

Application News

Liquid Chromatography Mass Spectrometry

Screening Analysis of Highly Polar Doping Agents in Urine Using 2DLC/MS/MS

No.C142A

The use of performance-enhancing drugs, or "doping," has been recognised for decades and since 1999 the World Anti-Doping Agency (WADA) has governed and harmonized the worldwide sports drug testing efforts. However, these needs are changing and the continuing. discovery of new doping strategies with naturally occurring substances, such as androgenic steroids, prohormones and related metabolites, peptide hormones,

as well as the emergence of designer drugs and the manipulation of blood and blood components results in sports drug testing methods which are capable of a range of tests. In this application news, we report the simultaneous analysis of highly polar doping agents including meldonium and adrenergic agents such as synephrine, norfenefrine, etilefrine, oxilofrine and octopamine using 2D LC/MS/MS.



Exact Mass: 146.1055

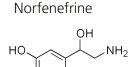


Exact Mass: 181.1103

[Adrenergic agents]

Synephrine

Formula: C9H13NO2 Exact Mass: 167.0946



Formula: C8H11NO2 Exact Mass: 153.079

Etilefrine

Formula: C10H15NO2 Exact Mass: 181.1103

Octopamine

Formula: C8H11NO2 Exact Mass: 153.079

Fig. 1 Structures of 6 Compounds

Table 1 Analytical Conditions

[LC] NexeraX2 System Analytical Column Trapping Column Mobile Phase

: Nucleodur HILIC (100 mm L. × 2 mm I.D., 1.8 μm) : Nucleodur HILIC (20 mm L. x 2 mm I.D., 3 µm)

: A: H₂O + 5 % buffer, B: Acetonitrile + 5 % buffer, C: Acetonitrile + 5 % buffer

(buffer: 200 mM Ammonium Acetate + 0.15 %

glacial acetic acid)

Column Oven Temp. 40 °C Injection Volume : 30 µL [MS] LCMS-8060 Ionization

: ESI (+/-) Nebulizing Gas Flow : 3.0 L/min. Drying Gas Flow : 15.0 L/min. Heating Gas Flow : 15.0 L/min. HB Temp. 500 °C 300 °C DL Temp. Interface Temp. : 400 °C

MRM parameter:

#	Name	Polarity	Q1	Q3	Q3	Ret. Time	CE	CE
#				Qualifier 1	Qualifier 2	(min)	Qualifier 1	Qualifier 1
1	Meldonium	+	147.20	58.25	59.25	8.18	-27	-18
2	Etilefrine	+	182.30	135.25	91.25	5.34	-20	-27
3	Norfenefrine	+	154.20	91.25	65.25	6.01	-21	-35
4	Octopamine	+	154.20	91.25	119.20	6.00	-21	-15
5	Oxilofrine	+	182.30	149.25	105.25	5.69	-20	-22
6	Synefrine	+	168.20	135.20	107.25	5.87	-20	-31
7	Meldonium-d3	+	150.20	62.25	60.25	8.18	-18	-30
8	Etilefrine sulphate	+	262.20	164.15		5.19	-19	
9	Synefrine sulphate	+	248.20	150.25	135.20	5.68	-15	-30
10	Norfenefrine sulphate	+	234.20	136.20	91.20	5.62	-18	-35
11	Etilefrine sulphate_neg	-	260.20	180.20	121.10	5.19	18	39
12	Oxilofrine sulphate_neg	-	260.20	77.10	178.20	5.49	26	12
13	Synefrine sulphate_neg	-	246.20	148.20	106.10	5.70	20	30
14	Norfenefrine sulphate_neg	-	232.20	152.20	121.15	5.69	17	36
15	Octopamine sulphate_neg	-	232.20	134.15	107.10	5.81	22	30

#7 : Internal Standard

#8 \sim 15 : Confirmation of Sulpho-conjugate

Compound list including MRM transitions for unchanged parent drug molecules and corresponding sulfonated metabolites. Rapid polarity switching was used during the analysis to confirm peak identification.

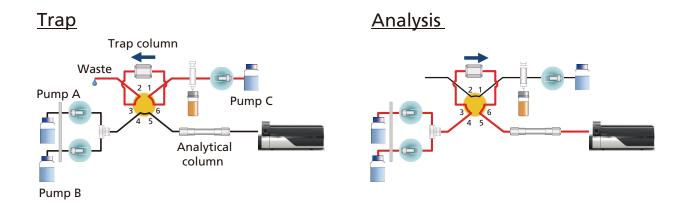


Fig. 2 Flow Diagram of 2D-HILIC System

Diluted urine samples were injected directly onto the 2D HILIC system using a HILIC trapping column for clean-up and pre-concentration followed by an effective HILIC analytical separation.

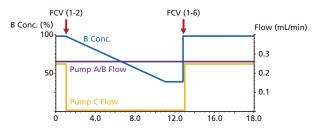


Fig. 3 Flow Rate and Gradient Program

■ Sample Preparation of Urine Sample

- 1. Centrifuge urine samples at 3,000 rpm for 10 min at room temperature.
- 2. Transfer $60~\mu L$ supernatant to new tube and add $10~\mu L$ IS solution (*) and $140~\mu L$ acetonitrile, mix the solution by vortex mixing.
- 3. Centrifuge at 13,000 rpm for 5 min.
- 4. Transfer 180 μL supernatant to vial.
 - (*) Meldonium-d3 in 200 mM Ammonium Acetate

■ Calibration Curves

Fig. 4 shows calibration curves of 6 compounds spiked into urine. Meldonium was included in the World Anti-Doping Agency (WADA) Prohibited List on 1 January 2016, the guidance for meldonium in urine samples collected after 30 September 2016 applies normal results management to samples above a concentration of 100 ng/mL. In this method, the urine calibration range between 1 to 200 ng/mL resulted in a linear response for all compounds with regression coefficients r2 > 0.997.

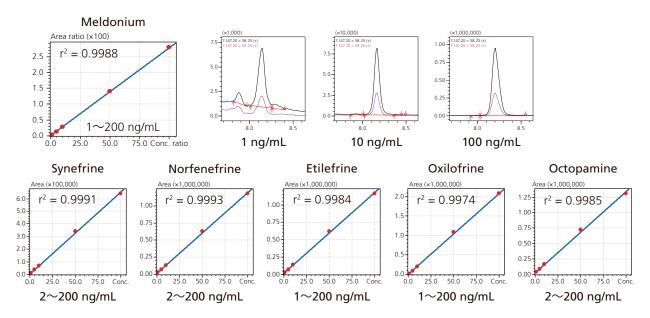


Fig. 4 Calibration Curves and MRM Chromatograms of 6 Compounds

■ Analysis of Synephrine, Etilefrine and Oxilofrineine in Urine

Each urine samples were collected from volunteers being separately administered with synephrine, etilefrine and oxilofrine were analyzed using 2D-HILIC System. In all samples, both the unchanged form and sulphated metabolites were detected.

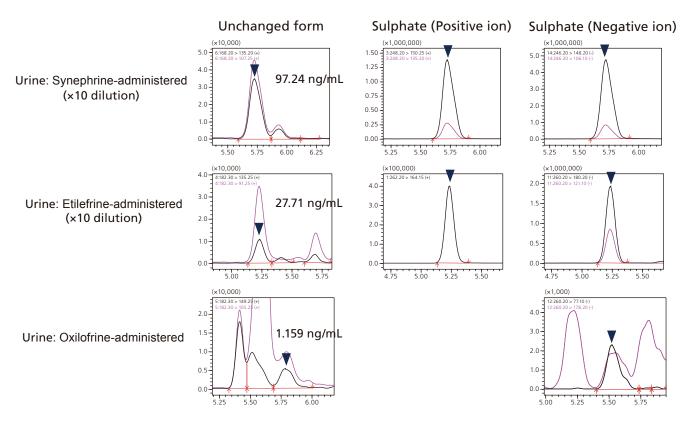
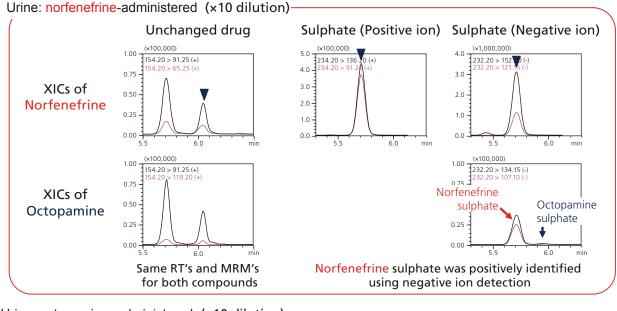


Fig. 5 Results of Urine: Synephrine, Etilefrine and Oxilofrine were separately administered

Distinguishing Norfenefrine and Octopamine in Urine

Norfenefrine is a positional isomer of octopamine resulting in the same retention time and MRM transitions for the unchanged parent drug molecule. However, by detecting the corresponding sulphate metabolite using rapid polarity switching enabled a positive identification.



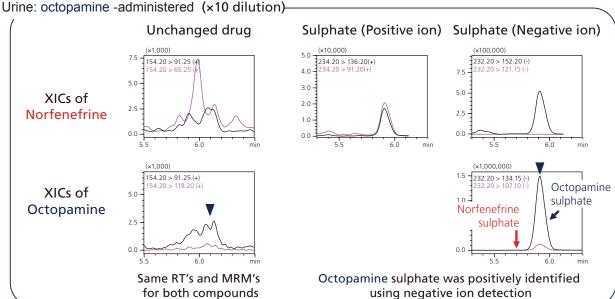


Fig. 6 Results of Urine: Norfenefrine and Octopamine were separately administered

The sample used for this analysis was provided by Anti-Doping Laboratory, LSI Medience Corporation, Tokyo, Japan References: Anal Bioanal. Chem. (2015), 407, 5354-5379

Drug Test. Analysis (2015), 7, 973–979

Notes: • The products mentioned in this article have not received approval for use as medical devices based on the Pharmaceutical and Medica Device Act.

The analytical methods mentioned in this article cannot be used for diagnostic purposes, for Research Use Only (RUO).

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