

Application News

No. C142A

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.

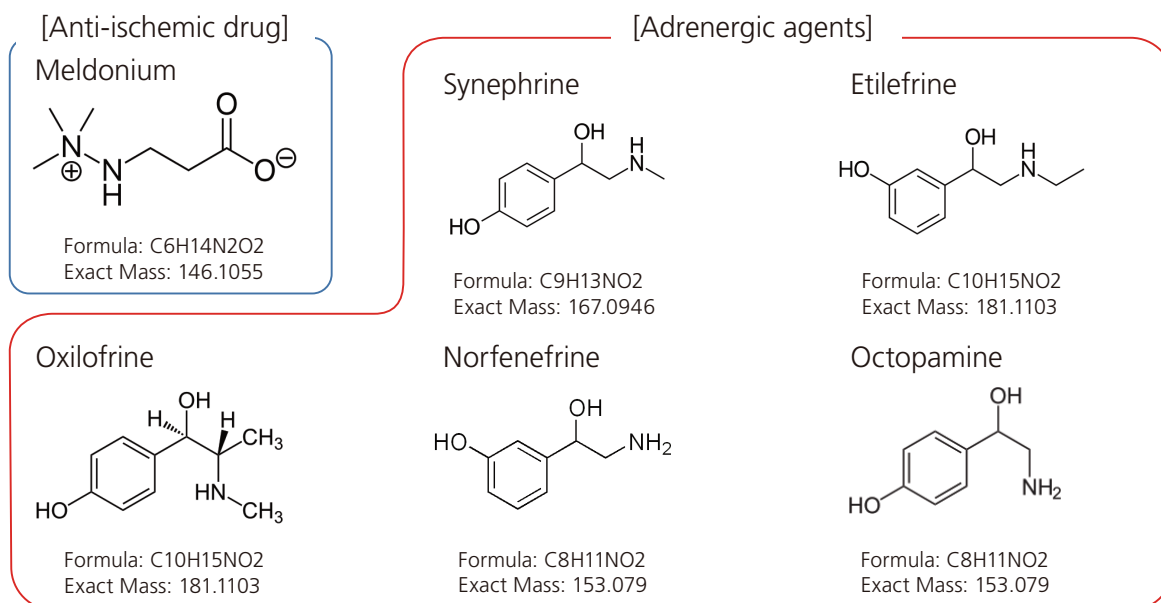


Fig. 1 Structures of 6 Compounds

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 ₂ O + 5 % buffer,	Drying Gas Flow : 15.0 L/min.
B: Acetonitrile + 5 % buffer,	Heating Gas Flow : 15.0 L/min.
C: Acetonitrile + 5 % buffer	HB Temp. : 500 °C
(buffer: 200 mM Ammonium Acetate + 0.15 % glacial acetic acid)	DL Temp. : 300 °C
Column Oven Temp. : 40 °C	Interface Temp. : 400 °C
Injection Volume : 30 μL	

MRM parameter:

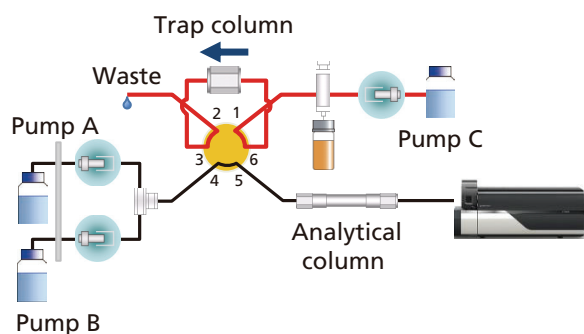
#	Name	Polarity	Q1	Q3 Qualifier 1	Q3 Qualifier 2	Ret. Time (min)	CE Qualifier 1	CE Qualifier 1
1	Meldonium	+	147.20	58.25	59.25	8.18	-27	-18
2	Etilefrine	+	182.30	135.25	91.25	5.34	-20	-27
3	Norfenefrine	+	154.20	91.25	65.25	6.01	-21	-35
4	Octopamine	+	154.20	91.25	119.20	6.00	-21	-15
5	Oxilofrine	+	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.

Trap



Analysis

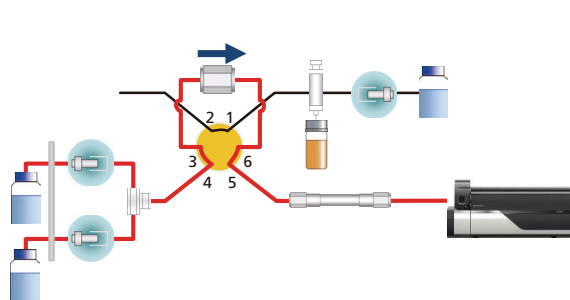


Fig. 2 Flow Diagram of 2D-HILIC System

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.

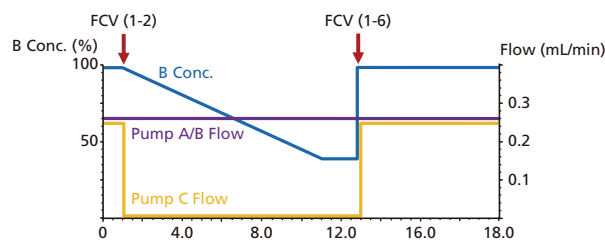


Fig. 3 Flow Rate and Gradient Program

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 $r^2 > 0.997$.

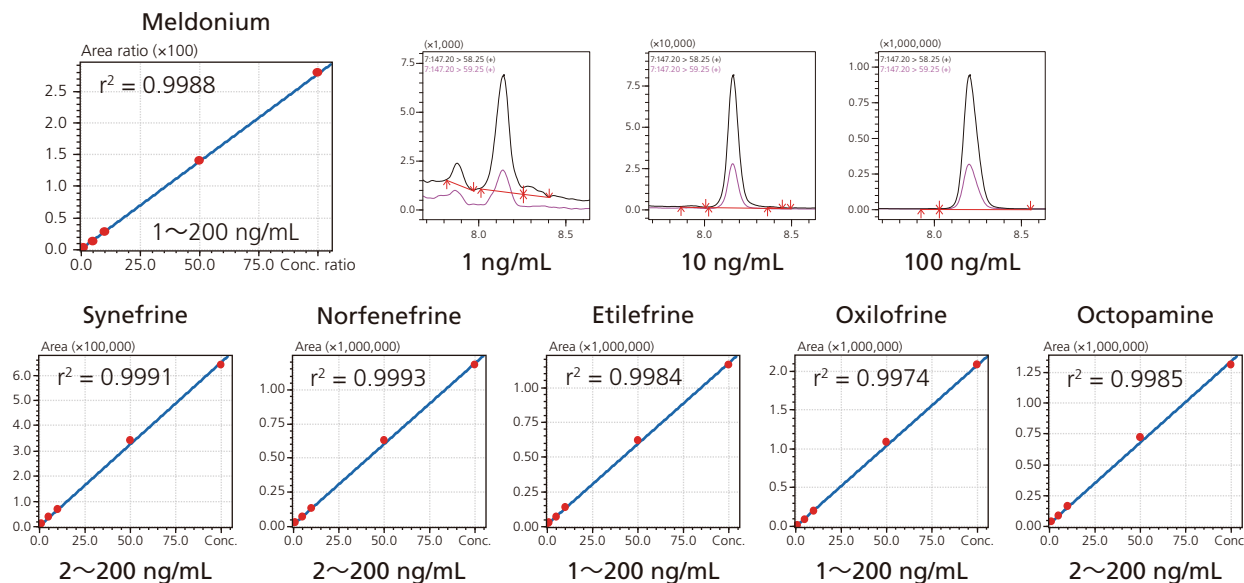


Fig. 4 Calibration Curves and MRM Chromatograms of 6 Compounds

■ Analysis of Synefrine, Etilefrine and Oxilofrine in Urine

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

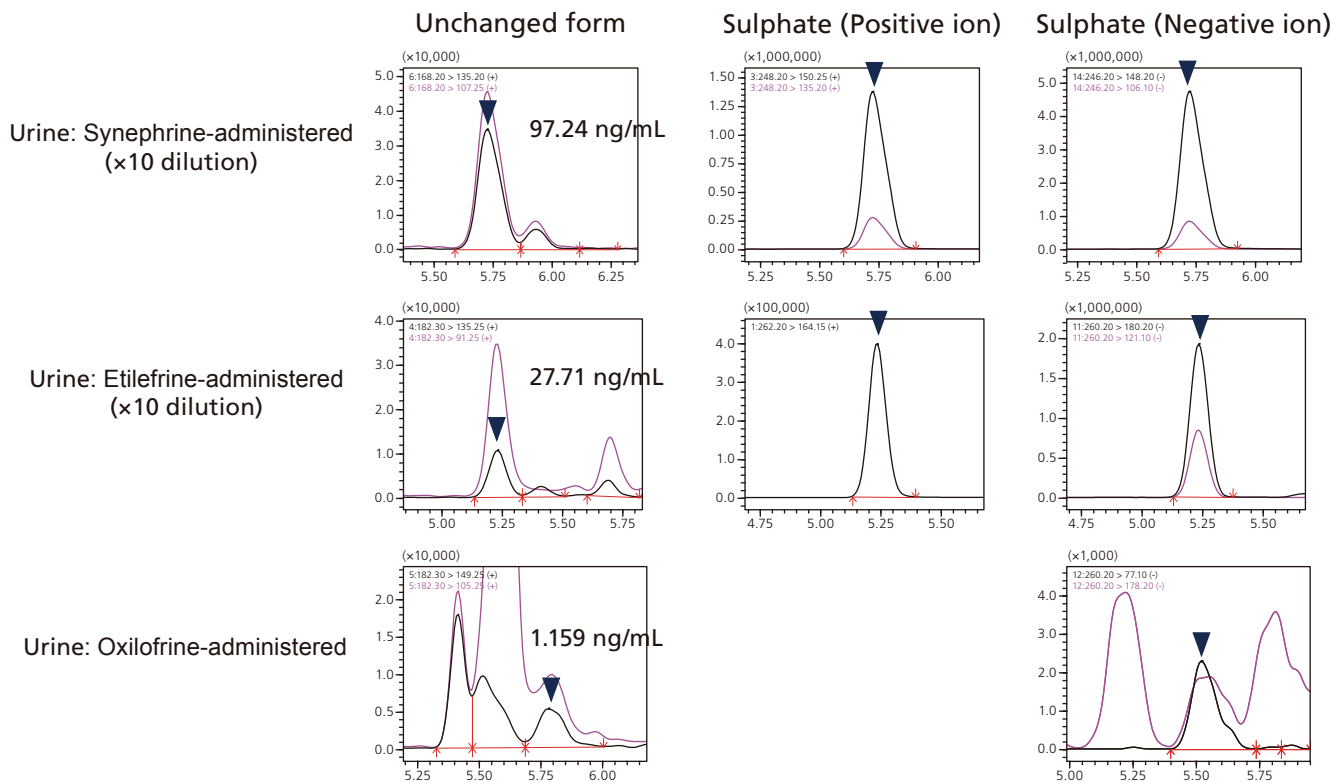
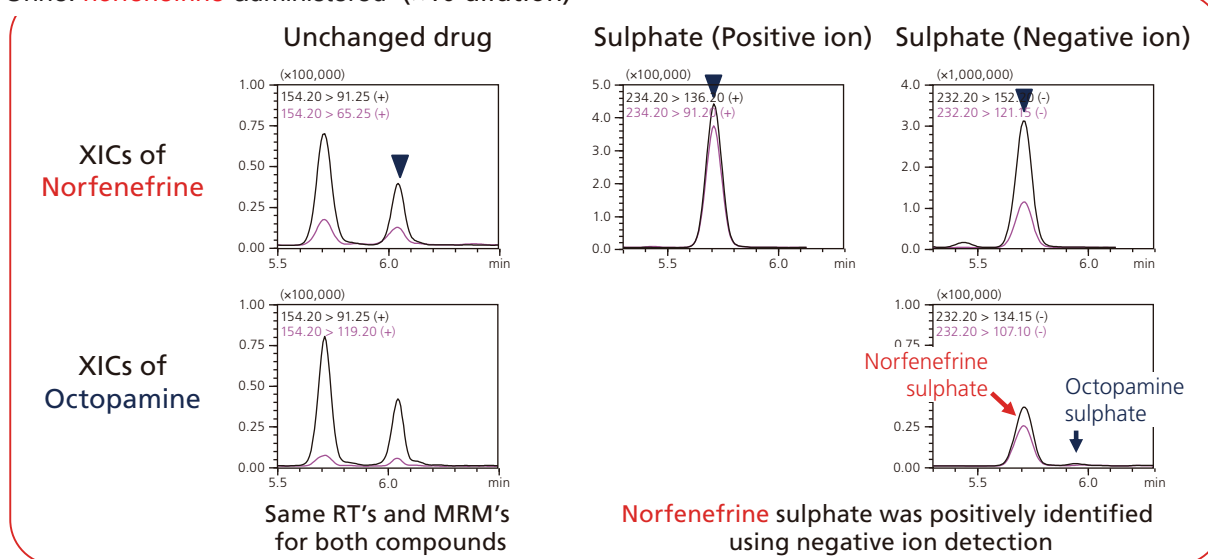


Fig. 5 Results of Urine: Synefrine, Etilefrine and Oxilofrine were separately administered

■ 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.

Urine: **norfenefrine**-administered (×10 dilution)



Urine: **octopamine** -administered (×10 dilution)

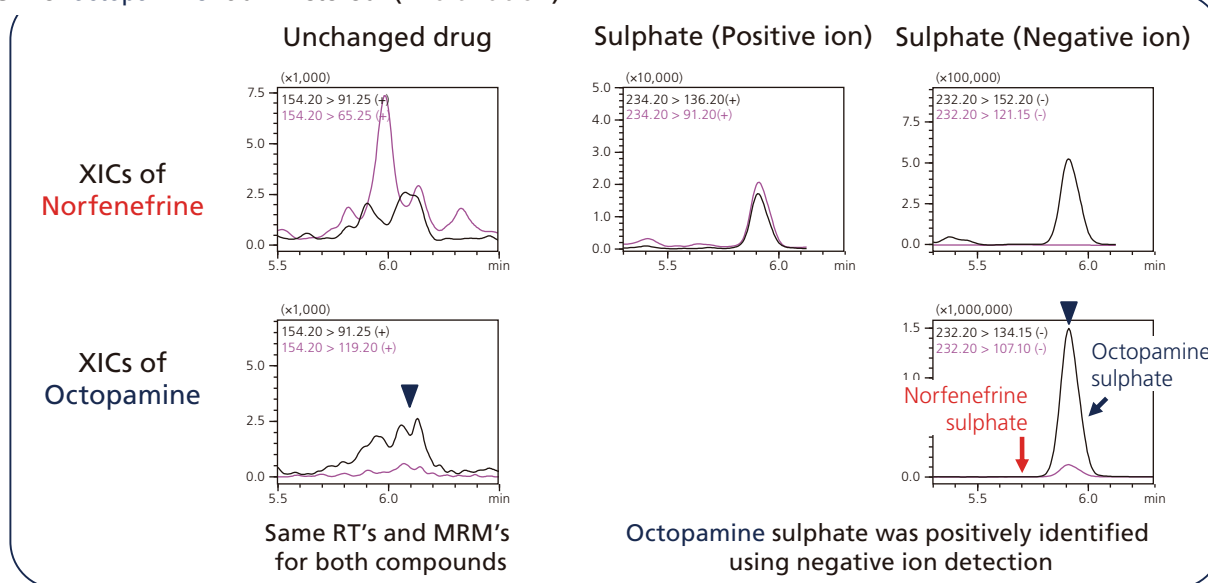


Fig. 6 Results of Urine: Norfenefrine and Octopamine were separately administered

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 Medical Device Act.

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

First Edition: Dec. 2016
Second Edition: Jan. 2017



For Research Use Only. Not for use in diagnostic procedures.

This publication may contain references to products that are not available in your country. Please contact us to check the availability of these products in your country.

The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu. Company names, product/service names and logos used in this publication are trademarks and trade names of Shimadzu Corporation or its affiliates, whether or not they are used with trademark symbol "TM" or "®". Third-party trademarks and trade names may be used in this publication to refer to either the entities or their products/services. Shimadzu disclaims any proprietary interest in trademarks and trade names other than its own.

The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.

© Shimadzu Corporation, 2016