drugs and metabolites were positively detected in human urine, 4 to 11 h post dose with no sample preparation and in 1.5 min per sample.

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 is possible without time-consuming sample preparation and chromatography. We have coupled paper spray technology to a high-resolution, accurate-mass (HRAM) Orbitrap mass spectrometer (MS) for the determination of drugs and metabolites in urine after administration of general anesthesia. The technique is being investigated for potential applications in areas of metabolism research and pharmaceutical drug development.

Methods

Sample Preparation

Human urine samples were collected at time intervals prior to and after administration of general anesthesia (pre and post dosing). Samples were collected at 1 h prior, 4, 6, 9, and 11 h after general anesthesia. Samples were stored at -30 ºC until analyzed. Seven microliters of undiluted urine were loaded directly on to paper spray cartridges and allowed to dry for 10 min. Solvent was automatically dispensed to the dried urine before analysis and an applied high voltage (3.2 kV) induced electrospray from the sharp tip of the paper. The extraction solvent was 90:10 acetonitrile/water, 100 ppm acetic acid (pH 4.5).

Mass Spectrometry

A prototype paper spray source (Prosolia, IN) was coupled to a Thermo Scientific™ Q Exactive™ MS. The experimental method consisted of two scan events: a Full scan MS followed by a Data Dependent MS^2 using a target list of 6–10 compounds. Resolving power was set at 140k (FWHM at *m/z* 200) throughout.

Data Analysis

Thermo Scientific™ Qual Browser software from the Thermo Scientific™ Xcalibur™ platform was used for initial spectra viewing. Further analysis was performed with Thermo Scientific™ Mass Frontier™ software version 7.0.

TABLE 1. Selection of drugs screened in urine sampled at various time points after administration of general anesthesia

Compound	Chemical Formula	*m/z*	Drug Structure	Total Dose
Fentanyl (opioid analgesic)	$C_{22}H_{28}N_2O$	337.2274		100 µg
Xylocaine (anesthetic and antiarrhythmic)	$C_{14}H_{22}N_2O$	235.1805		20 mg
Propofol (hypnotic/ anesthetic)	$C_{12}H_{18}O$	179.143		150 mg
Dexamethasone (anti-inflammatory / immunosuppressant)	$C_{22}H_{29}FO_5$	393.2072		12 mg
Metoclopramide (antiemetic / gastroprokinetic)	$C_{14}H_{22}ClN_3O_2$	300.1473		10 mg
Ondansetron (antiemetic)	$C_{18}H_{19}N_3O$	294.1601		4 mg

Results

Dosed Anesthetics Sampled from Urine

Common drugs administered during general anesthesia – fentanyl, lidocaine, propofol, dexamethasone, metoclopramide and ondansetron – were detected at 4, 6, 9, and 11 h post dose (Table 1). MEGX (monoethylglycinexylidide) and 6-β-OH dexamethasone, phase I metabolites for lidocaine and dexamethasone, respectively, were detected in the same time period. Compounds were identified by their exact *m/z* value (1–4 ppm mass accuracy) and confirmed by MS/MS and accurate mass of their fragments (≤5 ppm). Further details follow.

Xylocaine and Phase I Metabolite

Figure 1 shows the full MS spectra for xylocaine and its phase I metabolite, MEGX, at different time points pre and post dose. The pre dose spectrum shows small amounts (~2 orders of magnitude less intense) of interfering compounds in the *m/z* of interest. See inset figure. High mass accuracy of <1ppm and high resolving power of 140,000 (FWHM at *m/z* 200) are required tools when evaluating samples from complex biological matrix with no prior chromatography or clean-up step in order to discern between contaminants and drug/metabolite in a full MS spectrum.

FIGURE 1. Full scan MS spectra showing xylocaine (also known as lidocaine) and its metabolite, MEGX, present at various time points in urine analyzed post dose. Resolving power at 140k (FWHM at *m/z* 200). Mass accuracy is < 1ppm for both compounds.

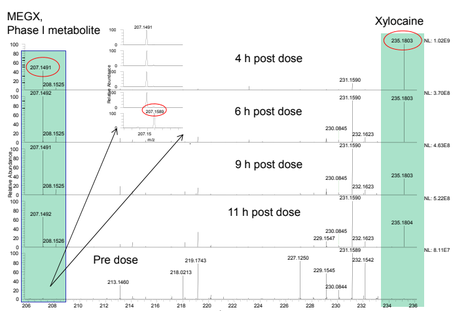
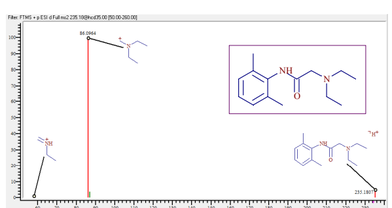


FIGURE 2. Xylocaine MS^2 spectrum (*m/z* 235.18, isolation width 2 amu). All fragments can be traced to the precursor ion. Analysis and annotation of the MS^2 data by Mass Frontier software. Resolving power 140k (FWHM at *m/z* 200).



Dosed Anesthetics – MS^2 Data

Ion chronograms (signal versus time) that are 35 s wide per sample allow versatility in the experimental setup. In this study, both scan events in the experiment were conducted with the Q Exactive MS at the highest resolving power setting of 140k (FWHM at *m/z* 200).

Figures 2 and 3 show the annotated fragmentation spectra for xylocaine and MEGX, respectively. The xylocaine MS^2 spectrum shows fragments that can be traced to the drug precursor ion alone (*m/z* 58.065 and 86.096). In Figure 3, fragments directly derived from the metabolite are identified as *m/z* 58.065 and 122.097. The most intense fragment at *m/z* 58.065 is a subsequent fragment from the metabolite, which is a one step process, as opposed to originating from the drug, where it goes through a two step process and is less abundant. Other fragments present in Figure 3 can be traced to background ions in the of 2 amu isolation window (± 1 amu around the precursor ion). In targeted analysis, isolation and fragmentation of the "windows" around the target analytes performed in a pre dose or blank sample (Figure 4) provide a background measure that can be compared against real sample MS^2.

FIGURE 3. Fragmentation of MEGX (MS^2 *m/z* 207.15). Ions from background matrix are shown by arrows. Analysis and annotation of the MS/MS data by Mass Frontier software. Instrument resolving power at 140k (FWHM at *m/z* 200).

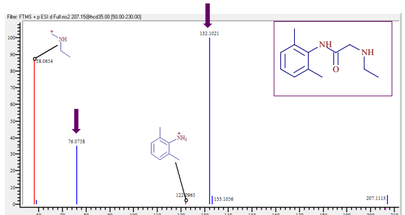


FIGURE 4. Background ions (arrows) in the pre dose sample contributing to the MS^2 spectrum of MEGX (MS^2 *m/z* 207.15) in Figure 3

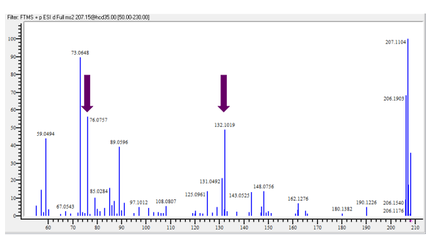


Figure 5 shows the full scan MS spectra zoomed to the region where the drug fentanyl appears (*m/z* 337.227). Because ultrahigh resolution is used, closely interfering peaks from the complex matrix separate enough to show the drug's presence. MS^2 spectra (Figure 6) confirms the presence of fentanyl and its absence in the pre dose sample. The *m/z* 188.143 peak accounts for the neutral loss of N-phenylpropanamide. Fentanyl has an extremely short half-life so the first sample will have most of the dose. Please note that the 'NL' number displayed in Figure 6 relates to intensities in which areas have not been normalized to an internal standard.

Figure 7 shows the full scan MS for the major metabolite of fentanyl in urine, norfentanyl, which forms by oxidative N-dealkylation. Fentanyl has a unique biphasic elimination with a longer terminal elimination, so the metabolite is detected in all time points sampled for this study

FIGURE 5. Full scan MS for the fentanyl ion. Resolving power 140k (FWHM at *m/z* 200).

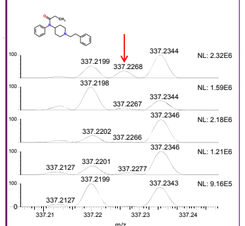


FIGURE 6. MS/MS fragmentation of fentanyl (*m/z* 337.227→188.143). Resolving power 140k (FWHM at *m/z* 200).

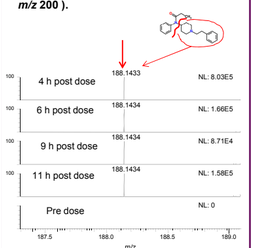


FIGURE 7. Full scan MS spectrum of norfentanyl, the major urinary fentanyl metabolite. Resolving power 140k (FWHM at *m/z* 200).

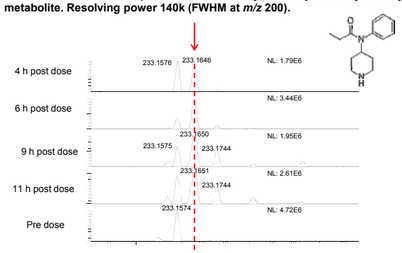


Table 2 provides a summary of all the anesthetic drugs and metabolites detected in the urine samples. Drugs such as neostigmine, glycopyrrolate and vecuronium were administered in very low dose or possess short half-time metabolic rates. This is probably the reason we were not able to detect neostigmine. Glycopyrrolate was only detected at 4 and 6 h post dose and vecuronium only at 4 h post dose.

TABLE 2. Summary of drugs and metabolites detected in urine sampled at various time points after administration of general anesthesia

Compound	Chemical Formula	*m/z*	Urine Metabolites, *m/z*	Detected in Urine? (Drug, Metabolite)
Fentanyl	$C_{22}H_{28}N_2O$	337.2274	Norfentanyl, 233.1648	Yes, Yes
Xylocaine (lidocaine)	$C_{14}H_{22}N_2O$	235.1805	MEGX, 207.1492	Yes, Yes
Propofol	$C_{12}H_{18}O$	179.143	Propofol β-D-glucuronide (neg mode)	Yes, NA[1]
Dexamethasone	$C_{22}H_{29}FO_5$	393.2072	6-β-OH dexamethasone, 409.2021	Yes, Yes
Metoclopramide	$C_{14}H_{22}ClN_3O_2$	300.1473	Dealkylation	Yes, NA[2]
Ondansetron	$C_{18}H_{19}N_3O$	294.1601	Glucuronide or sulfate conjugation	Yes , NA[1]
Neostigmine	$C_{12}H_{19}N_2O_2$	224.1519		No
Glycopyrrolate (glycopyrronium Br)	$C_{19}H_{28}NO_3^+$	318.2064	Glucuronide or sulfate conjugation	Yes (4, 6 hrs), NA[1]
Vecuronium	$C_{34}H_{57}N_2O_4^+$	557.4313		Yes (4 hrs only), NA[2]

[1] Glucuronide metabolites not analyzed in this study
[2] Metabolite not checked

Conclusion

- We have shown an easy-to-use technique (no sample preparation, no chromatography) that shows extraordinary potential for clinical and pharmaceutical research.
- The paper sample cassette allows for direct sample deposition, safe sample handling, and storage after drying. Once dry, the sample is stable for convenient shipping.
- Paper spray uses 7–12 µL of biofluid samples, making it ideal for microliter sample clinical research, such as those using mice as the animal model.
- The paper spray signal (35 s wide in our experiments) allowed for several experiments to be performed with ultrahigh resolution in both full scan MS and MS^2.
- High resolution and accurate mass are crucial techniques for analyzing complex samples by direct analysis MS and nicely complement the paper spray technique in the screening of drugs and metabolites from urine spots.
- Data collected in this application allows for retrospective analysis as a HRAM full scan MS is available for re-examination.
- Accurate mass MS^2 fragments collected at high resolving power are necessary when analyzing complex samples and required for drug/metabolite confirmation.
- The paper spray technique coupled with data processing using Mass Frontier software version 7.0 provides a compelling solution for drug and metabolism studies in clinical research.
- Future work will adapt Thermo Scientific node-based processing software for the analysis of non-chromatography traces generated with paper spray ionization to allow identification of unknown metabolites in biological samples using sophisticated structural identification tools.

References

1. N.E. Manicke *et al.* Assessment of paper spray ionization for quantitation of pharmaceuticals in blood spots. *International Journal of Mass Spectrometry*, **2011**, *300, 123–129.*

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