Phytronix

Application Note 0711

LDTD High-Throughput Screening and Quantification of Doping Agents in Human

Patrice Tremblay[1], Paule-Émilie Groleau[2], Christiane Ayotte[2], Pierre Picard[1] and Edith Viel[2]
[1]Phytronix Technologies, Québec, Canada, [2]INRS-Doping Control, Laval, Québec, Canada

Keywords: High-throughput, LDTD, Tandem mass spectrometry, Screening, Doping Agents , Human Urine

Overview

- High-throughput screening and quantification of hydroxythiazide, benzoylecgonine, and prednisone in human urine is performed by LDTD-MS/MS;
- Calibration range from 5 to 500 ng/mL.
- Sample-to-sample run time of 6 seconds;

Instrumentation

- Phytronix Technologies LDTD ion source (model T-960);
- Thermo Fisher Scientific TSQ® Quantum™ Ultra AM mass spectrometer.

Introduction

The detection of performance-enhancing drugs in sports is a great analytical challenge. Methods must achieve fast screening and accurate identification of a wide variety of compounds. LDTD-MS/MS is used for high throughput analysis of a diuretic (hydrochlorothiazide), a stimulant metabolites (benzoylecgonine) and a corticosteroid (prednisone). The innovative design of LDTD allows a rapid laser thermal desorption of the sample under atmospheric condition followed by an APCI process prior to introduction of the ions into the MS system.

LDTD ionization process

The LDTD ion source uses an infrared laser diode to desorbs sample that have been dried onto a well of a LazWell™ (96-well plate). The desorbed gas phase molecules are carried into a corona discharge region to undergo APCI, then they are transferred directly into the mass spectrometer.

Samples Preparation

Human urine was spiked with hydroxythiazide, benzoylecgonine, and prednisone. The urine sample was split in two sub-samples. Each sub-sample was treated by adding either a phosphate buffer (pH 5.0) or a carbonate buffer (pH 9.0) to saturation. Following a vortex agitation of 5 sec. 4 mL of ethyl acetate was added for liquid-liquid extraction. The extracts were combined and ISTD were added (paracetamol for APCI(+) and chloramphenicol for APCI(-)). A volume of 2.0 µL was manually transferred into a well of a LazWell™ and was allowed to dry at room temperature.

Results and Discussion

Calibration Curves

The calibration curves were evaluated over a nominal range of 5 ng/mL to 500 ng/mL (**Figure 1**). The desorption time for all compounds was 6 seconds. Blank analysis were used to calculate the LOD (3-time the signal value expressed in concentration) to be 0.4 ng/mL for hydrochlorothiazide and benzoylecgonine and 2 ng/mL for prednisone. The linearity was acceptable for all compounds.

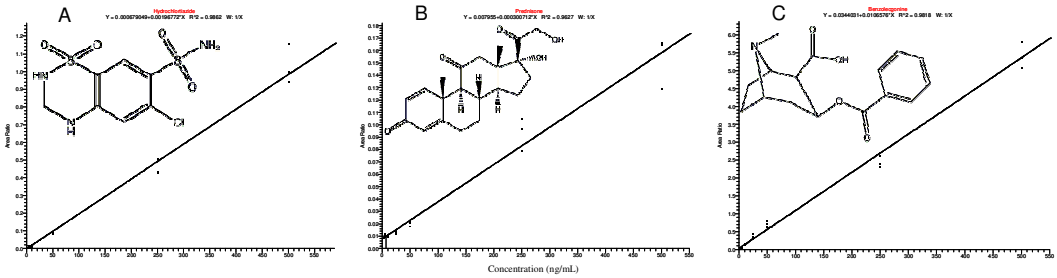

Figure 1 Calibration curve of A) hydrochlorothiazide, B) prednisone and C) benzoylecgonine in human urine.

Method Precision

The within-run accuracy and precision were evaluated on standard area ratio. All standards were injected in triplicates and RSD % on the area ratio (Compound area/ISTD area) were calculated. The mean RSD % obtained (for all standard concentrations) were 10.6±4.2 % for benzoylecgonine, 5.7±4.2 % for hydrochlorothiazide and 11.2±2.3 % for prednisone. For this study, two different ISTD not related to the target compounds were added. Instead, using a deuterated ISTD related to one of the target compound might have improved the precision on the analysis.

Blank Urine Sample Analysis

Five (5) negative control blank human urine samples were analyzed to evaluate potential interference. No interference from urinary matrix was detected over the limit of detection for the target compounds. Operating without chromatographic separation, the LDTD ion source allows high-throughput doping agents screening from different urine sample compositions without suffering from interferences.

LDTD-MS/MS for Unknows Quantification

Real positive urine samples were analyzed with the LDTD and the obtained concentrations are reported in **Table 1**. The samples were previously analyzed in accordance with INRS screening methods and 1 doping agent was found in each sample (concentrations not available). The LDTD method allows to determine positive sample for all samples.

Table 1 Doping agent concentrations in real positive urine samples with LDTD-MS/MS.

		Positive 1	Positive 2	Positive 3	Positive 4
GC-MS/MS	**Doping agent detected**[1]	Modafinil acid	Hydrochlorothiazide	Prednisone	Benzoylecgonine
LDTD-MS/MS	**Hydrochlorothiazide**	< LOD	4961[2] ng/mL	< LOD	< LOD
	Prednisone	< LOD	< LOD	140 ng/mL	< LOD
	Benzoylecgonine	< LOD	< LOD	< LOD	226 ng/mL

[1] Concentration not available, [2] Concentration higher then the limit of linearity

MS Parameters

Collision gas pressure	1.5 mTorr (Argon)
Collision energy (V)	Hydroxythiazide 28 Prednisone 25 Benzoylecgonine 18
Tube lens (V)	Hydroxythiazide 40 Prednisone 90 Benzoylecgonine 51
Scan time	0.050 s
Needle voltage	5000 V
Q1 width	0.70 amu
Q3 width	0.70 amu
Hydroxythiazide SRM transition	296 → 205 amu
Prednisone SRM transition	327 → 285 amu
Benzoylecgonine SRM transition	290 → 128 amu

LDTD Parameters

Laser power pattern	0 to 35 % in 3.0 s Decrease to 0 %
Carrier gas flow	3.0 L/min (Air)

Conclusions

LDTD-MS/MS allows the screening and the quantification of doping agents in human urine sample with a sample-to-sample run time of 8 seconds.

High-throughput screening and analysis with excellent linearity can be achieved using LDTD as ion source in mass spectrometry.

Phytronix Technologies
Parc technologique du Québec métropolitain
4535, boulevard Wilfrid-Hamel, suite 120, Québec (Qc) Canada G1P 2J7
www.phytronix.com