Analytica Chimica Acta 1152 (2021) 338257

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Ion mobility-high resolution mass spectrometry in anti-doping analysis. Part I: Implementation of a screening method with the assessment of a library of substances prohibited in sports

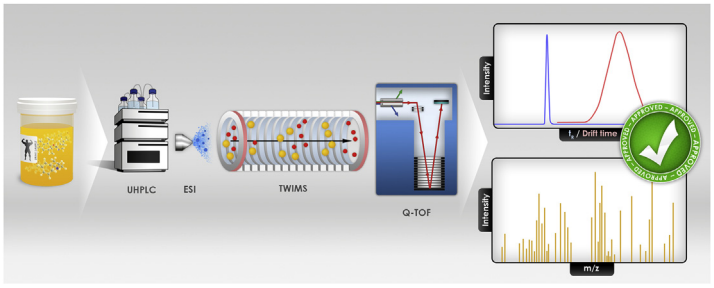
Kateřina Plachká [a], Julian Pezzatti [b, c], Alessandro Musenga [d], Raul Nicoli [d], Tiia Kuuranne [d], Serge Rudaz [b, c], Lucie Nováková [a], Davy Guillarme [b, c, *]

[a] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Heyrovského 1203, 500 05, Hradec Králové, Czech Republic
[b] School of Pharmaceutical Sciences, University of Geneva, CMU-Rue Michel Servet 1, 1211, Geneva 4, Switzerland
[c] Institute of Pharmaceutical Sciences of Western Switzerland (ISPSO), University of Geneva, CMU-Rue Michel Servet 1, 1211, Geneva 4, Switzerland
[d] Swiss Laboratory for Doping Analyses, University Center of Legal Medicine Lausanne and Geneva, Lausanne University Hospital and University of Lausanne, Switzerland

HIGHLIGHTS

- UHPLC-IM-HRMS method was developed for the analysis of 192 doping agents.
- A database including retention times, CCS values, and *m/z* ratios was obtained.
- The intraday, interday, and interweek RSD of retention times and CCS values were $\leq$2%.
- The spectra filtration by IMS dimension led to 86% less interfering peaks in average.
- Additional selectivity of IMS was beneficial for separation of isomeric/isobaric compounds.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 23 September 2020
Received in revised form
22 January 2021
Accepted 25 January 2021
Available online 31 January 2021

Keywords:
Ion mobility spectrometry
Ultra-high performance liquid chromatography
Doping analysis
Collision cross section
High resolution mass spectrometry

ABSTRACT

In this series of two papers, 192 doping agents belonging to the classes of stimulants, narcotics, cannabinoids, diuretics, β2-agonists, β-blockers, anabolic agents, and hormone and metabolic modulators were investigated, with the aim to assess the benefits and limitations of ion mobility spectrometry (IMS) in combination with ultra-high performance liquid chromatography (UHPLC) and high resolution mass spectrometry (HRMS) in anti-doping analysis.

In this first part, a generic UHPLC-IM-HRMS method was successfully developed to analyze these 192 doping agents in standard solutions and urine samples, and an exhaustive database including retention times, $^{TW}CCS_{N2}$ values, and *m/z* ratios was constructed. Urine samples were analyzed using either a simple "dilute and shoot" procedure or a supported liquid-liquid extraction (SLE) procedure, depending on the physicochemical properties of the compounds and sensitivity criteria established by the World Anti-Doping Agency (WADA) as the minimum required performance levels (MRPL). Then, the precision of the generic UHPLC-IM-HRMS method was assessed as intraday, interday as well as interweek variation of UHPLC retention times and $^{TW}CCS_{N2}$ values, for which RSD the values were always lower than 2% in urine

* Corresponding author. School of Pharmaceutical Sciences, University of Geneva, CMU-Rue Michel Servet 1, 1211, Geneva 4, Switzerland.
E-mail address: Davy.Guillarme@unige.ch (D. Guillarme).

https://doi.org/10.1016/j.aca.2021.338257

samples. The possibility to filter MS data using IMS dimension was also investigated, and in average, the application of IMS filtration provided low energy MS spectra with 86% less interfering peaks in both standard and urine samples. Therefore, the filtered MS spectra allowed for an easier interpretation and a lower risk of false positive result interpretations. Finally, IMS also offers additional selectivity to the UHPLC-HRMS enabling to separate isobaric and isomeric substances. Among the selected set of 192 doping agents, there were 30 pairs of isobaric or isomeric compounds, and only two pairs could not be resolved under the developed conditions. This illustrates the potential of adding ion mobility to UHPLC-HRMS in anti-doping analyses.

1. Introduction

Anti-doping analysis is challenging domain of analyses in many aspects. Each year, an updated version of the WADA Prohibited List [1] is released, with the inclusion of new substances and/or classes [2]. The discovery of emerging substances potentially misused in sport is the first step in the implementation of an anti-doping strategy and for an efficient detection, the advances in the analytical instrumentation and methods should be implemented for the analysis of anti-doping samples. As knowledge in anti-doping science expands, laboratories are obliged to update their analytical strategies. In recent years, doping laboratories have invested considerable resources to improve their methods, not just to improve the selectivity and to increase sample throughput, but also to include the new target substances in compliance with the Prohibited List [3].

The anti-doping analysis consist of initial testing procedures (ITP, "screening"), followed by confirmatory analyses of the suspect samples. Although only a limited number of samples require the dedicated confirmation procedure, each urine sample undergoes all the various ITPs that are designed to cover the entire prohibited list. Good performance of the ITPs increases productivity, also by limiting the number of time-consuming confirmatory analyses to a necessary minimum. For these reasons, method selectivity is a key aspect, not only to provide the certainty of identification for confirmatory procedures, but also to improve the performance of the screening procedures, reducing time and costs of the overall analytical process [4,5].

Over the years, liquid chromatography coupled to mass spectrometry (LC-MS) has gained increasing popularity in anti-doping analyses. Several multi-component studies including among 20–150 compounds were reported using ultra-high performance liquid chromatography (UHPLC) for high-throughput screenings in doping control [4]. Similarly to our method, most of them are carried out on reversed-phase stationary phase, particularly C18, within 9–12 min with MS/MS detection [6–10]. Despite the latest advances and the already widespread utilization of high resolution MS, additional selectivity is often desired [3–5]. To these demands, the introduction of ion mobility-high resolution mass spectrometry (IM-HRMS) could provide a viable response.

Ion mobility spectrometry (IMS) is a post-ionization separation technique that enables the separation of ions in a mobility cell according to their size, shape, and charge in the gas phase (generally nitrogen or helium) under the influence of an electric field. For some of the different commercially available IMS technologies, the drift times, i.e. mobility of the ions in the mobility cell, can be converted to collision cross section (CCS) values. While drift times are instrument dependent, CCS values represent a structural property of ionized molecules that is related to their shape in a specific gas, temperature, and reduced field strength environment. Therefore, they should be comparable between different instruments and/or laboratories operating in the same experimental conditions [11]. Several papers have already focused on the variability study of CCS values between different IMS techniques, for example TWIMS *vs* DTIMS [12] and between laboratories [13,14] showing variability within 1–2% for most of the compounds. To this extent, CCS values offer a supplementary structural information on the detected molecules along with retention time, exact mass, isotopic pattern, and tandem MS/MS information [15,16]. CCS values can be obtained by either drift tube ion mobility spectrometry (DTIMS), travelling wave ion mobility spectrometry (TWIMS) or trapped ion mobility spectrometry (TIMS) [16]. Primary DTIMS (i.e., stepped field CCS method) is classified as a primary IM method in which measured drift times can be directly converted into CCS values. Secondary DTIMS (i.e., single-field CCS method), TWIMS, and TIMS are classified as secondary IM methods and require an instrumental calibration using reference compounds to determine CCS values [17].

Since their arrival on the market, IM-HRMS instruments are becoming increasingly popular in many fields, particularly where the complexity of the target analytes or the matrices require an additional separation capability, such as food sciences, biopharmaceutical analysis, "omics" sciences (e.g. lipidomics, metabolomics, etc.), forensics, doping control, etc. [16,18–22]. In these different fields, IMS represents a complementary and powerful analytical technique to facilitate the identification and characterization of analytes of interest, responding to the increasing demand for additional selectivity.

The continuous need to update methods and the potential for retrospective analyses have contributed to wide spreading across anti-doping laboratories of the so-called untargeted screening methods, based on high-resolution mass spectrometry [23–29]. By operating in scan mode, these methods permit the full data acquisition. The subsequent inclusion of new substances does not require changes in the acquisition method, and the search for additional substances can be performed in pre-acquired samples by data reprocessing. Basically, current HRMS instruments enable two advanced data acquisition approaches [30], data dependent analysis (DDA) and data independent analysis (DIA). In DDA, an intensity threshold is set-up to trigger another data acquisition MS function, such as acquisition of MS/MS spectra of a selected precursor ion in most instances. However, the precursor selection is not always correct and may result in missing information for some relevant precursors. In DIA analysis, MS and MS/MS spectra are acquired for all present ions automatically within one run. These types of methods may also be designated as all ion fragmentation modes represented by MS^E (Waters) and SWATH (Sequential Windowed Acquisition of All Theoretical Fragment Ion, AB Sciex), where the product ions are generated without precursor ion selection, with the advantage of flexibility, but at the cost of specificity, unless there are tools to link a specific fragment ion to a certain parent.

IM-HRMS instruments can offer advantages in this respect. With IM-HRMS, not only peak capacity and dynamic range have been increased, but also the performance of all ion fragmentation data analysis can be improved [11,31]. LC peaks with similar retention times, peak shapes, precursor mass to charge ratio (*m/z*), and some similar fragments can be deconvoluted using IMS in AIF/MS^E mode, as it allows the drift time based separation of chromatographically coeluting LC precursor ions before fragmentation [15,31,32].

In anti-doping analysis, only limited investigation has been performed so far despite a great potential to adopt IMS on a wide scale. One advantage of IMS is the reduction of background noise by filtering the signal based on the mobility value, with the outcome of increased sensitivity. As an example, this has been successfully shown with steroid analytes using differential ion mobility [33,34] or with travelling wave drift tube ion mobility [35]. A similar approach was used for the analysis of larger molecules such as growth hormone releasing peptides and other low molecular mass peptides [36]. By filtering data according to their mobility values, interferences could be eliminated, thus increasing the overall sensitivity.

The benefit of IMS was also investigated to improve separation of the analytes of interest. In anti-doping analysis, steroids analysis has been the main field attracting interest for IMS application [37,38], particularly in attempts to resolve isomeric compounds [39], though this has proven challenging and not always possible, unless additional steps such as derivatization are applied [40]. In metabolite discovery, IMS was applied for the structural elucidation of metabolites of the anabolic agent stanozolol, as a complementary information to confirm the location of glucuronidation [41].

These studies are usually targeting a specific class of analytes or a specific analytical problem. The capability to analyze a very diverse cohort of substances remains one of the main challenges in anti-doping analysis. However, a thorough investigation of the potential of IMS over a large cohort of doping agents across different classes of compounds (Table 1) has not been performed so far.

The aim of this study was to present the first large retention time - $^{TW}CCS_{N2}$ (travelling wave collision cross section in nitrogen buffer gas) database for doping agents, as well as to discuss the potential of UHPLC-IM-HRMS at initial testing procedure stage of anti-doping analyses. The database covers almost 200 doping agents across the various classes of the WADA prohibited list, including stimulants, narcotics, cannabinoids, diuretics, β2-agonists, β-blockers, anabolic agents, and hormone and metabolic modulators. Robustness of the method was evaluated by taking into consideration the intraday, interday, and interweek variability of retention times and $^{TW}CCS_{N2}$ values of all target substances at different concentrations in mixtures of standards and in human urine samples. Moreover, spiked urine samples were used to assess the matrix effect on signal and $^{TW}CCS_{N2}$ variabilities. Finally, the capacity of IMS to filter the obtained precursor and product ion spectra was also evaluated.

2. Experimental part

2.1. Reagents and analytes

The analyzed compounds are listed in Table 2. All analytes as well as β-glucuronidase from *Escherichia coli* were provided by the Swiss Laboratory for Doping Analyses. Acetonitrile (ACN) and water of UHPLC/MS grade were obtained from Fisher (FisherScientific, Loughborough, UK). UHPLC-MS grade formic acid (FA) was supplied by Biosolve (Valkenswaard, Netherlands). Monopotassium phosphate, dipotassium phosphate, and methyl *tert*-butyl ether were purchased from Sigma-Aldrich (Buchs, Switzerland).

2.2. Sample preparation of biological samples

A pool of blank urines was prepared by mixing urine samples obtained from six healthy volunteers. Standard stock solutions were prepared in methanol and subsequently diluted by water. Urine aliquots of 1 mL were spiked with mixtures of target compounds in water to obtain six different concentration levels.

The analyzed doping agents were divided into two groups, based on the sensitivity requirements by WADA, the metabolism, and the sample preparation required. Group I contained 102 compounds including especially stimulants and narcotics. They were divided into three mixtures measured at 0.1, 1, 5, 10, 50, and 100 ppb. A “dilute and shoot” approach was used for sample preparation, using a 5-fold dilution with water. Group II contained 90 compounds including steroids, glucocorticoids, and hormone and metabolic modulators. They were also divided into three mixtures measured at 1, 5, 10, 50, 100, and 500 ppb. Supported liquid-liquid extraction (SLE) was used as the sample preparation method using the following protocol. Phosphate buffer (200 μL, 0.8 M, pH 7.0) and 30 μL of β-glucuronidase enzyme were added to 1 mL urine sample followed by 1 h incubation at 50 °C. A positive pressure manifold Biotage PRESSURE+ 96 with 3 psi (Biotage, Uppsala, Sweden) was used for the loading on Isolute SLE+ 48-well plates. After 5 min waiting time, the elution was carried out by percolating 3 mL methyl *tert*-butyl ether through the wells into a 48-well collection plate using positive pressure again for a few seconds. The extracted samples were evaporated using a SpeedVac (ThermoFisher, Langenselbold, Germany) at room temperature for 45 min. For the sample reconstitution, 100 μL ACN/water mixture (1:1 v/v) and 5 min agitation were used.

The same procedures were used for preparation of post-extraction spiked blank urine samples for the evaluation of the matrix effect (ME). In this case, blank urine was used for the SLE and the samples were then reconstituted in ACN/water (1:1 v/v) mixture spiked with the target substances at the required concentrations. These samples were used to calculate recoveries and matrix effects for each analyte belonging to Group II. Recoveries were calculated as the ratio of peak areas in pre-extraction spiked urine over those in post-extraction spiked urine, expressed as percentage. ME corresponded to the ratio of peak areas in post-extraction spiked urine over peak areas in standard solutions, expressed as percentage. For Group I analytes, since a simple dilution was used as the sample preparation, ME effect could easily be calculated from the ratio of the peak area observed in spiked urine samples vs the peak area obtained in standard solutions at the corresponding concentration, expressed as percentage (%).

From these values, relative matrix effects (ME_{rel}) were calculated using the equation: $ME_{rel} = ME - 100$ (%).

Table 1
Classes of prohibited substances by WADA.

Class	Definition
S1	Anabolic agents
S3	Beta-2 agonists
S4	Hormone and metabolic modulators
S5	Diuretics and masking agents
S6	Stimulants
S7	Narcotics
S8	Cannabinoids
S9	Glucocorticoids
P1	Beta-blockers

Table 2
Monoisotopic masses and chemical formulas of analyzed doping agents together with obtained average values from six measured sequences of retention times (t_r) and $^{TW}CCS_{N2}$ values in standard and urine samples. See Table 1 for WADA classes of substances.

Group	Substance	Chemical formula	Monoisotopic mass	Observed ions	t_r (min)	CCS STD	CCS urine	WADA class
Group I	**6-Hydroxy-bromantan**	$C_{16}H_{20}BrNO$	321.0728	$[M+H]^+$	5.50	168.89	168.84	S6
	Alfentanil	$C_{21}H_{32}N_6O_3$	416.2536	$[M+H]^+$	3.50	200.26	200.20	S7
	Amfepramone	$C_{13}H_{19}NO$	205.1467	$[M+H]^+$	2.68	147.16	147.40	S6
	Amiloride	$C_6H_8ClN_7O$	229.0479	$[M+H]^+$	2.13	146.53	146.32	S5
	Amphetamine	$C_9H_{13}N$	135.1048	91.0543	2.55	x	x	S6
	Atenolol	$C_{14}H_{22}N_2O_3$	266.1630	$[M+H]^+$	2.15	158.61	158.63	P1
	Bambuterol	$C_{18}H_{29}N_3O_5$	367.2107	$[M+H]^+$	3.16	190.25	189.77	S3
	Benzylpiperazine	$C_{11}H_{16}N_2$	176.1313	$[M+H]^+$	1.64	139.93	139.68	S6
	Bisoprolol	$C_{18}H_{31}NO_4$	325.2253	$[M+H]^+$	3.35	193.19	193.22	P1
	Buprenorphine	$C_{29}H_{41}NO_4$	467.3036	$[M+H]^+$	3.67	210.23	210.52	S7
	Cafedrine	$C_{18}H_{23}N_5O_3$	357.1801	$[M+H]^+$, $[M+H-H_2O]^+$	2.84	182.50	182.09	S6
	Canrenone	$C_{22}H_{28}O_3$	340.2038	$[M+H]^+$	5.00	186.03	185.65	S5
	Carphedon	$C_{12}H_{14}N_2O_2$	218.1055	$[M+H]^+$, $[M+Na]^+$	3.14	159.69	159.33	S6
	Cathine	$C_9H_{13}NO$	151.0997	$[M+H-H_2O]^+$	2.24	129.35	128.80	S6
	Cathinone	$C_9H_{11}NO$	149.0841	$[M+H]^+$, $[M+H-H_2O]^+$	2.26	124.99	125.61	S6
	Clobenzorex	$C_{16}H_{18}ClN$	259.1128	$[M+H]^+$	3.62	156.31	156.46	S6
	Clopamide	$C_{14}H_{20}ClN_3O_3S$	345.0914	$[M+H]^+$	3.47	181.33	181.61	S5
	Cocaine	$C_{17}H_{21}NO_4$	303.1471	$[M+H]^+$	3.14	169.02	169.26	–
	Benzoylecgonine	$C_{16}H_{19}NO_4$	289.1314	$[M+H]^+$	2.83	167.71	167.57	S6
	Methylecgonine	$C_10H_{17}NO_3$	199.1208	$[M+H]^+$	0.72	140.60	141.64	S6
	Codeine[a]	$C_{18}H_{21}NO_3$	299.1521	$[M+H]^+$	2.39	168.20	167.85	S7
	Cropropamide	$C_{13}H_{24}N_2O_2$	240.1838	$[M+Na]^+$	3.94	167.47	166.98	S6
	Crotethamide	$C_{12}H_{22}N_2O_2$	226.1681	$[M+Na]^+$	3.52	162.28	161.61	S6
	Cyclazodone	$C_{12}H_{12}N_2O_2$	216.0899	$[M+H]^+$, $[M+Na]^+$	3.50	163.79	163.84	S6
	Cyclopenthiazide	$C_{13}H_{18}ClN_3O_4S_2$	379.0427	$[M+H]^+$	4.52	188.23	189.43	S5
	Dextromoramide	$C_{25}H_{32}N_2O_2$	392.2464	$[M+H]^+$	4.02	194.25	194.37	S7
	EDDP	$C_{20}H_{23}N$	277.1830	$[M+H]^+$	3.81	166.26	165.79	S7
	Ephedrine	$C_{10}H_{15}NO$	165.1154	$[M+H]^+$, $[M+H-H_2O]^+$	2.36	133.25	133.05	S6
	Eplerenone	$C_{24}H_{30}O_6$	414.2042	$[M+H]^+$, $[M+Na]^+$	4.07	199.73	200.17	S5
	Esmolol	$C_{16}H_{25}NO_4$	295.1784	$[M+H]^+$	3.11	169.62	169.54	P1
	Ethamivan	$C_{12}H_{17}NO_3$	223.1208	$[M+H]^+$, $[M+Na]^+$	3.51	149.74	149.74	S6
	Ethylamphetamine	$C_{11}H_{17}N$	163.1361	$[M+H]^+$	2.77	136.73	136.61	S6
	Etilefrine	$C_{10}H_{15}NO_2$	181.1103	$[M+H]^+$, $[M+H-H_2O]^+$	1.82	138.66	138.25	S6
	Famprofazone	$C_{24}H_{31}N_3O$	377.2467	$[M+H]^+$	3.87	192.42	192.47	S6
	Fenbutrazate	$C_{23}H_{29}NO_3$	367.2147	$[M+H]^+$	4.22	189.47	189.38	S6
	Fencamfamine	$C_{15}H_{21}N$	215.1674	$[M+H]^+$	3.35	154.82	154.53	S6
	Fencamine	$C_{20}H_{28}N_6O_2$	384.2274	$[M+H]^+$	2.86	183.56	183.05	S6
	Fenetylline	$C_{18}H_{23}N_5O_2$	341.1852	$[M+H]^+$	2.99	177.88	177.80	S6
	Fenfluramine	$C_{12}H_{16}F_3N$	231.1235	$[M+H]^+$	3.42	149.89	149.60	S6
	Fenoterol	$C_{17}H_{21}NO_4$	303.1471	$[M+H]^+$	2.38	171.14	171.03	S3
	Fenproporex	$C_{12}H_{16}N_2$	188.1313	$[M+H]^+$	2.69	144.67	144.56	S6
	Fentanyl	$C_{22}H_{28}N_2O$	336.2202	$[M+H]^+$	3.53	183.04	182.85	S7
	Formoterol	$C_{19}H_{24}N_2O_4$	344.1736	$[M+H]^+$	2.96	178.87	178.99	S3
	Furfenorex	$C_{15}H_{19}NO$	229.1467	$[M+H]^+$	3.21	150.02	149.67	S6
	Heptaminol	$C_8H_{19}NO$	145.1467	$[M+H-H_2O]^+$	2.07	134.10	134.27	S6
	Hydromorphone	$C_{17}H_{19}NO_3$	285.1365	$[M+H]^+$	2.10	163.79	163.47	S7
	Hydroxymesocarb	$C_{18}H_{18}N_4O_3$	338.1379	$[M+H]^+$	3.82	183.13	183.24	S6
	Chlorphentermine	$C_{10}H_{14}ClN$	183.0815	$[M+H]^+$	3.16	x	x	S6
	Isometheptene	$C_9H_{19}N$	141.1517	$[M+H]^+$	2.97	135.62	135.30	S6
	Labetalol	$C_{19}H_{24}N_2O_3$	328.1787	$[M+H]^+$	3.27	177.12	177.21	P1
	Lixivaptan	$C_{27}H_{21}ClFN_3O_2$	473.1306	$[M+H]^+$, $[M+Na]^+$	5.63	221.97	221.96	S5
	MDA	$C_{10}H_{13}NO_2$	179.0946	$[M+H]^+$	2.58	125.11	126.66	S6
	MDMA	$C_{11}H_{15}NO_2$	193.1103	$[M+H]^+$	2.65	144.27	143.70	S6
	Mefenorex	$C_{12}H_{18}ClN$	211.1128	$[M+H]^+$	3.17	146.62	146.36	S6
	Mefentanil	$C_{23}H_{30}N_2O$	350.2358	$[M+H]^+$	3.72	185.38	185.21	S7
	Mephentermine	$C_{11}H_{17}N$	163.1361	$[M+H]^+$	7.17	126.37	124.41	S6
	Metamphetamine	$C_{10}H_{15}N$	149.1204	$[M+H]^+$	2.62	134.60	134.25	S6
	Metcathinone	$C_{10}H_{13}NO$	163.0997	$[M+H]^+$, $[M+H-H_2O]^+$	2.36	128.44	128.54	S7
	Methadone	$C_{21}H_{27}NO$	309.2093	$[M+H]^+$	4.05	177.38	177.06	S6
	Methedrone	$C_{11}H_{15}NO_2$	193.1103	$[M+H]^+$, $[M+H-H_2O]^+$	2.59	136.17	136.20	S6
	Methoxyphenamine	$C_{11}H_{17}NO$	179.1310	$[M+H]^+$	2.84	140.10	140.70	S6
	Methylephedrine	$C_{11}H_{17}NO$	179.1310	$[M+H]^+$, $[M+H-H_2O]^+$	2.44	139.60	139.16	S6
	Methylphenidate	$C_{14}H_{19}NO_2$	233.1416	$[M+H]^+$	3.02	153.82	153.69	S6
	Metoprolol	$C_{15}H_{25}NO_3$	267.1834	$[M+H]^+$	2.98	172.27	172.06	P1
	Modafinil	$C_{15}H_{15}NO_2S$	273.0823	$[M+Na]^+$	3.74	170.18	170.07	S6
	Morphine	$C_{17}H_{19}NO_3$	285.1365	$[M+H]^+$	1.88	163.51	163.77	S7
	6-Acetyl-morphine	$C_{23}H_{27}NO_9$	327.1471	$[M+H]^+$	2.53	174.80	174.84	S7
	Morphine-3β-D-glucuronide	$C_{19}H_{21}NO_4$	461.1686	$[M+H]^+$	1.43	206.61	207.20	S7
	Mozavaptan	$C_{27}H_{29}N_3O_2$	427.2260	$[M+H]^+$	3.51	217.49	217.46	S5
	Nadolol	$C_{17}H_{27}NO_4$	309.1940	$[M+H]^+$	2.57	172.93	172.54	P1
	N-ethylnicotinamide	$C_{10}H_{14}N_2O$	150.0793	$[M+H]^+$	1.95	134.48	134.62	S6
	Nikethamide	$C_8H_{10}N_2O$	178.1106	$[M+H]^+$	2.65	143.22	142.90	S6

Table 2 (*continued*)

Group	Substance	Chemical formula	Monoisotopic mass	Observed ions	t_r (min)	CCS STD	CCS urine	WADA class
	Norbuprenorphine	$C_{25}H_{35}NO_4$	413.2566	$[M+H]^+$	3.24	200.69	200.19	S7
	Norfenfluramine	$C_{10}H_{12}F_3N$	203.0922	$[M+H]^+$	3.21	147.32	147.57	S6
	Norfentanyl	$C_{14}H_{20}N_2O$	232.1576	$[M+H]^+$	2.83	156.42	155.81	S7
	Octopamine	$C_8H_{11}NO_2$	153.0790	$[M+H-H_2O]^+$	0.71	131.50	x	S6
	Ortetamine	$C_{10}H_{15}N$	149.1204	$[M+H]^+$	2.84	x	x	S6
	Oxilofrine	$C_{10}H_{15}NO_2$	181.1103	$[M+H-H_2O]^+$	1.25	137.41	137.01	S6
	Oxprenolol	$C_{15}H_{23}NO_3$	265.1678	$[M+H]^+$	3.24	161.52	160.95	P1
	Oxycodone	$C_{18}H_{21}NO_4$	315.1471	$[M+H]^+$, $[M+Na]^+$	2.52	173.50	173.61	S7
	Oxymorphone	$C_{17}H_{19}NO_4$	301.1314	$[M+H]^+$, $[M+Na]^+$	1.99	164.84	164.76	S7
	Pemoline	$C_9H_8N_2O_2$	176.0586	$[M+H]^+$, $[M+Na]^+$	2.79	151.31	150.94	S6
	Pentazocine	$C_{19}H_{27}NO$	285.2093	$[M+H]^+$	3.33	174.57	x	S7
	Pentetrazol	$C_6H_10N_4$	138.0905	$[M+H]^+$	2.74	127.87	127.51	S6
	Pethidine	$C_{15}H_{21}NO_2$	247.1572	$[M+H]^+$	3.17	158.18	158.52	S7
	Phendimetrazine	$C_{12}H_{17}NO$	191.1310	$[M+H]^+$	2.60	144.58	144.15	S6
	Piretanide	$C_{17}H_{18}N_2O_5S$	362.0936	$[M+H]^+$	4.53	182.36	182.55	S5
	Prolintane	$C_{15}H_{23}N$	217.1830	$[M+H]^+$	3.40	153.04	152.54	S6
	Propanolol	$C_{16}H_{21}NO_2$	259.1572	$[M+H]^+$	3.47	162.94	162.48	P1
	Propylhexedrine	$C_{10}H_{21}N$	155.1674	$[M+H]^+$	3.20	143.65	143.25	S6
	Ritalinic acid	$C_{13}H_{17}NO_2$	219.1259	$[M+H]^+$	2.78	149.40	149.22	S6
	Salbutamol	$C_{13}H_{21}NO_3$	239.1521	$[M+H]^+$	2.11	159.91	159.80	S3
	Salmeterol	$C_{25}H_{37}NO_4$	415.2723	$[M+H]^+$	4.13	202.88	203.23	S3
	Sotalol	$C_{12}H_{20}N_2O_3S$	272.1195	$[M+H]^+$, $[M+H-H_2O]^+$	2.16	166.49	166.22	P1
	Relcovaptan	$C_{28}H_{27}Cl_2N_3O_7S$	619.0947	$[M+H]^+$, $[M+Na]^+$	4.73	236.91	236.50	S5
	Sufentanil	$C_{22}H_{30}N_2O_2S$	386.2028	$[M+H]^+$	3.89	191.59	191.50	S7
	Terbutaline	$C_{12}H_{19}NO_3$	225.1365	$[M+H]^+$	2.09	155.54	x	S3
	Timolol	$C_{13}H_{24}N_4O_3S$	316.1569	$[M+H]^+$	2.89	175.49	175.62	P1
	Tolvaptan	$C_{26}H_{25}ClN_2O_3$	448.1554	$[M+H]^+$, $[M+Na]^+$	5.06	214.18	213.98	S5
	Torasemide	$C_{16}H_20N_4O_3S$	348.1256	$[M+H]^+$	3.57	187.96	187.77	S5
	Triamterene	$C_{12}H_{11}N_7$	253.1076	$[M+H]^+$	2.68	156.79	156.08	S5
	Trimetazidine	$C_{14}H_{22}N_2O_3$	266.1630	$[M+H]^+$	2.19	160.40	160.33	S4
Group II	**4-Hydroxy-clomifen**	$C_{26}H_{28}ClNO_2$	421.1809	$[M+H]^+$	4.25	211.17	212.03	S4
	Aminoglutethimide	$C_{13}H_{16}N_2O_2$	232.1212	$[M+H]^+$	2.56	167.70	168.43	S4
	Anamorelin	$C_{31}H_{42}N_6O_3$	546.3318	$[M+H]^+$, $[M+Na]^+$	4.07	233.81	233.84	S2
	Anastrozole	$C_{17}H_{19}N_5$	293.1640	$[M+H]^+$	4.14	184.73	184.58	S4
	Bazedoxifen	$C_{30}H_{34}N_2O_3$	470.2569	$[M+H]^+$	3.76	213.37	213.78	S4
	Beclomethasone	$C_{22}H_{29}ClO_5$	408.1704	$[M+H]^+$, $[M+H-H_2O]^+$, $[M+Na]^+$	4.16	190.03	190.50	S9
	Betamethasone	$C_{22}H_{29}FO_5$	392.1999	$[M+H]^+$, $[M+Na]^+$	4.06	216.21	216.25	S9
	Bolasterone	$C_{21}H_{32}O_2$	316.2402	$[M+H]^+$	5.03	180.53	180.60	S1
	Bolasterone metabolite[b]	$C_{21}H_{36}O_2$	320.2715	$[M+H-H_2O]^+$, $[M+H-2H_2O]^+$	5.27	172.37	x	S1
	Boldenone	$C_{19}H_{26}O_2$	286.1933	$[M+H]^+$, $[M+Na]^+$	3.74	165.18	165.69	S1
	Boldenone metabolite[c]	$C_{19}H_{28}O_2$	288.2089	$[M+H]^+$	4.97	173.10	172.37	S1
	Budesonide	$C_{25}H_{34}O_6$	430.2355	$[M+H]^+$, $[M+H-H_2O]^+$, $[M+Na]^+$	4.79	205.60	205.79	S9
	16α-Hydroxyprednisolone	$C_{21}H_{28}O_6$	376.1886	$[M+H]^+$, $[M+H-H_2O]^+$, $[M+Na]^+$	3.34	187.36	188.33	S9
	6β-Hydroxybudesonide	$C_{25}H_{34}O_7$	446.2305	$[M+H]^+$	3.95	206.10	209.55	S9
	Calusterone	$C_{21}H_{32}O_2$	316.2402	$[M+H]^+$	5.14	180.18	180.30	S1
	Calusterone metabolite[d]	$C_{21}H_{36}O_2$	320.2715	$[M+H-H_2O]^+$, $[M+H-2H_2O]^+$	5.42	172.88	172.60	S1
	Ciclesonide	$C_{32}H_{44}O_7$	540.3087	$[M+H]^+$	6.83	237.87	238.51	S9
	Desisobutyrylciclesonide	$C_{28}H_{38}O_6$	470.2668	$[M+H]^+$, $[M+H-H_2O]^+$	5.47	216.32	216.58	S9
	Clenbuterol	$C_{12}H_{18}Cl_2N_2O$	276.0796	$[M+H]^+$, $[M+H-H_2O]^+$	2.97	164.64	164.31	S1
	Clomifen	$C_{26}H_{28}ClNO$	405.1859	$[M+H]^+$	4.75	206.84	207.52	S4
	Clostebol metabolite[e]	$C_{19}H_{27}ClO_2$	322.1700	$[M+H-H_2O]^+$, $[M+H-2H_2O]^+$	5.46	168.84	169.20	S1
	6β-Hydroxymethandienone	$C_{20}H_{28}O_3$	316.2038	$[M+H]^+$, $[M+H-H_2O]^+$, $[M+H-2H_2O]^+$, $[M+Na]^+$	3.60	173.01	172.58	S1
	Methandienone metabolite[f]	$C_{20}H_{32}O_2$	304.2402	$[M+H-2H_2O]^+$	5.92	166.76	166.32	S1
	Desonide	$C_{24}H_{32}O_6$	416.2199	$[M+H]^+$, $[M+H-2H_2O]^+$	4.26	198.35	199.18	S9
	Dexamethasone	$C_{22}H_{29}FO_5$	392.1999	$[M+H]^+$, $[M+Na]^+$	4.09	216.38	216.62	S9
	Drostanolone metabolite[g]	$C_{20}H_{32}O_2$	304.2402	$[M+H-H_2O]^+$, $[M+H-2H_2O]^+$	5.90	172.73	171.63	S1
	Epitrenbolone	$C_{18}H_{22}O_2$	270.1620	$[M+H]^+$	4.40	165.63	164.72	S1
	Ethisterone	$C_{21}H_{28}O_2$	312.2089	$[M+H]^+$	4.88	179.50	178.69	S1
	17-Dihydro-exemestane	$C_{20}H_{26}O_2$	298.1933	$[M+H]^+$	4.75	172.57	170.97	S4
	Roxadustat (FG 4592)	$C_{19}H_{16}N_2O_5$	352.1059	$[M+H]^+$	5.44	185.92	186.22	S2
	Fludrocortisone	$C_{21}H_{29}FO_5$	380.1999	$[M+H]^+$	3.77	186.30	187.03	S9
	Flumethasone	$C_{22}H_{28}F_2O_5$	410.1905	$[M+H]^+$, $[M+Na]^+$	4.08	189.22	189.76	S9
	Flunisolide	$C_{24}H_{31}FO_6$	434.2105	$[M+H]^+$	4.28	200.28	200.51	S9
	Fluocortolone	$C_{22}H_{29}FO_4$	376.2050	$[M+H]^+$	4.38	188.97	189.58	S9
	Fluoxymesterone	$C_{20}H_{29}FO_3$	336.2101	$[M+H]^+$	4.18	177.87	177.46	S1
	Fluoxymesterone metabolite M1[h]	$C_{20}H_{31}FO_4$	354.2206	$[M+H]^+$, $[M+H-H_2O]^+$, $[M+Na]^+$	2.81	174.32	174.63	S1
	Fluoxymesterone metabolite M2[i]	$C_{20}H_{29}FO_4$	318.1995	$[M+H]^+$	5.45	176.82	177.06	S1
	6β-Hydroxy-fluoxymesterone	$C_{20}H_{27}FO_2$	352.2050	$[M+H]^+$	3.35	182.24	183.71	S1
	Fluticasone metabolite[j]	$C_{24}H_{30}F_2O_6$	452.2010	$[M+H]^+$	4.69	199.97	200.99	S9
	Fulvestrant	$C_{32}H_{47}F_5O_3S$	606.3166	$[M+H]^+$, $[M+Na]^+$	5.93	250.20	250.37	S4
	Gestrinone	$C_{21}H_{24}O_2$	308.1776	$[M+H]^+$	4.80	176.90	176.59	S1
	Ibutamoren	$C_{27}H_{36}N_4O_5S$	528.2406	$[M+H]^+$	3.82	223.44	223.99	S2

(*continued on next page*)

Table 2 (*continued*)

Group	Substance	Chemical formula	Monoisotopic mass	Observed ions	t_r (min)	CCS STD	CCS urine	WADA class
	JWH-018 5-pentanoic acid	$C_{24}H_{21}NO_3$	371.1521	$[M+H]^+$, $[M+H-H_2O]^+$, $[M+Na]^+$	5.17	188.76	189.66	S8
	JWH-018 4-hydroxypentyl	$C_{24}H_{23}NO_2$	357.1729	$[M+H]^+$, $[M+Na]^+$	5.29	188.50	188.53	S8
	JWH-073 4-butanoic acid	$C_{23}H_{19}NO_3$	357.1365	$[M+H]^+$, $[M+Na]^+$	5.03	185.98	185.74	S8
	JWH-073 3-hydroxybutylacid	$C_{23}H_{21}NO_2$	343.1572	$[M+H]^+$, $[M+Na]^+$	5.22	184.75	184.41	S8
	JWH-200 4-hydroxyindol	$C_{25}H_{24}N_2O_3$	400.1787	$[M+H]^+$	4.10	192.85	194.11	S8
	JWH-250 N-5-carboxypentyl	$C_{22}H_{23}NO_4$	365.1627	$[M+H]^+$, $[M+Na]^+$	4.87	189.43	188.84	S8
	JWH-250 N-4-hydroxypentyl	$C_{22}H_{25}NO_3$	351.1834	$[M+H]^+$, $[M+Na]^+$	4.98	187.76	188.07	S8
	Letrozole	$C_{17}H_{11}N_5$	285.1014	$[M+H]^+$	4.13	179.73	179.65	S4
	Metenolone	$C_{20}H_{30}O_2$	302.2246	$[M+H]^+$, $[M+H-H_2O]^+$, $[M+H-2H_2O]^+$	6.18	171.26	170.59	S1
	Methasterone	$C_{21}H_{34}O_2$	318.2559	$[M+H]^+$, $[M+H-H_2O]^+$, $[M+H-2H_2O]^+$	5.80	181.09	179.93	S1
	Methyldienolone	$C_{19}H_{26}O_2$	286.1933	$[M+H]^+$	4.59	166.84	165.06	S1
	Methylprednisolone	$C_{22}H_{30}O_5$	374.2093	$[M+H]^+$	4.03	185.83	185.82	S9
	Mibolerone	$C_{20}H_{30}O_2$	302.2246	$[M+H]^+$	4.86	177.90	176.78	S1
	18-Methylnandrolone metabolite M1[k]	$C_{19}H_{30}O_2$	290.2246	$[M+H]^+$, $[M+H-H_2O]^+$, $[M+H-2H_2O]^+$	5.61	167.97	167.73	S1
	18-Methylnandrolone metabolite M2[l]	$C_{19}H_{30}O_2$	290.2246	$[M+H]^+$, $[M+H-H_2O]^+$, $[M+H-2H_2O]^+$	5.21	163.20	161.78	S1
	Norbolethone metabolite M1[m]	$C_{21}H_{36}O_2$	320.2715	$[M+H]^+$, $[M+H-H_2O]^+$, $[M+H-2H_2O]^+$	5.92	177.55	177.96	S1
	Norbolethone metabolite M2[n]	$C_{21}H_{36}O_2$	320.2715	$[M+H]^+$, $[M+H-H_2O]^+$, $[M+H-2H_2O]^+$	5.75	173.63	173.67	S1
	Norethandrolone metabolite M1[o]	$C_{20}H_{34}O_2$	306.2559	$[M+H]^+$, $[M+H-H_2O]^+$, $[M+H-2H_2O]^+$	5.46	169.97	168.75	S1
	Norethandrolone metabolite M2[p]	$C_{20}H_{34}O_3$	322.2508	$[M+H]^+$, $[M+H-2H_2O]^+$, $[M+Na]^+$	4.45	171.02	170.55	S1
	Oxabolone metabolite[q]	$C_{18}H_{24}O_3$	288.1725	$[M+H]^+$	4.79	169.77	170.65	S1
	Oxandrolone	$C_{19}H_{30}O_3$	306.2195	$[M+H]^+$, $[M+H-H_2O]^+$, $[M+Na]^+$	4.58	171.30	171.23	S1
	Oxymesterone	$C_{20}H_{30}O_3$	318.2195	$[M+H]^+$, $[M+H-H_2O]^+$, $[M+H-2H_2O]^+$	4.95	177.23	175.65	S1
	Prednisolone	$C_{21}H_{28}O_5$	360.1937	$[M+H]^+$, $[M+H-H_2O]^+$, $[M+Na]^+$	3.72	181.01	181.34	S9
	Prednisone	$C_{21}H_{26}O_5$	358.1780	$[M+H]^+$	3.74	183.51	184.11	S9
	16β-Hydroxy-prostanozolol	$C_{20}H_{28}N_2O_2$	328.2151	$[M+H]^+$	3.36	187.86	188.97	S1
	3-Hydroxyprostanozolol	$C_{20}H_{28}N_2O_2$	328.2151	$[M+H]^+$	3.93	187.38	188.37	S1
	Raloxifen	$C_{28}H_{27}NO_4S$	473.1661	$[M+H]^+$	3.53	214.38	214.96	S4
	Desmethylsibutramine	$C_{15}H_{22}ClN$	265.1597	$[M+H]^+$	4.08	166.84	166.19	S6
	Bis-desmethylsibutramine	$C_{16}H_{24}ClN$	251.1441	$[M+H]^+$	4.03	165.75	164.09	S6
	Stanozolol	$C_{21}H_{32}N_2O$	328.2515	$[M+H]^+$	4.37	189.65	190.86	S1
	16β-Hydroxystanozolol	$C_{21}H_{32}N_2O_2$	344.2464	$[M+H]^+$	3.63	192.97	194.03	S1
	3′-Hydroxystanozolol	$C_{21}H_{32}N_2O_2$	344.2464	$[M+H]^+$	3.88	192.91	194.11	S1
	4α-Hydroxystanozolol	$C_{21}H_{32}N_2O_2$	344.2464	$[M+H]^+$, $[M+H-H_2O]^+$, $[M+H-2H_2O]^+$, $[M+Na]^+$	3.78	201.48	199.74	S1
	4β-Hydroxystanozolol	$C_{21}H_{32}N_2O_2$	344.2464	$[M+H]^+$, $[M+H-H_2O]^+$, $[M+H-2H_2O]^+$, $[M+Na]^+$	3.98	191.59	192.68	S1
	Tamoxifen	$C_{26}H_{29}NO$	371.2249	$[M+H]^+$	4.87	197.76	199.10	S4
	3-Hydroxy-4-methoxy-tamoxifen	$C_{27}H_{31}NO_3$	417.2304	$[M+H]^+$	4.51	203.20	203.84	S4
	Telmisartan[r]	$C_{33}H_{30}N_4O_2$	514.2369	$[M+H]^+$	3.98	231.38	231.67	–
	Tetrahydrotestolactone	$C_{21}H_{28}O_2$	304.2038	$[M+H]^+$, $[M+H-H_2O]^+$	4.59	166.92	167.62	S4
	Tetrahydrogestrinone	$C_{19}H_{28}O_3$	312.2089	$[M+H]^+$	5.24	179.38	178.98	S1
	Toremifene	$C_{26}H_{28}ClNO$	405.1859	$[M+H]^+$	4.77	204.34	204.85	S4
	Trenbolone	$C_{18}H_{22}O_2$	270.1620	$[M+H]^+$	4.27	165.49	164.48	S1
	Triamcinolone	$C_{21}H_{27}FO_6$	394.1792	$[M+H]^+$	3.35	187.47	188.80	S9
	Triamcinolone acetonide	$C_{24}H_{31}FO_6$	434.2105	$[M+H]^+$, $[M+Na]^+$	4.24	197.49	198.72	S9
	Triamcinolone hexacetonide	$C_{30}H_{41}FO_7$	532.2836	$[M+H]^+$	6.17	231.73	232.09	S9
	Dehydrochloromethyltestosterone	$C_{20}H_{27}ClO_2$	334.1700	$[M+H]^+$, $[M+Na]^+$	5.10	199.08	199.97	S1
	DHCMT metabolite[s]	$C_{20}H_{27}ClO_3$	350.1649	$[M+H]^+$, $[M+Na]^+$	4.09	203.11	204.67	S1
	Zeranol	$C_{18}H_{26}O_5$	261.1477	$[M+H]^+$, $[M+H-H_2O]^+$, $[M+Na]^+$	4.51	173.40	172.04	S1
	Zilpaterol	$C_{14}H_{19}N_3O_2$	322.1780	$[M+H]^+$, $[M+H-H_2O]^+$, $[M+H-2H_2O]^+$	2.09	162.00	162.29	S1

[a] Codeine is not prohibited but metabolized to morphine.
[b] Bolasterone metabolite: 7α,17α-dimethyl-5β-androstane-3α,17β-diol.
[c] Bolasterone metabolite: 5β-androst-1-en-17β-ol-3-one.
[d] Calusterone metabolite: 7β,17α-dimethyl-5β-androstane-3α,17β-diol.
[e] Clostebol metabolite: 4-chloro-androst-4-en-3α-ol-17-one.
[f] Methandienone metabolite: 17β-methyl-5β-androst-1-ene-3α,17α-diol.
[g] Drostanolone metabolite: 2α-methyl-5α-androstan-3α-ol-17-one.
[h] Fluoxymesterone metabolite M1: 9α-fluoro-17α-methyl-4-androsten-3α,16β,11β,17β-tetra-ol.
[i] Fluoxymesterone metabolite M2: 9α-fluoro-17,17-dimethyl-18-nor-androstan-4,13-diene-11β-ol-3-one.
[j] Fluticasone metabolite: fluticasone propionate-17β-carboxylic acid.
[k] 18-Methylnandrolone metabolite M1: 18-methyl-3α-hydroxy 5α-estran-17-one.
[l] 18-Methylnandrolone metabolite M2: 18-methyl-3β-hydroxy 5α-estran-17-one.
[m] Norbolethone metabolite M1: 13β,17α-diethyl-5α-gonane-3α,17β-diol.
[n] Norbolethone metabolite M2: 13β,17α-diethyl-5β-gonane-3α,17β-diol.
[o] Norethandrolone metabolite M1: 17α-ethyl-5β-estrane-3α,17β-diol.
[p] Norethandrolone metabolite M2: 7α-hydroxyethyl-5β-estrane-3α,17β-diol.
[q] Oxabolone metabolite: 4-hydroxyestr-4-ene-3,17-dione.
[r] Telmisartan was included in the study as a model compound, although not prohibited.
[s] DHCMT metabolite: 6β-hydroxy-4-chlorodehydromethyltestosterone.

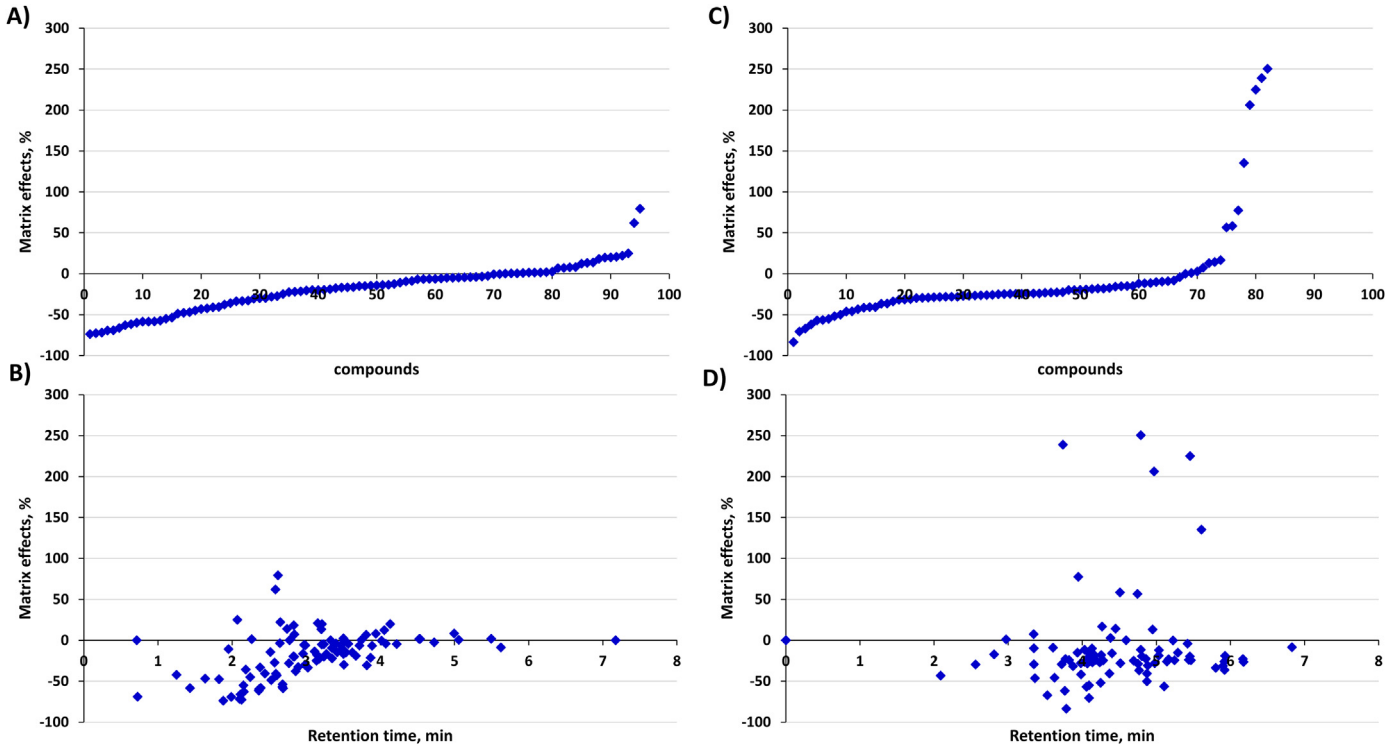


Fig. 1. Relative matrix effects evaluated for (A–B) Group I and (C–D) Group II doping agents.

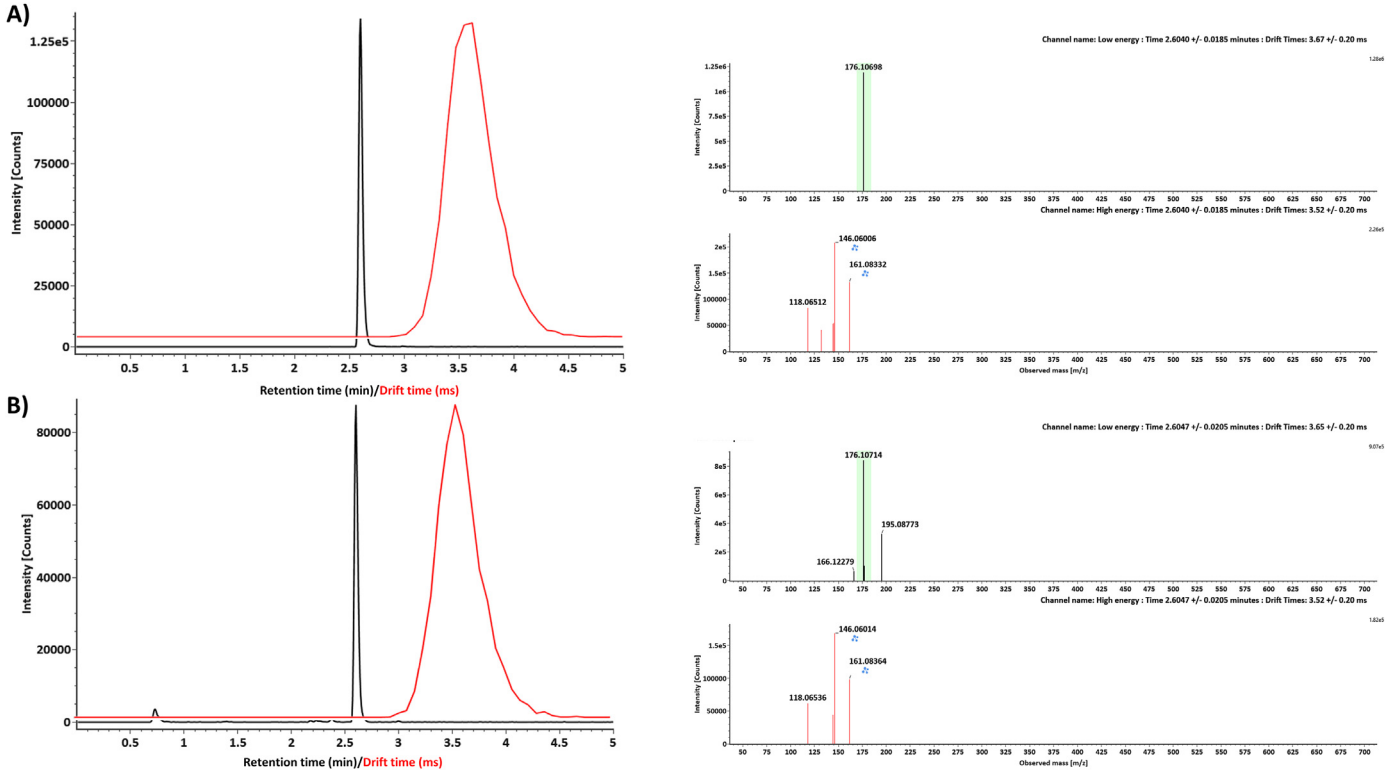


Fig. 2. Comparison of chromatograms, mobilograms, and low/high energy spectra of methedrone (Group I compound) at 100 ppb in (A) standard and (B) urine sample. MS Spectra are showing only peaks with intensity higher than 2000 counts. X-scales for retention time and drift time are numerically the same. Details of 3D spectra are described in Section 3.5.

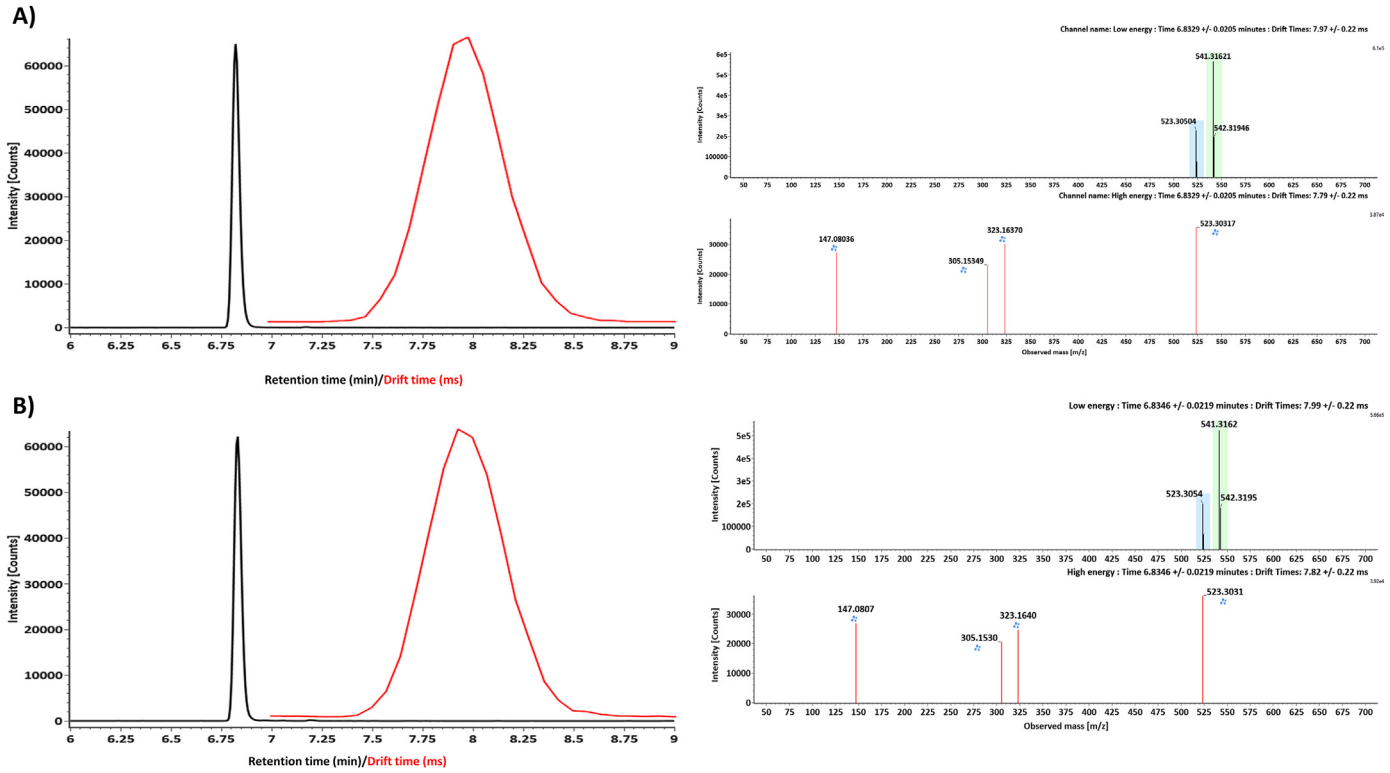


Fig. 3. Comparison of chromatograms, mobilograms, and low/high energy 3D spectra of ciclesonide (Group II compound) at 500 ppb in (A) standard and (B) urine sample. MS Spectra are showing only peaks with intensity higher than 2000 counts. X-scales for retention time and drift time are numerically the same. Details of 3D spectra are described in Section 3.5.

2.3. Ultra-high performance liquid chromatography ion mobility-high resolution mass spectrometry

The experiments were carried out using an Acquity Ultra Performance Liquid Chromatography H-class system from Waters (Milford, MA, USA) hyphenated to a Waters Vion TWIMS-quadrupole-time of flight (qTOF) mass spectrometer (Wilmslow, UK). The UHPLC system was equipped with a binary solvent manager, an autosampler, and a column manager composed of a pre-column eluent heater and a column oven set at 40 °C. A Waters Acquity UPLC BEH C18 column (100 × 2.1 mm, 1.7 μm) and the corresponding VanGuard precolumn were used for the separation. Solvent A, water, and solvent B, ACN, both containing 0.1% formic acid were used as mobile phases. The gradient profile was as follows: 2–98% of B in 6 min and then the conditions were returned in 0.1 min–2% B followed by 4 min equilibration of the column. The flow rate was 0.4 mL/min and the injection volume 5 μL.

HDMS^E (high definition MS^E) and MS^E data independent scan types were set-up for the experiments. Positive mode electrospray was used with the source temperature at 120 °C, capillary voltage 1.5 kV, and cone voltage 30 V. Nitrogen was used as the desolvation gas at 1000 L/h and 500 °C, and as cone gas at 50 L/h. The acquisition mass range was *m/z* 50–700, scan time 0.15 s, low energy 6 eV, and high energy consisted of a ramp from 28 to 56 eV. Internal calibration was carried out using 80 ng/mL leucine-enkephalin, external calibration using both CCS and mass scale calibration with Major Mix IMS/ToF Calibration Kit from Waters (Milford, MA, USA).

UNIFI software v1.9.3 was used for instrument control, data acquisition, and data processing. A compound library containing *m/z*, retention times, and $^{TW}CCS_{N2}$ values was prepared for each analyte Group, based on experimental data. All sequences were evaluated using a processing method with the following set-up: mass accuracy 5 ppm, high energy intensity threshold 50 counts, low energy intensity threshold 100 counts, $^{TW}CCS_{N2}$ tolerance <2%, and retention time identification tolerance <0.1 min.

2.4. Study design

Each measured sequence followed the same protocol, which can be found in the Supplementary data (S1). Analyses of all three mixtures of Group I or Group II compounds were carried out within one single sequence. Standards and urine samples of each mixture were prepared at the six specified concentration levels and measured consecutively. This sequence was repeated three times during week 1 and once on weeks 2, 3, and 4. During week 2, one sequence only in MS^E was also measured, and the results obtained are discussed in the second part of this study. Cleaning of the instrument followed by new external calibration and $^{TW}CCS_{N2}$ calibration was carried out each week directly before the start of the first sequence. The obtained data allowed for the evaluation of intraday, interday, and interweek variations of retention times and $^{TW}CCS_{N2}$ values.

3. Results and discussion

3.1. UHPLC-MS conditions and sample preparation

The chromatographic method used in this study is based on an established UHPLC-MS/MS method currently in use in Swiss Laboratory for Doping Analyses.

Adequate sensitivity for Group I compounds allowed for the use of a simple dilute and shoot procedure with 5-fold sample dilution. However, due to less efficient method performance of Group II

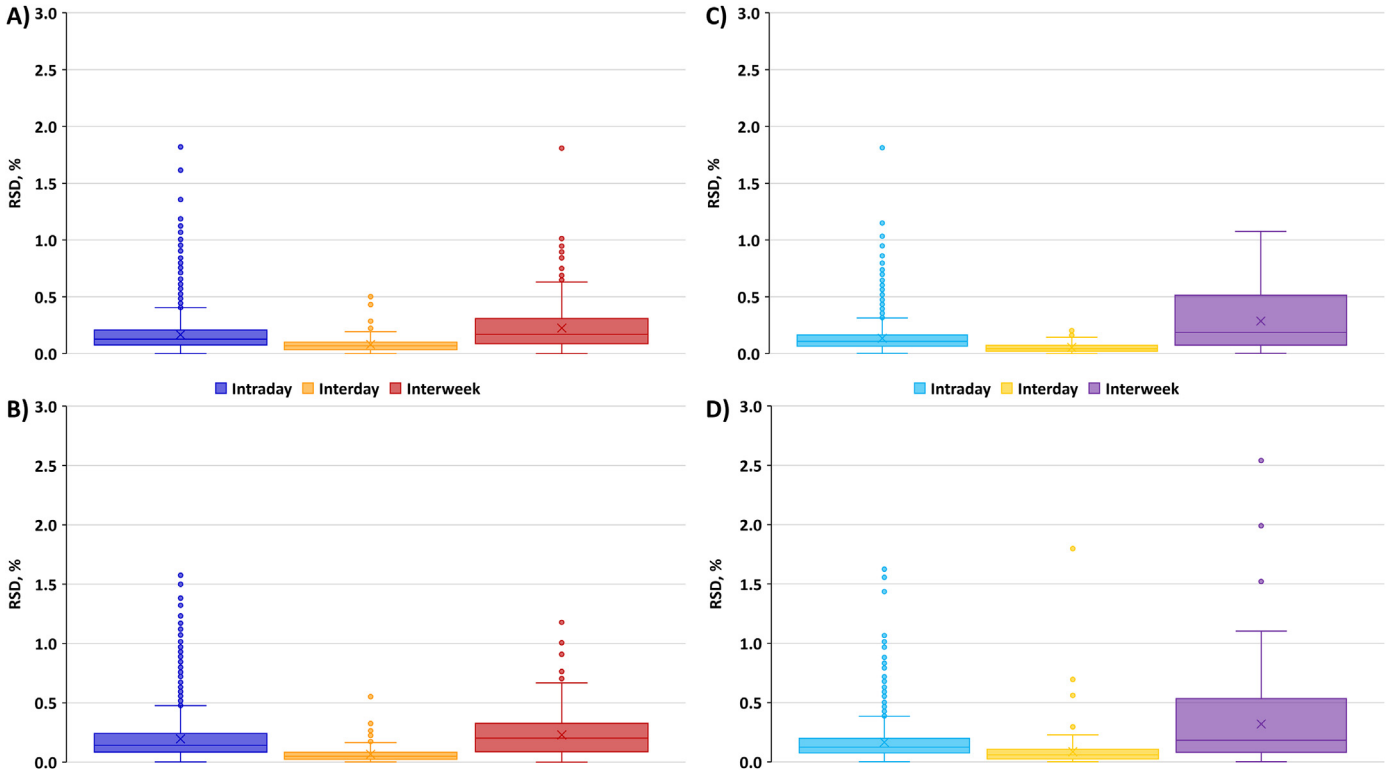


Fig. 4. Intraday, interday, and interweek variability of $^{TW}CCS_{N2}$ values of stimulants in (A) standard and (B) urine samples and steroids in (C) standard and (D) urine samples.

compounds and their more strict sensitivity criteria [42], a sample preparation method enabling preconcentration was necessary. Considering the rather lipophilic character of most of these compounds, SLE provides the required preconcentration as well as the possibility to clean-up the samples. A previously developed SLE procedure was used, with methyl *tert*-butyl ether as the elution solvent [43]. To increase the sensitivity of the method, the reconstitution volume was decreased from 200 to 100 μL. High recoveries (80–120%) were obtained for almost all compounds, with only two critical compounds, zilpaterol showing poor recovery (approx. 2%) and bolasterone metabolite being significantly affected by matrix effect, and another three compounds (aminoglutethimide, norbolethone metabolite M1 and fluoxymesterone metabolite M1) with recoveries ranging between 20 and 40% (for full names see Table 2).

Matrix effect (ME) values were determined using a sequence run on week 1 (see Supplementary data S1). For Group I compounds, ME was calculated at each concentration level as the peak area in the 5× diluted urine samples divided by the peak area in standard samples, expressed as percentage. Relative matrix effects (ME_{rel}) were calculated from the average of ME from all concentrations. Therefore, positive values in Fig. 1 directly indicate signal enhancement, while negative values correspond to signal suppression. ME_{rel} for 55% of Group I compounds were within ±20% limit, and only 18% of compounds had ME_{rel} higher than ±50%. Overall, most compounds in Group I were affected by signal suppression. Those compounds eluting at the beginning of the gradient and especially between 1.5 and 2.5 min (Fig. 1) were more susceptible to ME, as expected due to polar interferences from urine samples.

The calculation of ME for Group II doping agents was based on the post-extraction addition approach and ME_{rel} were calculated using the procedure described in Section 2.2. Overall, the ME_{rel} obtained were more significant than for Group I compounds (Fig. 1C and D). Only 31% of compounds had ME_{rel} within the ±20% limit and 24% had ME_{rel} higher than ±50%. Moreover, four critical compounds were determined, i.e. methyldienolone, 18-methylnandrolone metabolite M2, norbolethone metabolite M2, and oxandrolone. These compounds had acceptable ME around 10% at the three highest concentration levels, but at lower concentrations the ME was estimated up to 4000% due to the coelution of interfering compounds from urine matrix and problems imposed to the correct peak integration. Therefore, only the highest concentrations were applicable for the ME evaluation. The higher susceptibility to ME of Group II compounds could be partially explained by the preconcentration of the sample, contrary to dilution in Group I. Similarly to Group I, also compounds of Group II were mostly affected by signal suppression with the highest ME_{rel} around retention times of 4 min. The signal suppression was especially critical considering the low MRPL required by the WADA for the Group II compounds.

3.2. Creation of a database for m/z, retention times and $^{TW}CCS_{N2}$ values

Data obtained from the analysis of the samples were introduced in the database, which includes details of *m/z*, retention times, and $^{TW}CCS_{N2}$ values of the 192 doping agents. The data are presented in Table 2. Each doping agent was injected more than 100 times during the study and the average values were used for the data compilation. Once created, this database was used to identify doping agents in standard and urine samples. A comparison of the results obtained in standard and urine samples for two representative compounds of Group I and II is present in Figs. 2 and 3, respectively. Both figures show chromatograms, mobilograms, and low/high energy spectra obtained for methedrone (Group I) and ciclesonide (Group II). Overall, almost no differences were observed

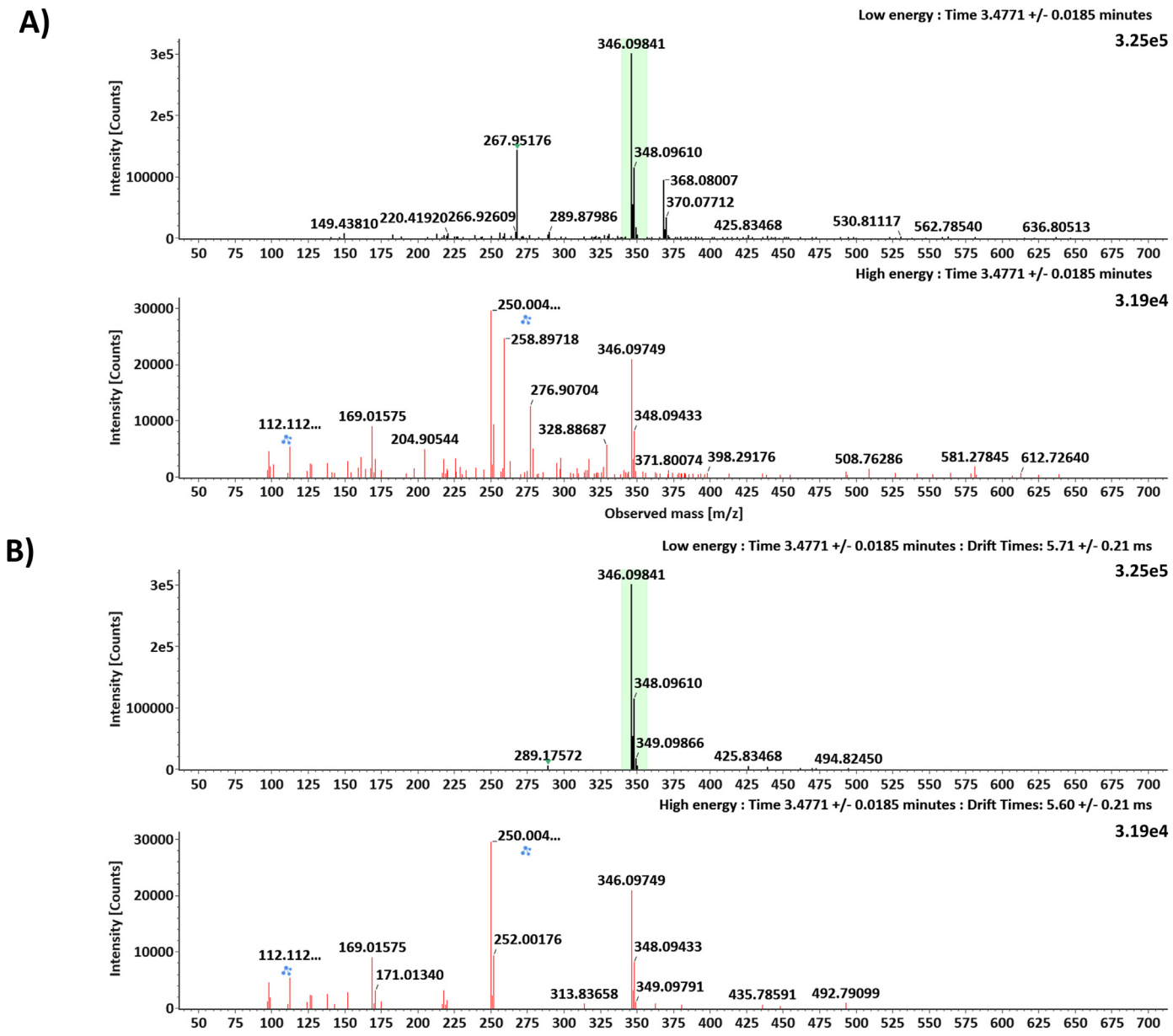


Fig. 5. Comparison of low energy and high energy 3D MS spectra measured in HDMS^E mode with and without IMS filtration in data processing. (A) UHPLC-HRMS spectra of clopamide filtered only by retention time ± 0.0185 min, (B) UHPLC-HRMS spectra of clopamide filtered by retention time ± 0.0185 min and drift time ± 0.21 ms.

between standard and urine samples in chromatograms and mobilograms. As expected, more peaks were detected in urine spectra, but usually in lower intensity counts. However, differences in intensities of parent peaks were also observed, which might be attributed to matrix effects (ME).

3.3. Variability of UHPLC retention times

Data obtained using the analysis protocol described in Supplementary data S1 allowed the determination of intraday, interday, and interweek variability of UHPLC retention times in standard and urine samples for both compound Groups. All measured concentration levels were used for the calculation of relative standard deviations (RSD) values.

The low variability of retention times proved the suitability and stability of the UHPLC conditions employed in this work over time. Indeed, intra- and interday RSD of retention times of Group II compounds were always <1%, while interweek RSD remained <2%. Due to very low retention times of some compounds from Group I (close to t_0), the variability was slightly higher in this case. Intra- and interday RSDs were always <2%, but several compounds exceeded the criteria of 1%. Considering the interweek variability, all RSD values were <2% except for three critical basic/polar compounds, i.e. oxilofrine, morphine-3β-D-glucuronide, and benzylpiperazine, with RSDs ranging between 3 and 5%. These three doping agents eluted at the beginning of the gradient, with retention times equal to 1.2, 1.3, and 1.5 min, respectively. This low retention could explain this higher variability.

3.4. Variability of IMS collision cross section values

The same workflow was applied for the calculation of variability of $^{TW}CCS_{N2}$ values. Except for the RSD value of one single compound (6β-hydroxybudesonide), all intraday, interday, and

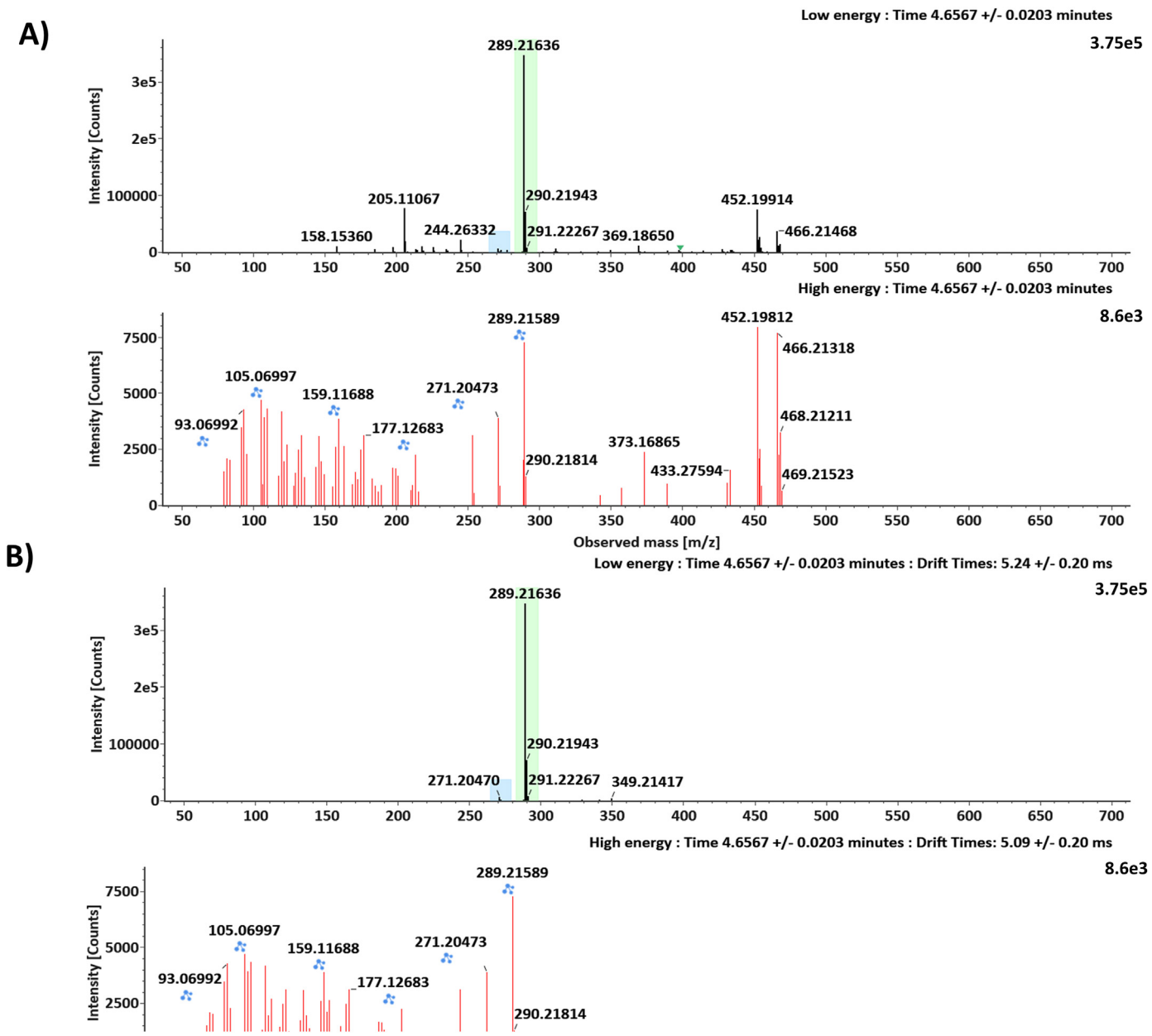


Fig. 6. Comparison of low energy and high energy 3D MS spectra measured in HDMS^E mode with and without IMS filtration in data processing. (A) UHPLC-HRMS spectra of oxandrolone filtered only by retention time ± 0.0203 min, (B) UHPLC-HRMS spectra of oxandrolone filtered by retention time ± 0.0203 min and drift time ± 0.2 ms.

interweek RSD values for both Groups of doping agents were <2% proving the stability of $^{TW}CCS_{N2}$ values across all the analyses even after performing a re-calibration of the IMS cell only once a week. Box-plots of the RSD values obtained for $^{TW}CCS_{N2}$ are shown in Fig. 4.

The influence of a biological matrix on the $^{TW}CCS_{N2}$ values of the doping agents was also examined. Overall, the variation of $^{TW}CCS_{N2}$ values between standard and urine samples was found to be negligible. Considering the prohibited substances from Group I, the differences in RSD values between $^{TW}CCS_{N2}$ in standard and urine samples was <1%, except for three compounds (MDA, cathine, mephentermine) with RSD in the range of 1.1–1.7%. Group II compounds offered even better match in RSD between $^{TW}CCS_{N2}$ in standards, pre-extraction, and post-extraction spiked samples, with values always lower than 0.7%, except for 6β-hydroxybudesonide, with RSD equal to 1.3%. Moreover, the differences between $^{TW}CCS_{N2}$ in standard and urine samples were also statistically evaluated using parametric paired *t*-test with corresponding p-value calculation ($\alpha = 0.05$). The two tested groups, i.e. $^{TW}CCS_{N2}$ in standards and $^{TW}CCS_{N2}$ in urine, showed no significant difference with the standard error of mean (SEM) of differences ± 0.09; n = 96; p = 0.9726 for Group I and SEM ± 0.09, n = 86; p = 0.1528 for Group II, respectively. The effectiveness of the pairing was then proven by correlation coefficient 0.9993 with $p < 0.0001$ for both Groups.

Overall, we demonstrated excellent robustness of the UHPLC-IM-HRMS method in terms of retention time and $^{TW}CCS_{N2}$ variability. This is fundamental for the potential use of the database for routine applications in initial testing procedures for anti-doping purposes. Moreover, these data have shown an excellent agreement of $^{TW}CCS_{N2}$ values with previously published data of Hines et al. for example for tamoxifen (197.2 vs 197.7), raloxifene (213.4 vs 214.4), and timolol (174.6 vs 175.5) [14].

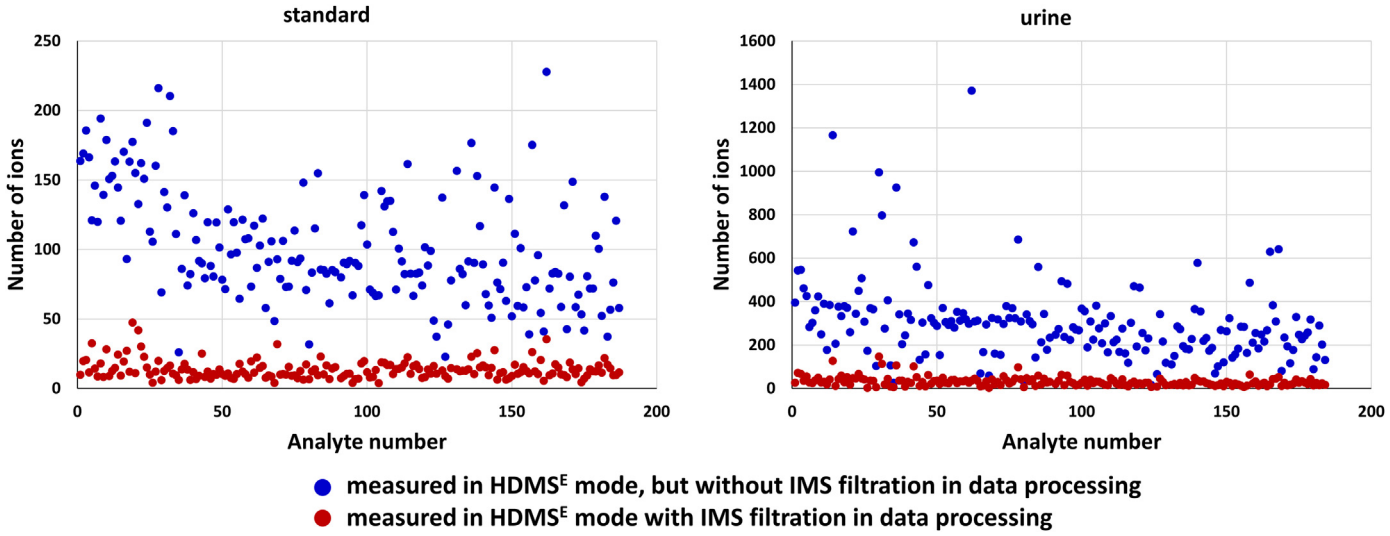


Fig. 7. Number of ions detected in the HDMS^E low energy spectra. 3D spectra filtered by peak apex retention time are shown with and without IMS filtration by drift time.

3.5. Effect of MS spectrum filtering by drift time

When adding an IMS dimension to the UHPLC-MS analysis, the MS spectra can be cleaner, and many interferences can be removed. The overall method sensitivity is increased due to lower background, which results from the filtration - not only by the retention time of the target compound but also by its drift time. The benefit from the addition of the IM dimension on top of the UHPLC separation is its power of filtering those ions from coeluting chromatographic peaks that do not match with the molecule of interest with respect to the drift time. By improved selectivity and simplified result interpretation, a number of "false alarms" of ITP can be reduced and an overall sample turnover improved. Moreover, by providing access to $^{TW}CCS_{N2}$ values, the IMS technology provides a gain of confidence in the doping agents identification.

UNIFI software version 1.9.3 was used for data processing. According to the instrument manufacturer [44,45], MS^E and HDMS^E data are processed in UNIFI using 3D peak detection. The software scans the entire set of data like a tridimensional plot (t_R and *m/z* on the horizontal dimension x and y, intensity of the signal on the vertical axis z) and identifies the summits (apexes) of the peaks. 3D peaks are then aligned (or grouped) based on their apex retention times. The same alignment principle applies between the high and low energy data. Peaks that are assigned to one candidate component based on this set of rules are reported into what is named the component spectrum (for the low energy and high energy). A component spectrum shows less ions than the corresponding extracted spectrum at the retention time of the analyte, since it only contains ions whose peak apexes have the same retention time as the candidate. When ion mobility is used, this additional fourth dimension is also added to the process of alignment (assigning ions to a specific candidate compound), usually resulting in even cleaner spectra.

Fig. 5 shows the spectra of a representative substance from Group I, namely clopamide, filtered only by its retention time (Fig. 5A), i.e. 3.48 ± 0.02 min, and by both its retention and drift time, i.e. 5.71 ± 0.21 ms (Fig. 5B). The same applies also for Fig. 6A and B showing the spectra of oxandrolone, a representative compound from Group II. As shown, a significantly lower number of ions was detected after the addition of the fourth dimension, i.e. the filtering by drift time. Therefore, the filtered spectra are much cleaner, with a reduced number of interferences, providing an easier interpretation and lower risk for false positive screening results. In this context, a detailed comparison of high energy spectra, ions and patterns of fragmentation of doping agents in IMS will be discussed in more detailed in the second part of this study. Here, we will focus only on low energy spectra, for which the number of detected ions was chosen as a parameter to enable the determination of the effect of IMS filtration. Fig. 7 shows number of detected ions in the low energy spectra HDMS^E with and without the IMS filtration during data processing. The percentage decrease in the number of ions in the MS spectra after IMS filtering was calculated in the range 54–98% for all doping agents. On average, 86% less ions were detected in the spectra of both standard and urine samples, after the IMS filtration.

3.6. Evaluation of isobaric and isomeric substances separation

It has been well described that the IMS can improve method selectivity by providing additional separation dimension, possibly allowing the separation of isobaric and isomeric compounds. The analyzed set of doping agents contained 30 pairs of isobaric or isomeric compounds which were listed in Table 3.

The developed UHPLC-IM-HRMS method here enabled the correct identification of most of these substances based on their differences in retention time and/or $^{TW}CCS_{N2}$. An example of the determination based on differences in $^{TW}CCS_{N2}$ is shown in Fig. 8. The pair of stanozolol metabolites (16β-hydroxystanozolol and 4α-hydroxystanozolol at 3.63 *vs.* 3.78 min) and especially the pair of methedrone and MDMA (2.59 *vs.* 2.65 min) have exactly the same *m/z* and very close retention times, which could lead to incorrect result interpretation for these substances. In these two examples, the differences in $^{TW}CCS_{N2}$ values are higher than those in retention times (192.97 *vs.* 201.48 Å^2 and 136.17 *vs.* 144.27 Å^2), therefore IMS could offer an easy way to improve the level of annotation of these doping agents.

Amongst the 30 pairs of isobaric/isomeric compounds analyzed in this work, only two pairs proved to be difficult to separate even with the IMS dimension. Flunisolide and triamcinolone acetonide, representatives from the group of glucocorticosteroids, have very similar retention times (4.27 min *vs.* 4.24 min). The difference in $^{TW}CCS_{N2}$ is quite large (198.19 *vs.* 205.52 Å^2), but due to the very limited resolution of the IMS instrumentation used in this work, the correct assignment of these compounds can only be obtained in

Table 3
Comparison of monoisotopic masses, retention times (t_r), and $^{TW}CCS_{N2}$ values of analyzed isobaric doping agents. See Table 2 for nomenclature.

Substance	chemical formula	monoisotopic mass	t_r	$^{TW}CCS_{N2}$
Cathinone	$C_9H_{11}NO$	149.0841	2.26	124.99
Methamphetamine	$C_{10}H_{15}N$	149.1204	2.62	127.90
Metcathinone	$C_{10}H_{13}NO$	163.0997	2.36	128.44
Ethylamphetamine	$C_{11}H_{17}N$	163.1361	2.77	136.73
Mephentermine	$C_{11}H_{17}N$	163.1361	2.77	126.37
Pemoline	$C_9H_8N_2O_2$	176.0586	2.79	151.31
Benzylpiperazine	$C_{11}H_{16}N_2$	176.1313	1.64	139.93
MDA	$C_{10}H_{13}NO_2$	179.0946	2.58	125.11
Methoxyphenamine	$C_{11}H_{17}NO$	179.1310	2.84	140.10
Methylephedrine	$C_{11}H_{17}NO$	179.1310	2.44	139.60
Oxilofrine	$C_{10}H_{15}NO_2$	181.1103	1.25	137.41
Etilefrine	$C_{10}H_{15}NO_2$	181.1103	1.82	138.66
Methedrone	$C_{11}H_{15}NO_2$	193.1103	2.59	136.17
MDMA	$C_{11}H_{15}NO_2$	193.1103	2.65	144.27
Amiloride	$C_6H_8ClN_7O$	229.0479	2.13	146.53
Furfenorex	$C_{15}H_{19}NO$	229.1467	3.21	150.02
Clobenzorex	$C_{16}H_{18}ClN$	259.1128	3.62	156.31
Propanolol	$C_{16}H_{21}NO_2$	259.1572	3.47	162.94
Atenolol	$C_{14}H_{22}N_2O_3$	266.1630	2.15	158.61
Trimetazidine	$C_{14}H_{22}N_2O_3$	266.1630	2.19	160.40
Morphine	$C_{17}H_{19}NO_3$	285.1365	1.88	163.51
Hydromorphone	$C_{17}H_{19}NO_3$	285.1365	2.1	163.79
Pentazocine	$C_{19}H_{27}NO$	285.2093	3.33	174.57
Cocaine	$C_{17}H_{21}NO_4$	303.1471	3.14	169.02
Fenoterol	$C_{17}H_{21}NO_4$	303.1471	2.38	171.14
Nadolol	$C_{17}H_{27}NO_4$	309.1940	2.57	172.93
Methadone	$C_{21}H_{27}NO$	309.2093	4.05	177.38
Bambuterol	$C_{18}H_{29}N_3O_5$	367.2107	3.16	190.25
Fenbutrazate	$C_{23}H_{29}NO_3$	367.2147	4.22	189.47
Epitrenbolone	$C_{18}H_{22}O_2$	270.1620	4.4	165.63
Trenbolone	$C_{18}H_{22}O_2$	270.1620	4.27	165.49
Oxabolone met.	$C_{18}H_{24}O_3$	288.1725	4.79	169.77
Boldenone met.	$C_{19}H_{28}O_2$	288.2089	4.97	173.10
18-Methylnandrolone metabolite M1	$C_{19}H_{30}O_2$	290.2246	5.61	167.97
18-Methylnandrolone metabolite M2	$C_{19}H_{30}O_2$	290.2246	5.21	163.20
Metenolone	$C_{20}H_{30}O_2$	302.2246	6.18	171.26
Mibolerone	$C_{20}H_{30}O_2$	302.2246	4.86	177.90
Ethisterone	$C_{21}H_{28}O_2$	312.2089	4.88	179.50
Tetrahydrogestrinone	$C_{19}H_{28}O_3$	312.2089	5.24	179.38
6β-Hydroxymethandienone	$C_{20}H_{28}O_3$	316.2038	3.6	173.01
Calusterone	$C_{21}H_{32}O_2$	316.2402	5.14	180.18
Bolasterone	$C_{21}H_{32}O_2$	316.2402	5.03	180.53
Fluoxymesterone metabolite M2	$C_{20}H_{29}FO_4$	318.1995	5.45	176.82
Oxymesterone	$C_{20}H_{30}O_3$	318.2195	4.95	177.23
Methasterone	$C_{21}H_{34}O_2$	318.2559	5.8	181.09
16β-Hydroxy-prostanozolol	$C_{20}H_{28}N_2O_2$	328.2151	3.36	187.86
3-Hydroxyprostanozolol	$C_{20}H_{28}N_2O_2$	328.2151	3.93	187.38
Stanozolol	$C_{21}H_{32}N_2O$	328.2515	4.37	189.65
16β-Hydroxystanozolol	$C_{21}H_{32}N_2O_2$	344.2464	3.63	192.97
4α-Hydroxystanozolol	$C_{21}H_{32}N_2O_2$	344.2464	3.78	201.48
4β-Hydroxystanozolol	$C_{21}H_{32}N_2O_2$	344.2464	3.98	191.59
3′-Hydroxystanozolol	$C_{21}H_{32}N_2O_2$	344.2464	3.88	192.91
Roxadustat (FG 4592)	$C_{19}H_{16}N_2O_5$	352.1059	5.44	185.92
Fluoxymesterone metabolite M1	$C_{20}H_{27}FO_2$	352.2050	3.35	182.24
JWH-073 4-butanoic acid	$C_{23}H_{19}NO_3$	357.1365	5.03	185.98
JWH-018 5-pentanoic acid	$C_{24}H_{23}NO_2$	357.1729	5.29	188.50
JWH-018 4-hydroxypentyl	$C_{24}H_{21}NO_3$	371.1521	5.17	188.76
Tamoxifen	$C_{26}H_{29}NO$	371.2249	4.87	197.76
6β-Hydroxybudesonide	$C_{21}H_{28}O_6$	376.1886	3.34	187.36
Fluocortolone	$C_{22}H_{29}FO_4$	376.2050	4.38	188.97
Betamethasone	$C_{22}H_{29}FO_5$	392.1999	4.06	216.21
Dexamethasone	$C_{22}H_{29}FO_5$	392.1999	4.09	216.38
Toremifene	$C_{26}H_{28}ClNO$	405.1859	4.77	204.34
Clomifen	$C_{26}H_{28}ClNO$	405.1859	4.75	206.84
Flunisolide	$C_{24}H_{31}FO_6$	434.2105	4.28	200.28
Triamcinolone acetonide	$C_{24}H_{31}FO_6$	434.2105	4.24	197.49
Bazedoxifen	$C_{30}H_{34}N_2O_3$	470.2569	3.76	213.37
Desisobutyrylciclesonide	$C_{28}H_{38}O_6$	470.2668	5.47	216.32

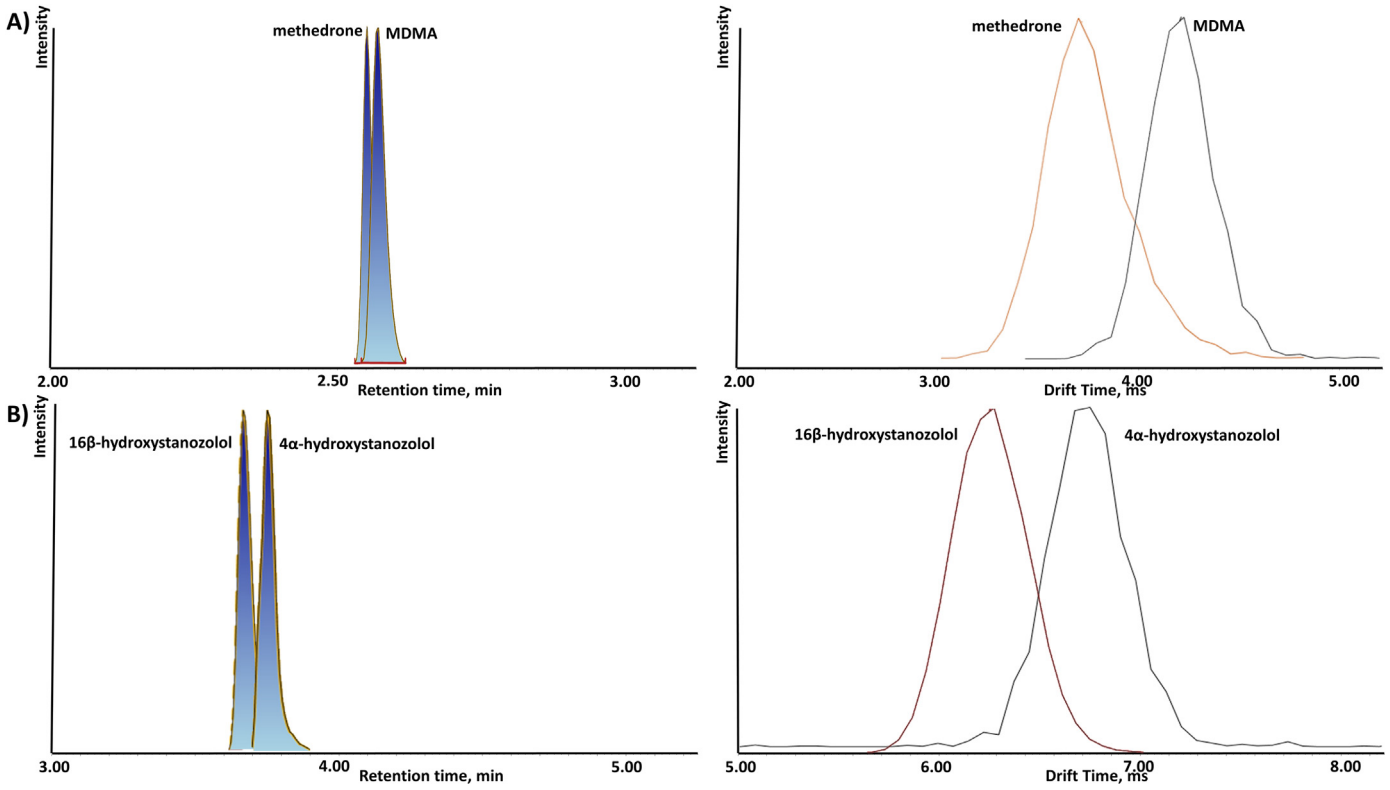


Fig. 8. Separation of isobaric and isomeric doping agents in the UHPLC and IMS dimensions. Overlay of chromatograms and mobilograms of (A) methedrone and MDMA and (B) 16β-hydroxystanozolol and 4α-hydroxystanozolol.

approximately 60% of the cases. Another exception is the determination of betamethasone and dexamethasone, glucocorticosteroids as well, with similar retention times (4.06 min *vs.* 4.07 min) as well as $^{TW}CCS_{N2}$ (219.02 *vs.* 220.84). In this case, the compounds were correctly identified in 70% of the cases for betamethasone but only 20% for dexamethasone. However, such an issue could be resolved with an IMS cell offering higher resolution [46,47]. Plot depicting the required resolving power to separate two compounds in ion mobility with a known difference in CCS values was presented by Dodds et al. [48]. So far, resolving power up-to 50 was reported for TWIMS technology. The differences between $^{TW}CCS_{N2}$ in our case were 1.8 and 0.4% for flunisolide/triamcinolone acetate and betamethasone/dexamethasone, respectively. Based on the plot [48], IMS with resolving power higher than 90 would be needed for 50% separation of the peaks of flunisolide and triamcinolone acetate. The same separation of betamethasone and dexamethasone would be achieved with IMS resolving power around 350. Considering 90% separation of peaks, resolving power >150 is necessary for the fluticasone and triamcinolone acetonide, while >500 would be needed for betamethasone and dexamethasone.

4. Conclusion

In this study, the benefits of IMS technology added to UHPLC-HRMS were explored for the analysis of 192 doping agents from the WADA Prohibited List. These substances were classified in two groups, namely Group I containing mainly stimulants and narcotics, and Group II containing mostly steroids, glucocorticoids, and hormone and metabolic modulators.

Based on the experiments performed with standard solutions and urine samples, a comprehensive database containing retention times (UHPLC information), $^{TW}CCS_{N2}$ values (IMS information), and *m/z* ratios (MS information) for the 192 doping agents was constructed. The robustness of the UHPLC-IM-HRMS method was also assessed by evaluating the intraday, interday, and interweek variability of retention times and $^{TW}CCS_{N2}$ values at different concentrations in standard and urine matrix. These results confirm the excellent robustness of the developed method, as the RSD values were always lower than 2%, except for the retention times of three weakly retained doping agents. IMS is also known to offer a filtering capability to obtain cleaner MS and MS/MS spectra, thus avoiding interferences and false positive screening results. This possibility was investigated here, and on average, 86% less peaks were detected in the low energy MS spectra of both standard and urine samples for the 192 doping agents. Finally, IMS also offers an additional separation dimension allowing a possible separation of isobaric compounds. Amongst the 192 doping agents, there were 30 pairs of isobaric or isomeric compounds, 28 of which were resolved using either the chromatographic or the IMS-HRMS dimensions, which can be considered as a significant improvement and a valuable asset to the anti-doping analysis.

CRediT authorship contribution statement

Kateřina Plachká: Writing - original draft, Methodology, Investigation. **Julian Pezzatti:** Methodology, Investigation, Writing - review & editing. **Alessandro Musenga:** Resources, Writing - review & editing. **Raul Nicoli:** Resources, Writing - review & editing. **Tiia Kuuranne:** Resources, Writing - review & editing. **Serge Rudaz:** Resources, Writing - review & editing. **Lucie Nováková:** Writing - original draft, Methodology, Investigation. **Davy Guillarme:** Supervision, Writing - review & editing, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors gratefully acknowledge the grant projects SVV No. 260 548, and the STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465) co-funded by ERDF. Authors wish to thank Prof. Jean-Luc Veuthey from the University of Geneva for his fruitful comments and discussions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2021.338257.

References

[1] WADA, Prohibited List, 2020 [Available from: https://www.wada-ama.org/en/resources/science-medicine/prohibited-list-documents.
[2] M. Thevis, T. Kuuranne, H. Geyer, Annual banned-substance review - analytical approaches in human sports drug testing, Drug Test. Anal. 12 (1) (2020) 7–26.
[3] M. Thevis, K. Walpurgis, A. Thomas, Analytical approaches in human sports drug testing: recent advances, challenges, and solutions, Anal. Chem. 92 (1) (2020) 506–523.
[4] F. Badoud, D. Guillarme, J. Boccard, E. Grata, M. Saugy, S. Rudaz, et al., Analytical aspects in doping control: challenges and perspectives, Forensic Sci. Int. 213 (1) (2011) 49–61.
[5] R. Nicoli, D. Guillarme, N. Leuenberger, N. Baume, N. Robinson, M. Saugy, et al., Analytical strategies for doping control purposes: needs, challenges, and perspectives, Anal. Chem. 88 (1) (2016) 508–523.
[6] M. Mazzarino, F. Botrè, A fast liquid chromatographic/mass spectrometric screening method for the simultaneous detection of synthetic glucocorticoids, some stimulants, anti-oestrogen drugs and synthetic anabolic steroids, Rapid Commun. Mass Spectrom. 20 (22) (2006) 3465–3476.
[7] J.O. Thörngren, F. Ostervall, M. Garle, A high-throughput multicomponent screening method for diuretics, masking agents, central nervous system (CNS) stimulants and opiates in human urine by UPLC-MS/MS, J. Mass Spectrom. 43 (7) (2008) 980–992.
[8] R.J.B. Peters, J.E. Oosterink, A.A.M. Stolker, C. Georgakopoulos, M.W.F. Nielen, Generic sample preparation combined with high-resolution liquid chromatography–time-of-flight mass spectrometry for unification of urine screening in doping-control laboratories, Anal. Bioanal. Chem. 396 (7) (2010) 2583–2598.
[9] F. Badoud, E. Grata, L. Perrenoud, L. Avois, M. Saugy, S. Rudaz, et al., Fast analysis of doping agents in urine by ultra-high-pressure liquid chromatography-quadrupole time-of-flight mass spectrometry I. Screening analysis, J. Chromatogr. A 1216 (20) (2009) 4423–4433.
[10] G.J. Murray, J.P. Danaceau, Simultaneous extraction and screening of diuretics, beta-blockers, selected stimulants and steroids in human urine by HPLC-MS/MS and UPLC-MS/MS, J Chromatogr B Analyt Technol Biomed Life Sci 877 (30) (2009) 3857–3864.
[11] G. Paglia, J.P. Williams, L. Menikarachchi, J.W. Thompson, R. Tyldesley-Worster, S. Halldórsson, et al., Ion mobility derived collision cross sections to support metabolomics applications, Anal. Chem. 86 (8) (2014) 3985–3993.
[12] V. Hinnenkamp, J. Klein, S.W. Meckelmann, P. Balsaa, T.C. Schmidt, O.J. Schmitz, Comparison of CCS values determined by traveling wave ion mobility mass spectrometry and drift tube ion mobility mass spectrometry, Anal. Chem. 90 (20) (2018) 12042–12050.
[13] L.C. Nye, J.P. Williams, N.C. Munjoma, M.P.M. Letertre, M. Coen, R. Bouwmeester, et al., A comparison of collision cross section values obtained via travelling wave ion mobility-mass spectrometry and ultra high performance liquid chromatography-ion mobility-mass spectrometry: application to the characterisation of metabolites in rat urine, J. Chromatogr. A 1602 (2019) 386–396.
[14] K.M. Hines, D.H. Ross, K.L. Davidson, M.F. Bush, L. Xu, Large-scale structural characterization of drug and drug-like compounds by high-throughput ion mobility-mass spectrometry, Anal. Chem. 89 (17) (2017) 9023–9030.
[15] M. Hernández-Mesa, V. D'Atri, G. Barknowitz, M. Fanuel, J. Pezzatti, N. Dreolin, et al., Interlaboratory and interplatform study of steroids collision cross section by traveling wave ion mobility spectrometry, Anal. Chem. 92 (7) (2020) 5013–5022.
[16] V. D'Atri, T. Causon, O. Hernandez-Alba, A. Mutabazi, J.L. Veuthey, S. Cianferani, et al., Adding a new separation dimension to MS and LC-MS: what is the utility of ion mobility spectrometry? J. Separ. Sci. 41 (1) (2018) 20–67.
[17] V. Gabelica, A.A. Shvartsburg, C. Afonso, P. Barran, J.L.P. Benesch, C. Bleiholder, et al., Recommendations for Reporting Ion Mobility Mass Spectrometry Measurements, vol. 38, 2019, pp. 291–320, 3.
[18] M. Hernandez-Mesa, D. Ropartz, A.M. Garcia-Campana, H. Rogniaux, G. Dervilly-Pinel, B. Le Bizec, Ion mobility spectrometry in food analysis: principles, current applications and future trends, Molecules 24 (15) (2019).
[19] A. Kaufmann, P. Butcher, K. Maden, S. Walker, M. Widmer, Practical Application of in Silico Fragmentation Based Residue Screening with Ion Mobility High-Resolution Mass Spectrometry, vol. 31, 2017, pp. 1147–1157, 13.
[20] G. Paglia, G. Astarita, Metabolomics and lipidomics using traveling-wave ion mobility mass spectrometry, Nat. Protoc. 12 (4) (2017) 797–813.
[21] C.B. Mollerup, M. Mardal, P.W. Dalsgaard, K. Linnet, L.P. Barron, Prediction of collision cross section and retention time for broad scope screening in gradient reversed-phase liquid chromatography-ion mobility-high resolution accurate mass spectrometry, J. Chromatogr. A 1542 (2018) 82–88.
[22] Drouin N, Mielcarek A, Wenz C, Rudaz S. Evaluation of ion mobility in capillary electrophoresis coupled to mass spectrometry for the identification in metabolomics. Electrophoresis.n/a(n/a), https://doi.org/10.1002/elps.202000120.
[23] A. Lommen, A. Elaradi, A. Vonaparti, M. Blokland, M.W. Nielen, K.A. Saad, et al., Ultra-fast retroactive processing of liquid chromatography high-resolution full-scan Orbitrap mass spectrometry data in anti-doping screening of human urine, Rapid Commun. Mass Spectrom. : RCM. 33 (20) (2019) 1578–1588.
[24] M. Polet, W. Van Gansbeke, P. Van Eenoo, Development and validation of an open screening method for doping substances in urine by gas chromatography quadrupole time-of-flight mass spectrometry, Anal. Chim. Acta 1042 (2018) 52–59.
[25] A. Musenga, D.A. Cowan, Use of ultra-high pressure liquid chromatography coupled to high resolution mass spectrometry for fast screening in high throughput doping control, J. Chromatogr. A 1288 (2013) 82–95.
[26] B. Han, H. Min, M. Jeon, B. Kang, J. Son, A rapid non-target screening method for determining prohibited substances in human urine using liquid chromatography/high-resolution tandem mass spectrometry, Drug Test. Anal. 11 (3) (2019) 382–391.
[27] V.F. Sardela, M.E.P. Martucci, A.L.D. de Araujo, E.C. Leal, D.S. Oliveira, G.R.A. Carneiro, et al., Comprehensive analysis by liquid chromatography Q-Orbitrap mass spectrometry: fast screening of peptides and organic molecules, J. Mass Spectrom. : JMS 53 (6) (2018) 476–503.
[28] W. Abushareeda, A. Vonaparti, K.A. Saad, M. Almansoori, M. Meloug, A. Saleh, et al., High resolution full scan liquid chromatography mass spectrometry comprehensive screening in sports antidoping urine analysis, J. Pharmaceut. Biomed. Anal. 151 (2018) 10–24.
[29] C. Gorgens, S. Guddat, A. Thomas, P. Wachsmuth, A.K. Orlovius, G. Sigmund, et al., Simplifying and expanding analytical capabilities for various classes of doping agents by means of direct urine injection high performance liquid chromatography high resolution/high accuracy mass spectrometry, J. Pharmaceut. Biomed. Anal. 131 (2016) 482–496.
[30] Pappireddi N, Martin L, Wühr MA-O. A Review on Quantitative Multiplexed Proteomics. (1439-7633 (Electronic)).
[31] X. Ma, J. Liu, Z. Zhang, T. Bo, Y. Bai, H. Liu, Drift Tube Ion Mobility and Four-Dimensional Molecular Feature Extraction Enable Data-independent Tandem Mass Spectrometric 'omics' Analysis without Quadrupole Selection, vol. 31, 2017, pp. 33–38, 1.
[32] P. Dwivedi, A.J. Schultz, H.H.H. Jr, Metabolic profiling of human blood by high-resolution ion mobility mass spectrometry (IM-MS), Int. J. Mass Spectrom. 298 (1) (2010) 78–90.
[33] S. Guddat, M. Thevis, J. Kapron, A. Thomas, W. Schänzer, Application of FAIMS to Anabolic Androgenic Steroids in Sport Drug Testing vol. 1, 2009, pp. 545–553, 11-12.
[34] J.A. Ray, M.M. Kushnir, R.A. Yost, A.L. Rockwood, A. Wayne Meikle, Performance enhancement in the measurement of 5 endogenous steroids by LC–MS/MS combined with differential ion mobility spectrometry, Clin. Chim. Acta 438 (2015) 330–336.
[35] G. Kaur-Atwal, J.C. Reynolds, C. Mussell, E. Champarnaud, T.W. Knapman, A.E. Ashcroft, et al., Determination of testosterone and epitestosterone glucuronides in urine by ultra performance liquid chromatography-ion mobility-mass spectrometry, Analyst 136 (19) (2011) 3911–3916.
[36] A. Thomas, C. Görgens, S. Guddat, D. Thieme, F. Dellanna, W. Schänzer, et al., Simplifying and Expanding the Screening for Peptides <2 kDa by Direct Urine Injection, Liquid Chromatography, and Ion Mobility Mass Spectrometry, vol. 39, 2016, pp. 333–341, 2.
[37] M. Hernández-Mesa, B. Le Bizec, F. Monteau, A.M. García-Campaña, G. Dervilly-Pinel, Collision cross section (CCS) database: an additional measure to characterize steroids, Anal. Chem. 90 (7) (2018) 4616–4625.
[38] A.L. Rister, E.D. Dodds, Steroid analysis by ion mobility spectrometry, Steroids 153 (2020) 108531.
[39] C.D. Chouinard, C.R. Beekman, R.H.J. Kemperman, H.M. King, R.A. Yost, Ion mobility-mass spectrometry separation of steroid structural isomers and epimers, Int. J. Ion Mobil. Spectrometr. 20 (1) (2017) 31–39.
[40] L. Ahonen, M. Fasciotti, G.B. af Gennas, T. Kotiaho, R.J. Daroda, M. Eberlin, et al., Separation of steroid isomers by ion mobility mass spectrometry, J. Chromatogr. A 1310 (2013) 133–137.

[41] M. Thevis, J. Dib, A. Thomas, S. Hoppner, A. Lagojda, D. Kuehne, et al., Complementing the characterization of in vivo generated N-glucuronic acid conjugates of stanozolol by collision cross section computation and analysis, Drug Test. Anal. 7 (11–12) (2015) 1050–1056.
[42] WADA, Technical Document – TD2019MRPL, 2019.
[43] L. Novakova, V. Desfontaine, F. Ponzetto, R. Nicoli, M. Saugy, J.L. Veuthey, et al., Fast and sensitive supercritical fluid chromatography - tandem mass spectrometry multi-class screening method for the determination of doping agents in urine, Anal. Chim. Acta 915 (2016) 102–110.
[44] J. Goshawk, D. Eatough, M. Wood, Compenzation Following 3-D Peak Detection in the UNIFI Scientific Information System, Waters Corporation, 2015, 720005480EN AW-PC.
[45] Componentization. Waters Corporation.720005480EN AW-PC.
[46] K. Giles, J. Ujma, J. Wildgoose, S. Pringle, K. Richardson, D. Langridge, et al., A cyclic ion mobility-mass spectrometry system, Anal. Chem. 91 (13) (2019) 8564–8573.
[47] D. Ropartz, M. Fanuel, J. Ujma, M. Palmer, K. Giles, H. Rogniaux, Structure determination of large isomeric Oligosaccharides of natural Origin through multipass and multistage cyclic traveling-wave ion mobility mass spectrometry, Anal. Chem. 91 (18) (2019) 12030–12037.
[48] J.N. Dodds, J.C. May, J.A. McLean, Correlating resolving power, resolution, and collision cross section: unifying cross-platform assessment of separation efficiency in ion mobility spectrometry, Anal. Chem. 89 (22) (2017) 12176–12184.