High Resolution and High Mass Accuracy: A New Approach for Screening in Doping Control Analysis

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Introduction

Liquid chromatography–mass spectrometry (LC-MS/MS) technology has revolutionized the types of detection assays used in doping control analysis over the last decade. Triple quadrupole or tandem mass spectrometers have been used most frequently in this area and provide accurate identification, confirmation, and quantification of prohibited compounds in a single analysis. In addition, ion trap and quadrupole time-of-flight mass spectrometers have been useful for screening and confirming results. However, these technologies cannot address all of the main requirements of doping control analysis such as:

- Data re-interrogation
- Unlimited number of compounds scanned during the analysis
- Fast and easy method development
- High resolution to efficiently separate analytes from interferences present in the matrix
- High mass accuracy to identify compounds by exact mass

Here we present a screening approach that uses high mass accuracy and high resolution (R = 50,000) in positive and negative polarities for the accurate screenThermo Scientific Exactive mass spectrometer. More than 120 analytes are ing of illicit substances in urine matrix using the screened using this method. Unequivocal compound identification and confirmation are made using the exact mass of the analytes in positive and negative mode (if available) and the retention time.

Goal

To demonstrate a new approach using high mass accuracy and high resolution (> 50,000) for the accurate screening of illicit substances in a urine matrix using the Exactive™ mass spectrometer, a new benchtop instrument equipped with OrbiTrap™ technology.

Experimental

Sample preparation

Solid phase extraction (SPE) was used for sample pre-treatment and clean up. The details of the procedure are described below.

- To 5 mL of urine add 25 µL of hydrocortisone-d3 at 10 µg/mL
- Add 1 mL of phosphate buffer
- Add 50 µL of β glucuronidase and 50 µL of protease
- Incubate for 1 hour at 55 °C
- Centrifuge at 4000 rpm for 30 minutes
- Transfer the supernatant to a tube
- Add 5 mL of water
- Condition the C18-HF cartridge (Varian, Les Ulis, France) with 3 mL of methanol and 3 mL of water
- Load the sample and wash the cartridge with 3 mL of water and 3 mL of hexane
- Elute with 3 mL of a mixture containing dichloromethane and ethanol
- Evaporate to dryness
- Reconstitute with 100 µL of a mixture containing water and acetonitrile (80/20)

Instrumentation Method

HPLC conditions

Chromatographic analyses were performed using Shimadzu binary pumps LC-20ADxr (Champs sur Marne, France). The chromatographic conditions were as follows:

- Column: Reversed-phase, silica-based C18 (3.5 µm, 150 x 2.1 mm) column
- Flow rate: 0.3 mL/min
- Injection volume: 10 µL
- Mobile phase:
  - A: Water containing 0.1% formic acid
  - B: Acetonitrile containing 0.1% formic acid
- Gradient Mode

Mass Spectrometry conditions

MS analysis was carried out on a Thermo Scientific Exactive benchtop mass spectrometer with an electrospray ionization (ESI) source. The MS conditions were as follows:

- Ion Polarity: Polarity switching scan dependent experiment
- Spray Voltage: 4500 V in positive mode and -3900 V in negative mode
- Sheath gas pressure (N_2): 45 (arbitrary units)
- Auxiliary gas pressure (N_2):3 (arbitrary units)
- Capillary temperature: 300 °C
- Resolution: 50,000
- Automatic Gain Control: Target value 500,000

Results and Discussion

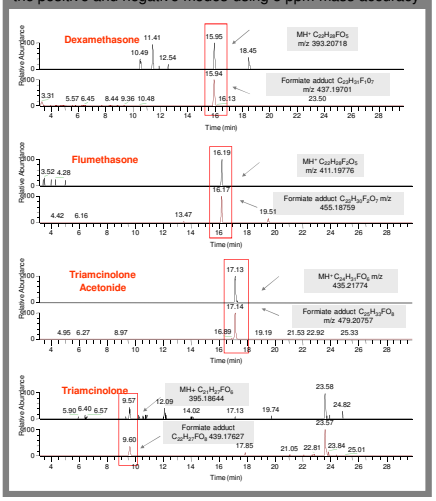
The screening method was set up for the identification and confirmation of more than 120 molecules, including anabolic agents, steroids, anesthetics, anti-inflammatory agents, and diuretics, as listed in Table 1.

Table 1 : List of compounds detected in the screening.

20 Beta dihydrocortisol	Diazoxide	Naftidrofuryl
4 Methylamino antypirine	Dichlorisone	Niketamide
5' Hydroxy Omeprazole	Diphenydramine	Nimesulide
Acepromazine	Diphylline	Nordazepam
Acide ethacrynic	Etamiphylline	Omeprazole
Althiazide	Etophylline (Etofylline)	Oxazepam
Ambroxol	Fenspiride	Oxyphenbutazone
Amcinonide	Fludrocortisone	Paramethasone
Amitryptylline	Flufenamic acid	Pentoxyphylline
Antipyrine (phenazone)	Flumethasone	Petidine (meperidine)
Beclomethasone	Flunisolid	Phenobarbital
Bendroflumethiazide	Flunixin	Phenylbutazone
Benzocaine	Fluocinolone acetonide	Phenytoin
Benzoylecgonine	Fluocinonide	Piroxicam
Benzydamine	Fluorometholone	Prednisolone
Betamethasone	Fluoroprednisolone	Prednisone
Budesonide	Flurandrenolide	Probenicid
Buflomedil	Fluticasone propionate	Procaine
Bumetanide	Furosemide	Prolintane
Bupivacaine	Guaifenesin	Promazine
Butorphanol	Halcinonide	Pyrilamine
Caffeine	Hydrochlorothiazide	Ranitidine
Capsaicine	Hydroflumethiazide	Sildenafil
Carbetapentane	Hydroxy Lidocaine	Sildenafil hydroxy
Chlorothiazide	Hydroxy Meloxicam	Sulindac
Chlorpheniramine	Hydroxy Piroxicam	Tenoxicam
Chlorpromazine	Hydroxy Tenoxicam	Tetracaine
Chlorthalidone	OH-Triamcinolone Aceto.	Tetrahydrogestrinone
Cimetidine	Imipramine	Tetramisole
Clenbuterol	Indapamide	Theobromine
Clobetasol	Isoflupredone	Theophylline
Cortisol	Ketamine	Timolol
Cortisol d3	ketoprofen	Tixocortol pivalate
Cortivazol	Ketorolac	Tramadol
Cyclothiazide	Lidocaine	Triamcinolone
Dantrolene	Meloxicam	Triamcinolone acetonide
Dantrolene hydroxy	Mepivacaine	hexacetonide
Desonide	Meprednisone	Trichlormethiazide
Desoximethasone	Methyl phenidate	Tripelennamine
Dexamethasone	Metocarbamol	Xipamide
Diazepam	Morphine	Xylazine

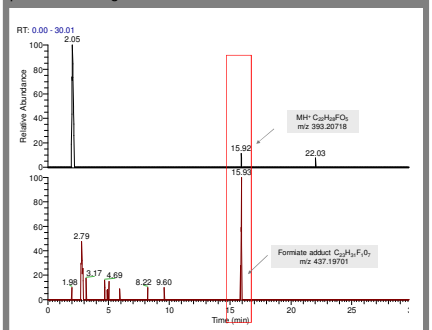
Acquisition was performed in the full scan mode, in both positive and negative mode, using external calibration. All data were reprocessed using 5 ppm mass accuracy. Figure 1 shows the sensitivity obtained for a urine sample spiked with 4 molecules: dexamethasone, flumethasone, triamcinolone acetonide, and triamcinolone.

Figure 1 : Extracted ion chromatograms for dexamethasone, flumethasone, triamcinolone acetonide, and triamcinolone in the positive and negative modes using 5 ppm mass accuracy



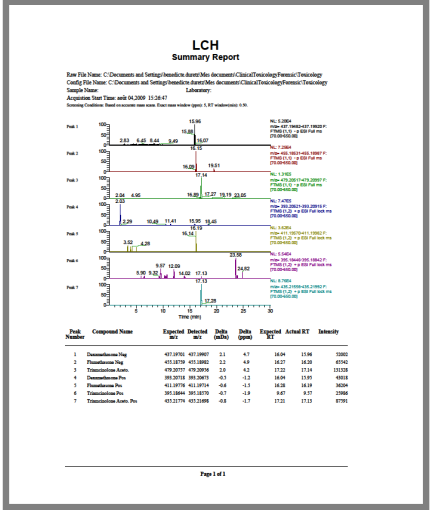
The injected concentrations were 50 pg/ml for dexamethasone and flumethasone, and 1 ng/ml for triamcinolone and triamcinolone acetonide. In the positive mode, the analytes were identified as protonated species and in the negative mode, as formate adducts.
Figure 2 shows an example of a real sample that has been analyzed using this method.

Figure 2: Dexamethasone identified in a real sample in positive and negative mode.



All data have been processed using Thermo Scientific ToxID automated compound screening software. ToxID™ for Exactive processes data and identifies the compounds using the mass accuracy and retention time of the analytes. An example of the automatically-generated report can be seen in Figure 3.

Figure 3: ToxID report – short summary style



Conclusion

The Exactive benchtop mass spectrometer is an ideal system for screening illicit substances in the doping control laboratory. Equipped with ToxID software, it demonstrates unequivocal analyte identification and confirmation in real urine samples using high resolving power, high mass accuracy, and retention time. Method development is fast and simple with the ability to configure screening for an unlimited number of compounds during the analysis. As the analysis is done in full scan mode, data re-interrogation can easily be done without the need for sample re-injection.

Currently, over 120 compounds are screened routinely and succesfully on thousands of real urine laboratory samples to date with this approach.

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