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Application News

No.C142

Liquid Chromatography Mass Spectrometry

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

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, pro-hormones 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.

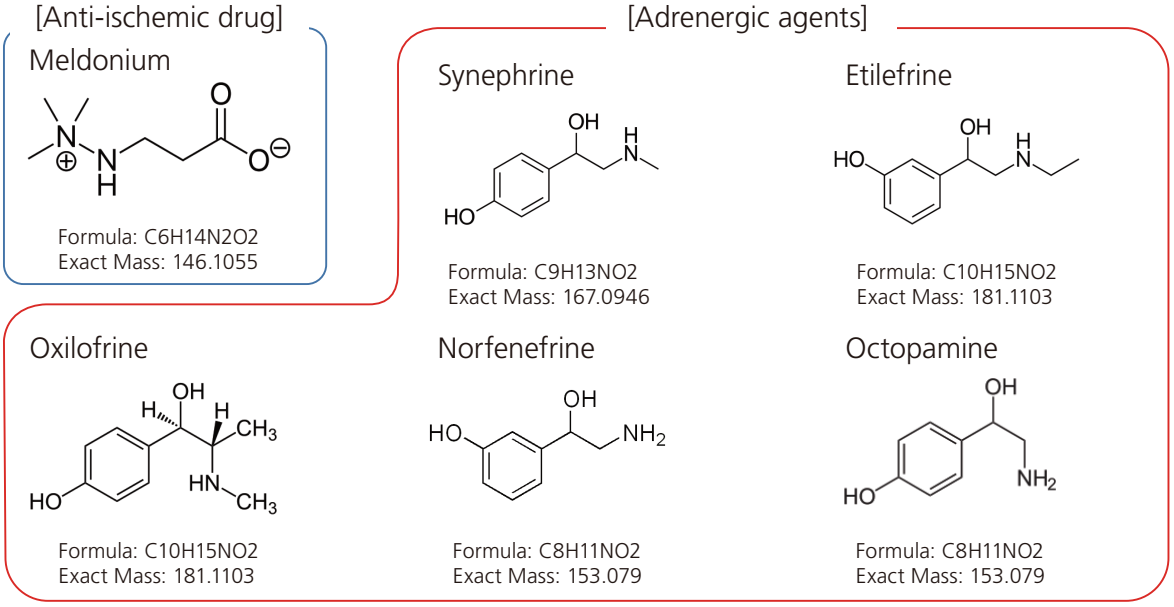

Fig. 1 Structures of 6 Compounds

Table 1 Analytical Conditions

[LC] NexeraX2 System		[MS] LCMS-8060	
Analytical Column	: Nucleodur HILIC (100 mm L. × 2 mm I.D., 1.8 µm)	Ionization	: ESI (+/-)
Trapping Column	: Nucleodur HILIC (20 mm L. × 2 mm I.D., 3 µm)	Nebulizing Gas Flow	: 3.0 L/min.
Mobile Phase	: A: H_2O + 5 % buffer, B: Acetonitrile + 5 % buffer, C: Acetonitrile + 5 % buffer (buffer: 200 mM Ammonium Acetate + 0.15 % glacial acetic acid)	Drying Gas Flow	: 15.0 L/min.
		Heating Gas Flow	: 15.0 L/min.
		HB Temp.	: 500 °C
		DL Temp.	: 300 °C
		Interface Temp.	: 400 °C
Column Oven Temp.	: 40 °C		
Injection Volume	: 30 µL		

MRM parameter:

#	Name	Polarity	Q1	Q3 Qualifier 1	Q3 Qualifier 2	Ret. Time (min)	CE Qualifier 1	CE 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 ～ 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.

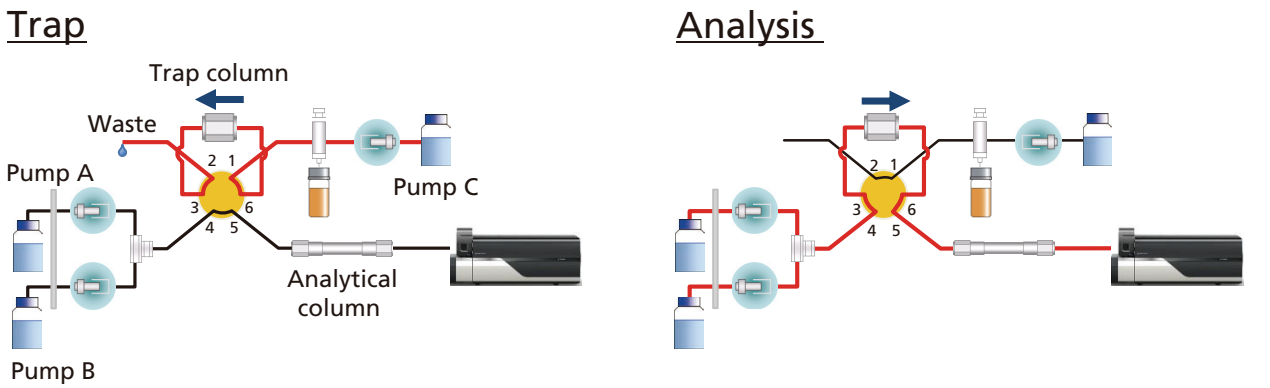

Fig. 2 Flow Diagram of 2D-HILIC System

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.

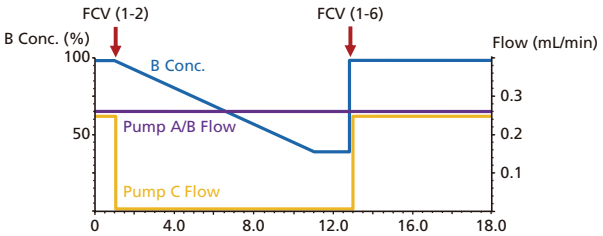

Fig. 3 Flow Rate and Gradient Program

Sample Preparation of Urine Sample

1. Centrifuge urine samples at 3,000 rpm for 10 min at room temperature.
2. Transfer 60 µL supernatant to new tube and add 10 µL IS solution (*) and 140 µL 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.

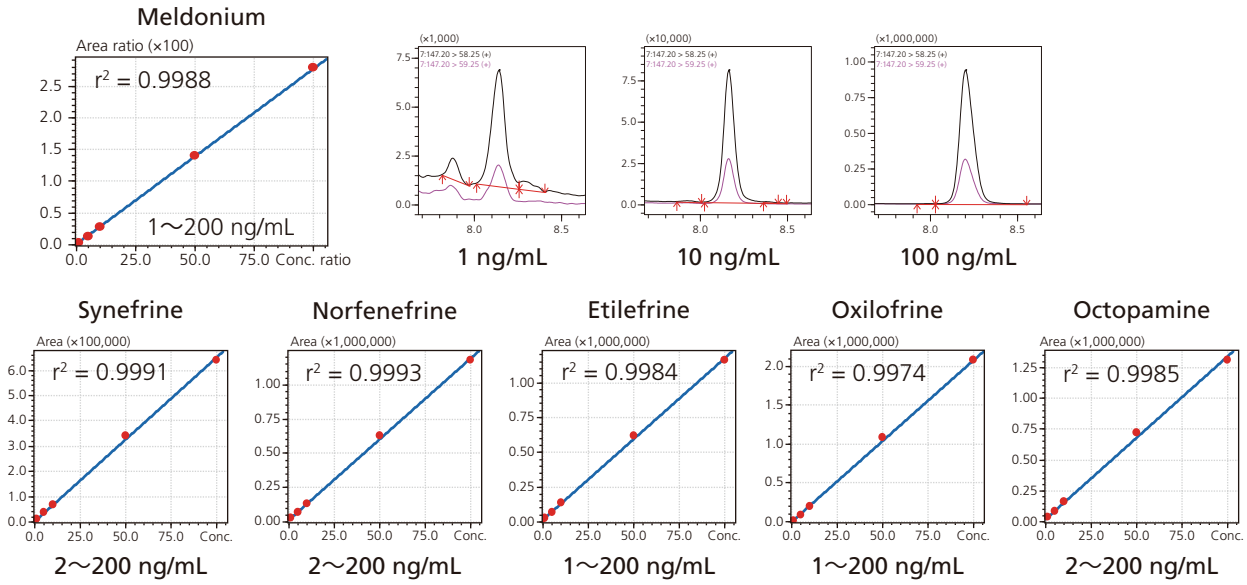

Fig. 4 Calibration Curves and MRM Chromatograms of 6 Compounds

Analysis of Synephrine, Etilefrine and Oxilofrineine in Urine

Urine samples spiked with synephrine, etilefrine and oxilofrine were analyzed using 2D-HILIC System. In all samples, both the unchanged form and sulphated metabolites were detected.

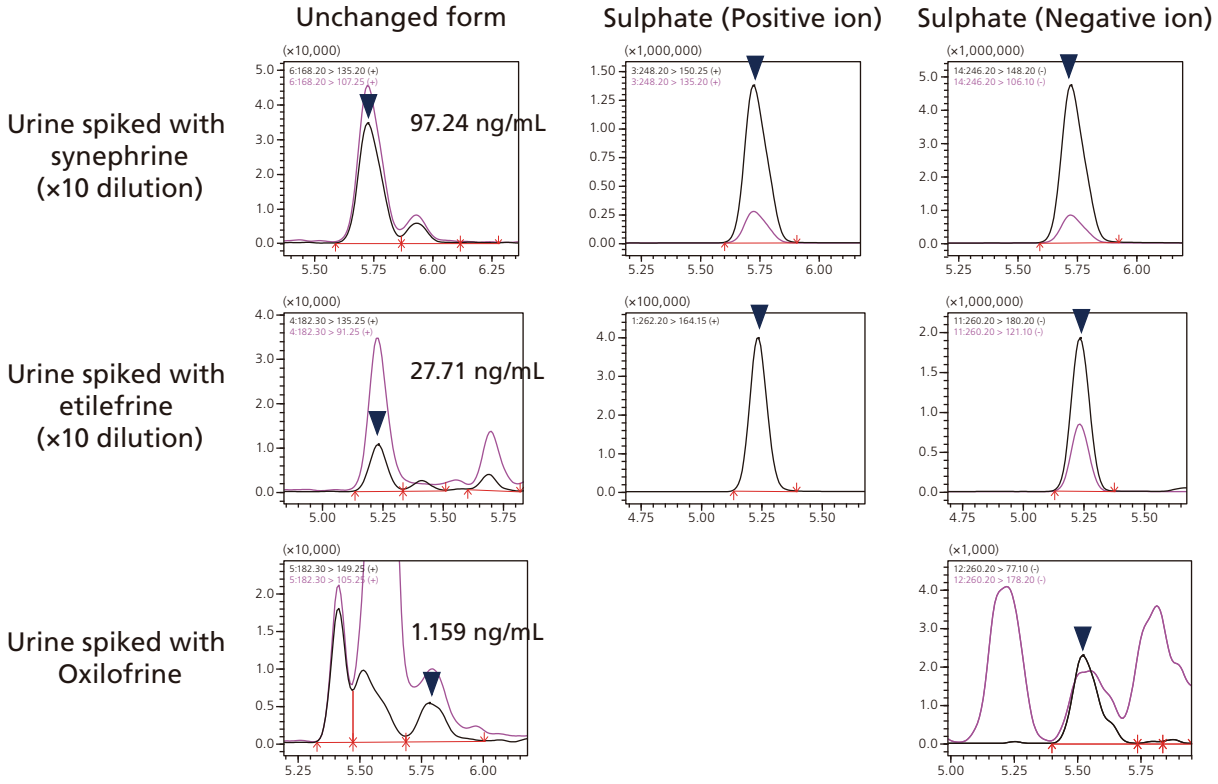

Fig. 5 Results of Urine Dosed with Synephrine, Etilefrine and Oxilofrine

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.

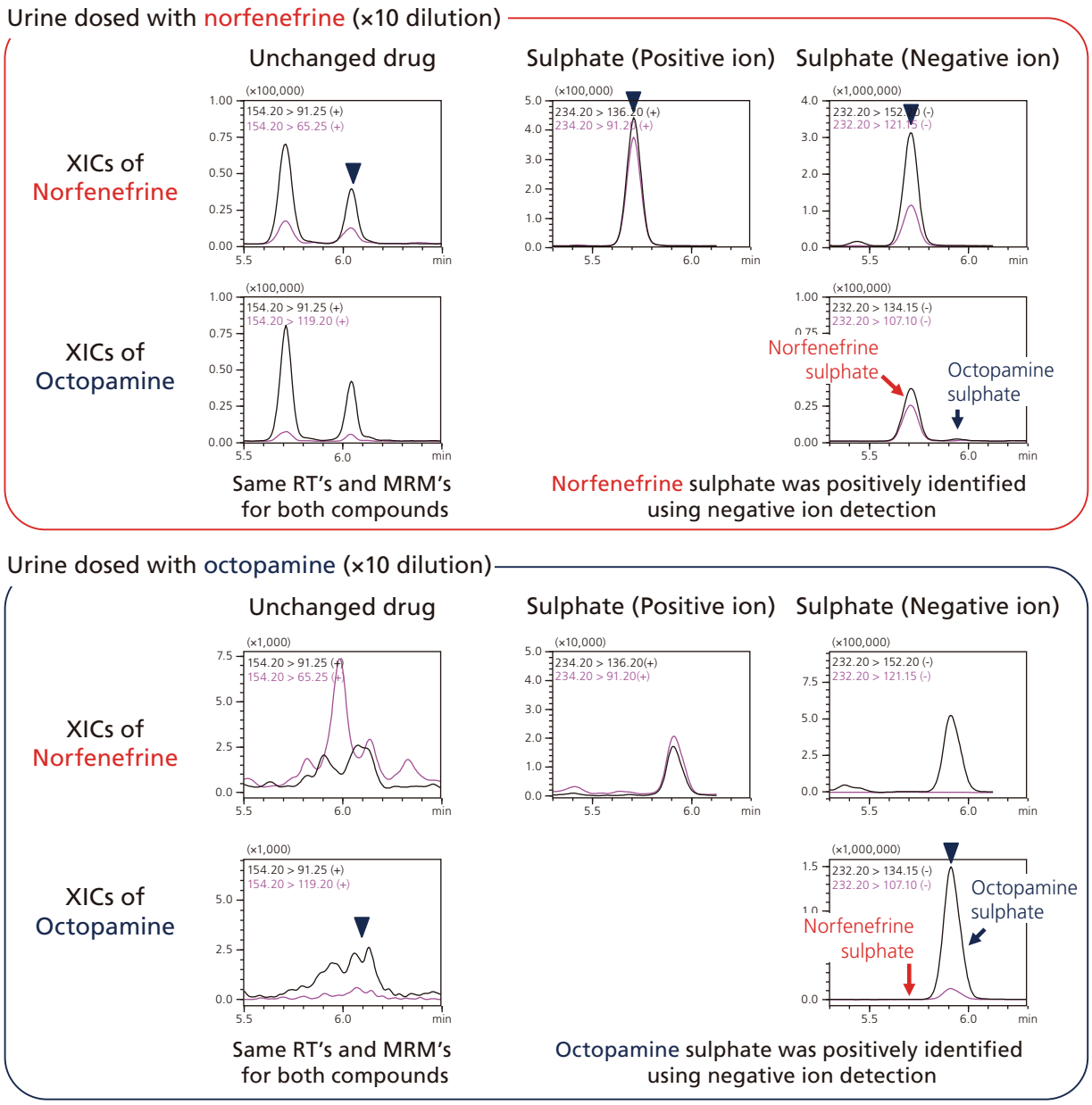

Fig. 6 Results of Urine Dosed with Norfenefrine and Octopamine

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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