

Quantitative LC-MS Screening for Illicit Drugs Using Ultrahigh Resolution Mass Analysis and Accurate Mass Confirmation

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Forensic toxicology use only.

Introduction

There is increasing demand to rapidly identify and quantify illicit drugs in human samples for forensic purposes. By using ultrahigh resolution, accurate mass spectrometry detection coupled to liquid chromatography separation, a large number of both expected and unexpected compounds can be easily screened and quantified without prior knowledge.

Goal

To evaluate a simple LC-MS method using a benchtop ultrahigh resolution mass spectrometer to quantitatively screen for 46 illicit drugs of abuse in urine with little sample preparation.

Experimental

Sample Preparation

Blank human urine diluted with 25% acetonitrile was spiked with varying concentrations of 46 drugs of abuse and their corresponding isotopically-labeled standards at 50 ng/mL.

HPLC

HPLC analyses were performed using a Thermo Scientific Accela liquid chromatography system. Gradient elution with a Thermo Scientific Hypersil GOLD PFP column (100 x 2.1 mm; 3 μ m) was used at a flow rate of 350 μ L/min. The injection volume was 10 μ L.

Mass Spectrometry

MS detection was carried out on a Thermo Scientific Exactive benchtop LC-MS system with a heated electrospray ionization (HESI) source in positive ion mode at a mass resolution of 50,000 FWHM via external mass calibration.

Results and Discussion

LC-MS quantification of 46 drugs of abuse was accomplished via the calculated area ratios of the compound to its heavy-labeled internal standard. Table 1 gives a listing of the targeted drugs of abuse, their limits of quantitation (LOQ), and their measured mass errors at the LOQ in urine.

Example extracted ion chromatograms for methamphetamine and benzoylecgonine in urine are shown in Figure 1. At a mass resolution of 50,000 FWHM, at least 10 data points were obtained across the LC peaks. Applying extracted ion chromatograms of ± 5 ppm, along with the isotopically-labeled internal standards for confirmation, all drugs of abuse were easily identified.

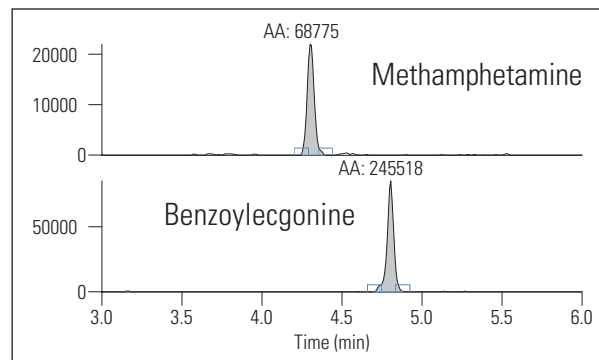


Figure 1: Example extracted ion chromatograms (± 5 ppm) at LOQs in urine

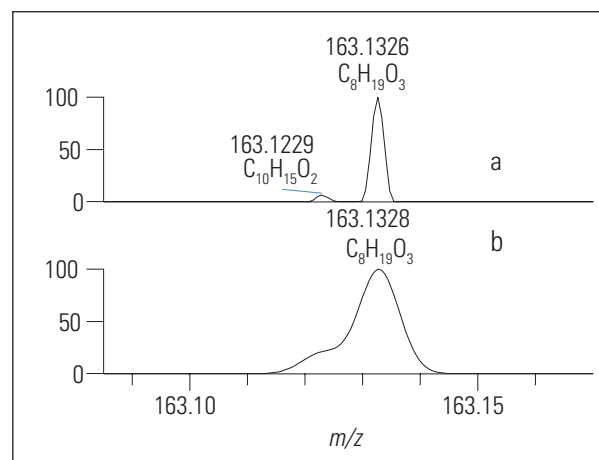


Figure 2: Nicotine mass spectrum in urine (a) and simulated spectrum (b) at 20,000 FWHM

Figure 2 shows the nicotine mass spectrum in urine (a) with an isobaric interference at m/z 163.1326 and a simulated spectrum (b) at 20,000 FWHM. Although nicotine and its isobaric interference have a large relative mass difference of ~ 60 ppm, the absolute mass difference is only 0.0097 u. As seen in the simulated spectrum (b) at 20,000 FWHM, the nicotine ion is not resolved from the interference. Only ultrahigh mass resolution of 50,000 FWHM provides the necessary selectivity to resolve these compounds and therefore allows for confident identification and quantification.

Conclusion

The Exactive benchtop LC-MS system provides easy confirmatory and quantitative analysis of 46 illicit drugs at LOQs of 0.5 – 5 ng/mL in urine for forensic toxicology. Owing to the sensitivity of the Exactive system, urine samples require only dilution with solvent to achieve this level of performance.

Key Words

- Exactive LC-MS
- Accela UHPLC
- Drug Screening
- Drug Quantitation
- Forensic Toxicology

Table 1: List of drugs of abuse monitored

| Drug of Abuse | RT (min) | Exact m/z | Measured m/z | Error (ppm) | LOQ (ng/mL) |
|--------------------------|----------|-----------|--------------|-------------|-------------|
| Nicotine | 1.40 | 163.12298 | 163.12288 | -0.6 | 0.5 |
| Cotinine | 1.55 | 177.10224 | 177.10204 | -1.2 | 0.5 |
| Morphine | 2.35 | 286.14377 | 286.14362 | -0.5 | 1.25 |
| Hydromorphone | 3.19 | 286.14377 | 286.14374 | -0.1 | 1.25 |
| Ephedrine | 3.23 | 166.12264 | 166.12256 | -0.5 | 1.25 |
| Amphetamine | 3.82 | 136.11208 | 136.11203 | -0.4 | 2.5 |
| Codeine | 3.91 | 300.15942 | 300.15891 | -1.7 | 1.25 |
| Noroxycodone | 4.17 | 302.13868 | 302.13828 | -1.3 | 1.25 |
| Methamphetamine | 4.32 | 150.12773 | 150.12761 | -0.8 | 1.25 |
| MDA | 4.36 | 180.10191 | 180.10181 | -0.6 | 0.5 |
| Oxycodone | 4.36 | 316.15433 | 316.15384 | -1.5 | 0.5 |
| 6-Acetylmorphine | 4.44 | 328.15433 | 328.15384 | -1.5 | 1.25 |
| Hydrocodone | 4.64 | 300.15942 | 300.15891 | -1.7 | 1.25 |
| MDMA | 4.69 | 194.11756 | 194.11742 | -0.7 | 0.5 |
| Norketamine | 4.77 | 224.08367 | 224.08351 | -0.7 | 1.25 |
| 7-Amino-clonazepam | 4.75 | 286.07417 | 286.07367 | -1.7 | 0.5 |
| Benzoylcegonine | 4.81 | 290.13868 | 290.13806 | -2.1 | 1.25 |
| Ketamine | 5.01 | 238.09932 | 238.09877 | -2.3 | 0.5 |
| Norfentanyl | 5.03 | 233.16484 | 233.16470 | -0.6 | 0.5 |
| MDEA | 5.31 | 208.13321 | 208.13304 | -0.8 | 0.5 |
| 7-Amino-flunitrazepam | 5.73 | 284.11937 | 284.11929 | -0.3 | 0.5 |
| Normeperidine | 6.26 | 234.14886 | 234.14865 | -0.9 | 0.5 |
| Meperidine | 6.45 | 248.16451 | 248.16428 | -0.9 | 0.5 |
| Cocaine | 6.62 | 304.15433 | 304.15405 | -0.9 | 0.5 |
| Norbuprenorphine | 6.74 | 414.26389 | 414.26257 | -3.2 | 2.5 |
| alpha-Hydroxymidazolam | 7.29 | 342.08039 | 342.07971 | -2.0 | 1.25 |
| Oxazepam | 7.30 | 287.05818 | 287.05768 | -1.7 | 5 |
| alpha-Hydroxytriazolam | 7.41 | 359.04609 | 359.04562 | -1.3 | 2.5 |
| alpha-Hydroxyalprazolam | 7.42 | 325.08507 | 325.08447 | -1.8 | 2.5 |
| Cocaehtylene | 7.43 | 318.16998 | 318.16962 | -1.1 | 0.5 |
| Lorazepam | 7.45 | 321.01921 | 321.01797 | -3.9 | 2.5 |
| PCP | 7.44 | 244.20598 | 244.20518 | -3.3 | 0.5 |
| Nitrazepam | 7.54 | 282.08732 | 282.08658 | -2.6 | 2.5 |
| 2-Hydroxyethylflurazepam | 7.57 | 333.08006 | 333.07889 | -3.5 | 1.25 |
| Midazolam | 7.64 | 326.08548 | 326.08493 | -1.7 | 0.5 |
| Nordiazepam | 7.71 | 271.06327 | 271.06265 | -2.3 | 0.5 |
| Clonazepam | 7.72 | 316.04835 | 316.04791 | -1.4 | 2.5 |
| Temazepam | 7.83 | 301.07383 | 301.07349 | -1.1 | 2.5 |
| Fentanyl | 7.90 | 337.22744 | 337.22635 | -3.2 | 2.5 |
| Alprazolam | 8.00 | 309.09015 | 309.08957 | -1.9 | 1.25 |
| Triazolam | 7.99 | 343.05118 | 343.05103 | -0.4 | 0.5 |
| Flunitrazepam | 8.01 | 314.09355 | 314.09293 | -2.0 | 1.25 |
| Buprenorphine | 8.12 | 468.31084 | 468.31006 | -1.7 | 1.25 |
| Diazepam | 8.24 | 285.07892 | 285.07819 | -2.6 | 0.5 |
| EDDP | 8.58 | 278.19033 | 278.18991 | -1.5 | 0.5 |
| Methadone | 8.81 | 310.21654 | 310.21628 | -0.8 | 0.5 |

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