methods and protocols

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Article: Supplementary Materials

An Ultra-High-Performance Liquid Chromatography Coupled with Tandem Mass Spectrometry Method with Online Solid-Phase Extraction Sample Preparation for the High-Throughput and Sensitive Determination of Ostarine in Human Urine

Kristián Slíž [1,2], Juraj Piešťanský [3] and Peter Mikuš [1,2,*]

[1] Department of Pharmaceutical Analysis and Nuclear Pharmacy, Faculty of Pharmacy, Comenius University Bratislava, Odbojárov 10, 832 32 Bratislava, Slovakia; kristian.sliz@uniba.sk
[2] Toxicologic and Antidoping Centre, Faculty of Pharmacy, Comenius University Bratislava, Odbojárov 10, 832 32 Bratislava, Slovakia
[3] Department of Galenic Pharmacy, Faculty of Pharmacy, Comenius University Bratislava, Odbojárov 10, 832 32 Bratislava, Slovakia; piestansky@fpharm.uniba.sk
* Correspondence: mikus@fpharm.uniba.sk; Tel.: +421-2-50-117-243

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Table S1. Comparison of UHPLC-MS/MS methods for the analysis of ostarine in human urine.

Sample preparation	Column	Detector	LOD (ng/mL)	Ref.
Dilute-and-shoot	Nucleoshell RP18 Plus (2.0 × 50 mm, 2.7 μm)	Q-Orbitrap	< 0.1	[16]
SPE	Kinetex C18 (2.1 × 100 mm, 1.7 μm)	Orbitrap	–	[17]
Enzym. + SPE	Cortecs C18 (2.1 x 100 mm, 2.7 μm	QqQ	0.005	[18]
Enzym. + LLE	Luna Omega Polar C18 (2.1 × 100 mm, 1.6 μm)	QqQ	0.005	[19]
Enzym. + LLE	Nucleodur C18 Pyramid (2.0 × 50 mm, 1.8 μm)	Q-Orbitrap	0.02	[20]
Enzym. + LLE	Ascentis C18 (2.1 x 150 mm, 2.7 μm)	QqQ	0.1	[21]
DLLME	Kinetex C18 (2.1 × 100 mm, 1.7 μm)	QqQ	0.05	[22]
Dilute-and-shoot + Online-SPE	Kinetex EVO C18 (2.1 × 100 mm, 2.6 μm)	QqQ	0.0005	This work

LOD, limit of detection; Ref., reference; Enzym., enzymatic hydrolysis; SPE, solid-phase extraction; LLE, liquid-liquid extraction; DLLME, dispersive liquid-liquid microextraction; Online-SPE, online solid-phase extraction; Q, quadrupole; QqQ, triple quadrupole.

Table S2. Mass transitions and optimized mass spectrometer parameters applied for mass reaction monitoring (MRM).

Substance	MRM transition (*m*/*z*)	Species of the parent ion	Frag (V)	CE (V)
Ostarine	390.1 → 120.2 (quantifier)	$[M + H]^+$	140	36
Ostarine	390.1 → 193.2 (qualifier)	$[M + H]^+$	140	40
Andarine (IS)	442.1 → 108.1 (quantifier)	$[M + H]^+$	140	40
Andarine (IS)	442.1 → 148.1 (qualifier)	$[M + H]^+$	140	32

Frag, fragmentor voltage; CE, collision energy; IS, internal standard.

Table S3. WADA's chromatographic identification criteria: retention time comparison.

No.	Retention time: IS (min)			Retention time: ostarine (min)		
	Sample	Reference	Max. tolerated diff. ± 1%	Sample	Reference	Max. tolerated diff. ± 1%
1	18.80	18.78	+ 0.1%	18.88	18.86	+ 0.1%
2	18.80		+ 0.1%	18.88		+ 0.1%
3	18.80		+ 0.1%	18.88		+ 0.1%
4	18.80		+ 0.1%	18.88		+ 0.1%
5	18.80		+ 0.1%	18.88		+ 0.1%
6	18.80		+ 0.1%	18.88		+ 0.1%
7	18.80		+ 0.1%	18.88		+ 0.1%
8	18.80		+ 0.1%	18.88		+ 0.1%
9	18.81		+ 0.2%	18.89		+ 0.2%
10	18.81		+ 0.2%	18.89		+ 0.2%

No, sample number; IS, internal standard; Diff., difference.

Table S4. WADA's mass spectrometric identification criteria: relative abundance of two diagnostic ions comparison.

No.	Diagnostic ion (m/z)	RA (%)		Difference (%)	Tolerance window (%)
		Sample	Reference		
1	390.1 → 120.2	100	100	- 1.2	34.83–52.25 (± 20%)
	390.1 → 193.2	43.02	43.54		
2	390.1 → 120.2	100	100	- 10.4	
	390.1 → 193.2	39.02	43.54		
3	390.1 → 120.2	100	100	- 13.9	
	390.1 → 193.2	37.50	43.54		
4	390.1 → 120.2	100	100	- 6.5	
	390.1 → 193.2	40.70	43.54		
5	390.1 → 120.2	100	100	- 7.7	
	390.1 → 193.2	41.18	43.54		
6	390.1 → 120.2	100	100	- 2.4	
	390.1 → 193.2	42.5	43.54		
7	390.1 → 120.2	100	100	+ 6.7	
	390.1 → 193.2	46.46	43.54		
8	390.1 → 120.2	100	100	+ 6.7	
	390.1 → 193.2	46.46	43.54		
9	390.1 → 120.2	100	100	- 1.8	
	390.1 → 193.2	42.75	43.54		
10	390.1 → 120.2	100	100	- 5.9	
	390.1 → 193.2	40.95	43.54		

No, sample number; RA, relative abundance.

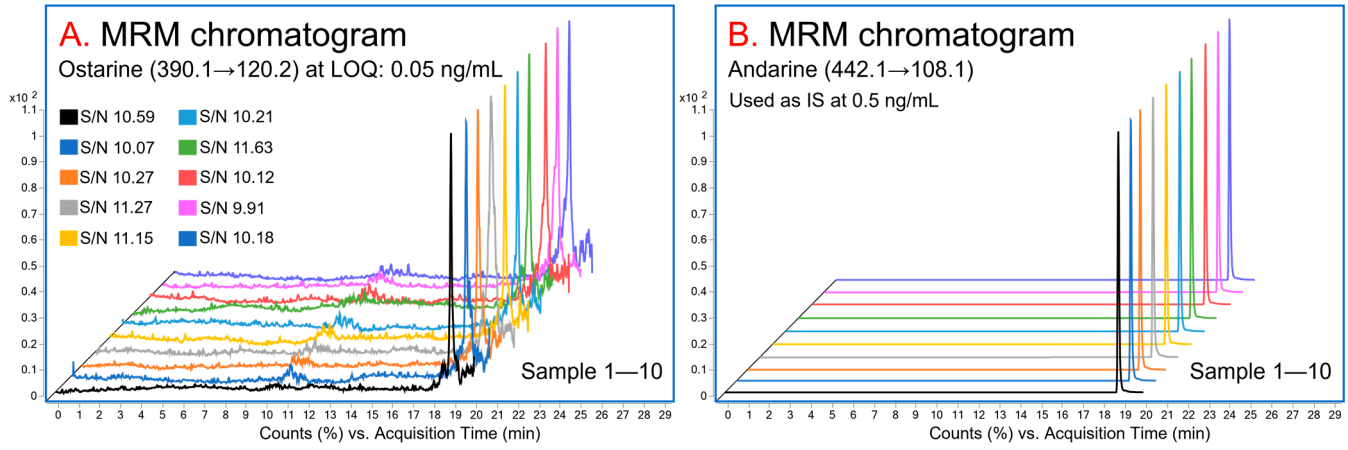

Figure S1. Illustrative chromatograms of ten different human urine samples measured at the LOQ level of ostarine demonstrating variability of sample fractions after online-SPE heart-cut, UHPLC analytical separation and MS/MS detection.