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Routine application of SFC-MS in doping control: Analysis of 3 × 1000 urine samples using three different SFC-MS instruments

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Abstract

Supercritical fluid chromatography-mass spectrometry (SFC-MS) has proved to be a beneficial tool for sample analysis for a wide variety of compounds and, as such, has recently gained the attention of the anti-doping community. We have tested the applicability of SFC-MS for routine doping control analysing approximately 3 × 1000 identical anti-doping samples utilising SFC-MS instruments from three different vendors: Agilent Technologies, Waters Corporation and Shimadzu Corporation. A 'dilute and inject' approach either without or after hydrolysis of glucuronide metabolites was applied. Most of the compounds included in our study demonstrated excellent chromatography, whereas some showed co-elution with endogenous interferences requiring MS discrimination. Retention times typically were very stable within batches (%CV ≤ 0.5%), although this appeared to be analyte and column dependent. Chromatographic peak shape was good (symmetrical) and stable over the period of the testing without any change of column. Our results suggest that SFC-MS is a sensitive, reproducible and robust analytical tool ready to be used in anti-doping laboratories alongside the currently applied techniques such as gas and liquid chromatography coupled to mass spectrometry. Even if instruments are designed slightly differently, all three setups demonstrated their fitness for the purpose in anti-doping testing.

KEYWORDS
doping control, robustness, SFC-MS

1 | INTRODUCTION

Gas and liquid chromatography coupled to mass spectrometry (GC–MS, LC–MS) are the two main analytical techniques employed by World Anti-Doping Agency (WADA) accredited laboratories for testing for the presence of compounds prohibited in sport.[1,2] Introduced to the anti-doping laboratories in 2013,[3] supercritical fluid chromatography-mass spectrometry (SFC-MS) has gained attention among researchers in the anti-doping community and several papers have demonstrated and justified its applicability.[4–10]

At first glance, SFC resembles normal phase chromatography and employs supercritical CO_2 as the mobile phase. Supercritical CO_2 is a non-polar solvent and is kept at or above its critical point, which represents the temperature and pressure conditions under which a liquid and its vapour co-exist. Alcohols (methanol, ethanol or propan-2-ol) are often added as co-solvents to modify the polarity of supercritical CO_2 which, together with the appropriate stationary phase, allow the separation of various types of analyte. Coupling of SFC to a mass spectrometer (MS) seems to be the most desirable choice for bioanalysis because of the mass spectrometer's sensitivity, selectivity, mass accuracy (especially with high resolution, high mass accuracy instrumentation), and the possibility of performing qualitative and quantitative analysis. Successful SFC-MS hyphenation of the high-pressure SFC column with the atmospheric pressure ion source of the MS requires a suitable back-pressure regulator to maintain the CO_2 in a supercritical state. The design of SFC-MS interfaces has been improved over the years and, with advances in column technology, especially sub-2 μm particle size ultra-high performance columns, SFC-MS technology has proven to be a good analytical tool.[11,12] There are examples of employing SFC-MS for the analysis of pesticides, pharmaceuticals, metabolites and metabolomic studies.[13–20]

With respect to the growing interest and the potential use in doping control, we undertook a study to determine whether SFC-MS would be a practical alternative, or at least complementary, to the widely applied routine reversed-phase liquid chromatography–mass spectrometry (LC–MS) techniques currently used by WADA accredited laboratories. Despite its versatility, reversed-phase LC–MS is not the ultimate technique in any anti-doping laboratory and some of its limitations are related to the analysis of polar and basic compounds, such as ephedrine type stimulants. Hydrophilic interaction liquid chromatography (HILIC) columns have been proposed to overcome these limitations.[21–23] Good retention, separation and sensitivity can be achieved provided sufficient equilibration time is used and the injection solvents are compatible with the mobile phases. These limitations of HILIC may prevent a practical solution, especially in anti-doping laboratories when a fast turnaround time and efficient use of expensive LC–MS instruments are required. In our view, a potential alternative is SFC-MS. In our previous study, we have demonstrated that approximately 200 compounds of various types of drugs from the WADA Prohibited List (e.g. stimulants, glucocorticoids and β-blockers) could easily be analysed by SFC-MS. Furthermore, we comment on the ease of SFC-LC switching, making the approach even more cost-effective enabling the WADA accredited laboratory to invest in an LC and an SFC attached to the same mass spectrometer.[24]

A SFC-MS screening method for the analysis of approximately 110 various drugs prohibited in sport including anabolic steroids, hormone modulators, stimulants, narcotics, synthetic cannabinoids and glucocorticoids has been published.[4–7] These authors optimised the choice of stationary phase as well as the composition of mobile phases and MS conditions to enable chromatographic separation and resolution and to ensure maximised MS response.

Losacco et al. studied the retention time stability on a single SFC-MS instrument over a period of 4 months[25] using a single pool of urine samples obtained from three male and female volunteers that was divided into six aliquots, which were spiked with 51 compounds at three different analyte concentrations (0.5, 5 and 50 ng/mL). Good retention time stability was demonstrated and with this current study we have also reported a similar experiment using different equipment, but with nearly 200 analytes. Also, we undertook a semi-quantitative calibration using four different concentrations from 25% MRPL (minimum required performance level) to 200% MRPL where we diluted with one blank urine all analytes in our quality controls (QCs; of 54 analytes). Generally, linearity was good (meeting WADA criteria) and seems to be a useful means of a WADA accredited laboratory providing an indicative estimate of concentration for assisting the results management authority's review of an anti-doping violation.[24] A comprehensive review about the application of SFC-MS for anti-doping analysis has recently been provided by Parr and Botrè.[26]

Our study shows practical reliability and readiness of SFC-MS to be used as a tool for routine sample analysis in anti-doping laboratories. The developed SFC-MS method was compared to a conventional LC–MS/MS method and was found to be sufficiently sensitive to perform screening analysis for doping control. We studied the performance using approximately 3 × 1000 identical anti-doping samples in total on SFC-MS instruments from three different vendors: Agilent Technologies, Waters Corporation and Shimadzu Corporation. Two of the vendors used acquisition methods based on previously published work.[4,8] Analytes were chosen to reflect various categories of compounds from the WADA Prohibited List. However, instead of using solid-phase extraction as a standard sample preparation procedure, a simple 'dilute and inject' approach was employed by all. We investigated the reliability of different SFC-MS instruments for their use in the routine anti-doping laboratory and assessed the results against each other. We impartially employed SFC-MS instruments available on the market leaving the choice of instruments to each laboratory.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Escherichia coli (*E. coli*) β-glucuronidase K 12, d_3-ephedrine (as hydrochloride 1 mg/mL methanolic solution in ampoule) and d_6-DHEA sulphate (3β-hydroxy-androst-5-en-17-one-3-sulphate 2,2,3,4,4,6-d_6, as sodium salt, 1 mg) were purchased from

Sigma–Aldrich (Gillingham, UK). d_3-salbutamol (as hydrochloride, 1 mg) and d_3-testosterone glucuronide (as sodium salt, 1 mg) were purchased from the National Measurement Institute (Canberra, Australia). Mefruside was purchased from Bayer (Leverkusen, Germany). Analytes used to prepare QC samples were purchased as reference materials from various vendors. Methanol was purchased either from Fisher Scientific (Loughborough, UK) or from Merck KGaA (Darmstadt, Germany). LC–MS grade methanol, acetonitrile, anhydrous tetrahydrofuran (THF), carbon dioxide, ammonia, ammonium formate and ammonium acetate were already acquired by vendors and were used from in-house inventories.

2.2 | Urine samples

One thousand urine samples obtained from athletes who had consented for their samples to be used for research purposes on the sample collection document (Doping Control Form) were collected for this study. Ethical permission for the study was obtained from King's College London Research Ethics Committee (LRS-17/18-7119). Once the samples had been analysed by the routine liquid chromatography-high resolution mass spectrometry (LC-HRMS) method in the Drug Control Centre (DCC), samples were stored at −20°C for at least 3 months after the negative anti-doping analytical report was issued, as required by WADA. Then samples were transferred from the coded bottles to separate plastic containers to anonymise all samples. Each sample was then further divided into three identical batches each of 1000 samples at the DCC, frozen at −20°C and shipped to the three different vendors' locations where they were stored frozen until ready for analysis.

2.3 | Internal standard solution

Mefruside solution (1 mL, 1 mg/mL), d_3-ephedrine and d_3-salbutamol ampoules, d_6-DHEA sulphate and d_3-testosterone glucuronide vials were (quantitatively) transferred to a 100 mL volumetric flask and diluted with methanol to make the final concentration of 10 µg/mL. Aliquots of this solution were supplied to all three vendors.

2.4 | Quality control samples (QCs)

Two QC solutions, QC 1 and QC 2, were prepared by spiking a blank urine sample with the methanolic solutions of the compounds at the concentrations shown in Table 1, and aliquots were supplied to all three vendors.

2.5 | Agilent Technologies sample preparation and instrument setup

The sample preparation was adapted from the publication by Parr et al.[8] Urine (200 µL) was aliquoted into an Eppendorf® LoBind tube (Wesseling-Berzdorf, Germany) and internal standard solution (10 µL) was added as well as *E. coli* β-glucuronidase solution (25 µL) to samples, QC urines and blanks (drug-free urine treated in the same way as samples) but not to double blanks (drug-free urine without the addition of internal standard). Samples were briefly vortexed, placed on an Eppendorf® Thermomixer (Wesseling-Berzdorf, Germany) and gently agitated for 1 h at 50°C to hydrolyse the samples. After the hydrolysis, samples were cooled to room temperature prior to the addition of 765 µL THF. Samples were cooled to 4°C for 30 min before centrifugation at a relative centrifugal force (rcf) of 13,400 g for 8 min using an Eppendorf® MiniSpin centrifuge (Wesseling-Berzdorf, Germany). The supernatant was transferred into glass autosampler vials prior to SFC-MS analysis.

The analysis was performed on an Infinity II 1290 SFC-LC hybrid system coupled to an Agilent Ultivo triple quadrupole mass spectrometer (Agilent Technologies GmbH, Waldbronn, Germany). The Jetstream electrospray ionisation (ESI) source conditions were the following: sheath gas temperature 375°C, sheath gas flow 12 L/min, gas temperature 150°C, gas flow 5 L/min, nebuliser 30 psi, capillary voltage 4000 V and nozzle voltage 500 V (positive mode) or 1000 V (negative mode). Nitrogen was used as the sheath and nebuliser gas, and compounds were analysed using dynamic multiple reaction monitoring mode (MRM) with positive and negative ionisation depending on the analysed compound. A minimum of two MRM transitions were acquired for each analyte. Please refer to Table S1 for analyte specific details.

The chromatographic separation was achieved with an ACQUITY UPC^2® BEH 2-EP column 100 mm × 3 mm, 1.7 µm (Waters, GmbH, Eschborn, Germany). Injection volume was 5 µL. Mobile phase A was supercritical CO_2 (back pressure regulator 150 bar, 60°C) and mobile phase B 10 mM ammonium acetate dissolved in methanol: water (96.5:3.5, *v:v*). The segmented linear gradient with different slopes started with 2% B at 0 min, 5% B at 3 min, then 20% B at 8 min, 50% B at 11 min, 60% B at 12 min and 62.5% B at 13.5 min. The post-run time was 2.5 min and flow rate was 1.4 mL/min. As a make-up, solvent of the same composition as mobile phase B was used at a flow rate of 0.4 mL/min. Mass Hunter software version 10 was used for data acquisition and data analysis. MassHunter Optimizer and Source Optimizer 1.1 were used for compound and method optimisation, respectively. Study Manager version 1.1 was used to chain worklists and batches for a seamless operation. Sample analysis was conducted within 10 weeks from receiving the samples and considering instrument availability to mimic a reasonable time scale of operation of the instrument with minimum intervention in an anti-doping control laboratory.

2.6 | Waters sample preparation and instrument setup

Sample preparation was performed following the published method by Novákova et al.[5] The internal standard solution (10 µL) was pipetted into an Eppendorf® LoBind tube (Stevenage, UK) followed by 200 µL urine and 790 µL acetonitrile. The contents of the tube

TABLE 1 Composition of QC 1 and QC 2.

QC 1	Concentration (ng/mL)	QC 2	Concentration (ng/mL)
Amiloride	100	1,3-Dimethylbutylamine	100
Amphetamine	100	2-Amino-6-methylheptane	100
p-Hydroxy amphetamine	100	Bendroflumethiazide[b]	100
Atenolol	100	Dexamethasone	30
Benzoylecgonine	100	Oxilofrine	100
Betamethasone	30	Probenecid	100
Buprenorphine	5	Salmeterol	20
Cathine	100	Tuaminoheptane	100
Codeine	50	Cortisol	30
Ephedrine	100	Prednisone	30
Etilefrine	100	20β-Hydroxy prednisone	20
Fenoterol	20	THC–COOH[a]	150
Fentanyl	2	Norfenefrine	100
Formoterol	20	Octopamine	1000
FPCAM[a]	30	Pseudoephedrine	100
Hydrochlorothiazide[b]	100	19-Norandrosterone sulphate	50
Ketoconazole	50	Androsterone sulphate	200
Methamphetamine	100	DHEA sulphate[a]	200
Morphine	50	5-DHT sulphate[a]	50
Nikethamide	100	Ethyl sulphate	500
Oxymorphone	50	19-Noretiocholanolone sulphate	50
Prednisolone	30	Meldonium	200
Propranolol[b]	50	Octopamine sulphate	500
Ritalinic acid	100	Nandrolone sulphate	50
Salbutamol	500	Epitestosterone sulphate	50
Tramadol	50	Testosterone sulphate	50
Cortisone	30		
20β-Hydroxy prednisolone	30		

[a]FPCAM, fluticasone propionate-17-carboxylic acid; DHEA, dehydroepiandrosterone; DHT, 5α-dihydrotestosterone; THC–COOH, 11-nor-9-carboxy-Δ9-tetrahydrocannabinol.
[b]Compound spiked at 50% MRPL, all others at their MRPL (TD2019MRPL).[27] Salbutamol was spiked at 50% threshold and THC–COOH at the threshold (WADA TD2017DL).[28] Threshold is the maximum permissible level of the concentration for a threshold substance in a sample.[29]

were briefly vortexed and then centrifuged for 5 min at a rcf of 5000 g using an Allegra 25R centrifuge (Beckman Coulter, Wycombe, UK). The supernatant was transferred to a 96-well sample collection plate, 2 mL square well (P/N 186002482, Waters Corporation) prior to the analysis.

A Waters ACQUITY UPC$^{2®}$ system (Waters Corporation) was coupled to a Xevo TQ-XS tandem quadrupole mass spectrometer with an ESI source (Waters Corporation). The chromatographic separation was achieved using a Torus Diol column, 100 mm × 3 mm, 130 Å, 1.7 µm (Waters Corporation) thermostated at 35°C. Gradient elution was performed using supercritical CO_2 (mobile phase A) and methanol with 0.1% aqueous ammonia solution (mobile phase B, organic modifier). Methanol was used as a make-up solvent at a flow rate of 0.2 mL/min. The Automatic Back Pressure Regulator (ABPR) was set to 2000 psi. A flow splitter was used to coordinate flow to the MS and the ABPR from the column manager. The run time was 7 min with a 1.2 mL/min flow rate. The gradient started with 10% organic modifier which was held for 1 min then increased to 50% over the next 3 min, 56.7% by 4.5 min and maintained for 0.5 min. At 5.1 min, the modifier was returned to 10% which was then maintained until the end of the run. Methanol was used as a seal and needle wash. The injection volume was 2 µL.

Target compounds were analysed in the positive and negative ionisation mode using the following source conditions: capillary voltage ±2 kV, source temperature 150°C, desolvation temperature 600°C, desolvation gas flow 1000 L/h, cone gas flow 150 L/h and

nebuliser gas flow setting of 7 bar. Nitrogen was used as a desolvation and nebuliser gas, whereas argon was used as the collision gas. Mass spectrometry analysis was performed in the MRM mode with one or two transitions for each analyte. Cone, collision energies and dwell times were adjusted individually for each MRM transition. Please refer to Table S2 for analyte specific details. MassLynx™ software version 4.2 was utilised for data acquisition; data processing was achieved using the integral TargetLynx™ application manager. Once received, samples were analysed within 2 weeks with approximately 1 month in between. For each operational week, the instrument was working 24/7.

2.7 | Shimadzu sample preparation and instrument setup

An aliquot of each urine sample (200 µL) was mixed with methanol (800 µL) and internal standard solution (10 µL). Samples with sediment that appeared cloudy were centrifuged prior to analysis. To perform the sample analysis, a Shimadzu Nexera-UC SFC [CBM-20A, LC-30ADSF (CO_2 pump), LCMS-30 AD (modifier pump), SIL-30 AC (5 µL loop), SFC-30A (back pressure regulator] coupled to a Shimadzu triple quadrupole mass spectrometer LCMS-8060 was used. Mobile phases A (supercritical CO_2) and B (10 mM ammonium formate, 1% water in methanol) were employed for the analysis. The gradient started with 5% B, reaching 30% B at 8 min, with a further increase to 50% B at 8.25 min and remaining at 50% B until 10.25 min and decreasing to 5% B at 11 min. The total run time was 13.5 min and the flow rate was 2 mL/min. The backpressure regulator (SFC-30A) was set at 150 bar, with a temperature of 50°C. The chromatographic separation was achieved with an Excel CN 100 mm × 4.6 mm, 3 µm column (ACE, Aberdeen UK) thermostated to 40°C. The injection volume was 5 µL, using a fixed 5 µL injection loop. Samples were held in the autosampler at 4°C.

The ESI source contained a dedicated coated ESI needle for the SFC-MS analysis. The default settings for the ESI source were applied: nebulising gas flow 3 L/min, heated gas flow 10 L/min, drying gas flow 10 L/min, interface temperature 300°C, desolvation line temperature 250°C and heat block temperature 400°C. The analysis was performed using a LCMS 8060 instrument. Mass spectrometry analysis was performed in the MRM mode with one or sometimes two transitions for each analyte. Please refer to Table S3 for analyte specific details. The data were acquired and analysed using LabSolutions software version 5.97 and Insight version 3.5, respectively. Sample analysis was conducted within 4 months from receiving the samples and considering instrument availability.

2.8 | Statistical analysis

Chromatographic variables such as retention time (t_R) and peak symmetry were considered for statistical analysis using Minitab software version 19.

3 | RESULTS AND DISCUSSION

3.1 | Sample preparation

The analysis was conducted with almost three identical portions of 1000 real anti-doping samples. QC compounds represented a selection of chemicals available to cover drug categories on the WADA 2019 Prohibited List,[30] effective at the time of our study. The selection of the QC composition was based on the aim to cover as many different chemical structures, physicochemical properties and inclusion of different classes of compounds from the WADA Prohibited List as reasonable. Table 1 lists the 54 compounds selected. The QC concentrations were set to the minimum required performance levels (MRPL) given in WADA Technical Document TD2019MRPL.[27] The MRPL is defined as the minimum concentration at which anti-doping laboratories must consistently detect and confirm a prohibited substance (either parent or metabolite).[29] WADA technical document TD2019MRPL was in force and used as a guide. Even with the recently updated technical document TD2022MRPL,[31] our conclusions are not jeopardised and our study still demonstrates appropriate SFC-MS sensitivity. We used four deuterated chemicals (d_3-ephedrine, d_3-salbutamol, d_3-testosterone glucuronide and d_6-DHEA sulphate) and mefruside as internal standards. Each manufacturer received the same internal standard mix, but not every internal standard component was included in the acquisition method because it often happened that the concentration in the internal standard was not sufficient to perform the MS optimisation.

Samples were prepared using a 'dilute and inject' approach adapting the methods already published. However, there were subtle differences in the approach among vendors such as diluting solvent and volume of injections. Discussions with each manufacturer were undertaken regarding any preference or experience they could share with respect to sample preparation prior to SFC-MS analysis. It was decided that different methods for sample preparation could be utilised, but QCs and internal standard solution were identical for all three manufacturers. With the much-improved coupling of SFC to MS and MS sensitivity, in our study a 'dilute and inject' sample preparation approach has been shown to work for SFC-MS. Thanks to more sensitive MS instruments the 'dilute and inject' approach is now being successfully employed in the analysis of drugs prohibited in sport by LC–MS[23,32] as well. It represents a more cost-effective and quicker approach to sample preparation than solid-phase or liquid–liquid extraction. However, at present, our work demonstrated that the 'dilute and inject' approach might not be sufficiently sensitive to detect some analytes such as metabolites of the anabolic steroids (e.g. 3′-hydroxy stanozolol metabolite) where WADA has set an MRPL of 2 ng/mL in TD2019MRPL (in force at the time of doing our study), further reduced to 1 ng/mL in the recently published TD2022MRPL. Sample extraction and concentration might still be required for the analysis of anabolic steroids pending further advances in MS sensitivity. For this reason, we chose to include fewer anabolic steroids in our study. On the other hand, several anabolic steroid sulphates have been included. Currently, these phase II metabolites do not have any

associated WADA MRPL (which refers so far to the non-conjugated compounds), and the concentration spike was determined by instrument sensitivity. Furthermore, our QC list included some very polar compounds such as ethyl sulphate and meldonium. As expected, we demonstrated with all three instruments good retention and chromatography for both analytes (t_R > 2 min, Gaussian peak shape), as well as of other early eluting compounds in reversed-phase LC–MS (e.g. morphine). SFC-MS offered a good degree of orthogonality with LC–MS. The SFC column chemistries utilised for this study, 2-ethylpyridine, high density diol and cyano stationary phases (BEH 2-EP, Torus Diol and Excel CN columns) in synergy with mobile phase composition enabled the separation of ephedrine and pseudoephedrine. Almost baseline separation occurred with the Excel CN column (ephedrine $t_R = 3.38$ min and pseudoephedrine $t_R = 3.50$ min). Nevertheless, the separation of these two diastereoisomers was not the aim of this study.

The internal standard mixture was chosen to provide one sulphate and one glucuronide in order to be able to observe the performance of these conjugates, and in the case of the glucuronide, d_3-testosterone glucuronide was used to monitor hydrolysis when undertaken. Deuterated ephedrine and salbutamol were included to assist with quantitative estimates of these two analytes. Only one vendor hydrolysed samples (deglucuronidation) before analysis. The inclusion or exclusion of hydrolysis in sample preparation produced no observable difference in the performance of the three different instruments. Glucuronides generally appear to be particularly sensitive to the stationary phase selected.[33–35] In our experience, glucuronides showed tailing when analysed using the BEH 2-EP column, while a more Gaussian peak shape was achieved with the Torus Diol column. Similar to routine LC–MS screening methods, SFC-MS routine screening methods developed in any anti-doping laboratory would include a large variety of compounds. For superior separation and to be able to include as many compounds as possible in one method, the column choice often balances the column chemistry and the chemical diversity of compounds included in the application.

Using 3′-hydroxy stanozolol and tamoxifen as model compounds to evaluate peak shape, Nováková et al. tested Torus Diol, 2-picolylamine (2-PIC), diethylamine (DEA), 1-aminoanthracene (1-AA), BEH, BEH 2-ethylpyridine (2-EP), CSH fluoro-phenyl (PFP) and HSS C18 SB columns[4] with the same 100 mm column length as used by us (3 mm column diameter and with particle sizes of 1.7 µm, except for the HSS C18 SB column which has 1.8 µm particles). Of all of them, the best peak shape was achieved on the Torus Diol column. Therefore, this stationary phase was selected for their SFC-MS study, which assessed the performance of relatively few compounds compared with our study. Considering the number of samples included in our study as well as chemical variety of QC compounds, each vendor achieved very good results just by using one column throughout the whole study. It appears that SFC has a wider range of phases that can be used compared with the most popular LC stationary phases. This has the advantage of selectivity, but the disadvantage that the laboratory has to decide which to use because there does not appear to be one favoured stationary phase compared with the C18 reversed-phase column chemistry commonly used in LC. Further optimisation is achievable by selecting a better suited column for a specific compound.

In general, many of the QC compounds demonstrated good Gaussian peak shape and met chromatographic and mass spectrometric criteria defined in various WADA Technical Documents such as TD2015IDCR and TD2017DL,[28,36] which were in force at the time of conducting this study. In the meantime, TD2021IDCR and TD2022DL, newer versions of the technical documents have been issued by WADA.[37,38] However, we believe this should not have a significant impact on our results and conclusions. It was noticed that some compounds co-eluted with endogenous components. It is believed this is related to the 'dilute and inject' approach used for which, even though adapted from the literature, very little optimisation regarding choice of diluent was undertaken. Similarly, the chromatographic conditions to achieve optimal separation were very little optimised because this was not the purpose of our study. Other issues that were not clearly linked to endogenous co-elution such as peak shoulders were also observed. Whether this could be improved was not evaluated in this study and was not considered to be a limitation of the SFC-MS technique but related to the column used and optimisation of solvent conditions. Although carryover was occasionally observed, one of the vendors reduced or removed this issue by optimisation of the washing solvents used. We estimated carryover generally to be less than 1%.

3.2 | Sample analysis

We have anonymised vendors' names and marked them simply as vendors A, B and C in order to discuss and present results impartially. Multi-analyte acquisition methods were set up to demonstrate the applicability of SFC-MS for routine anti-doping analysis. Table 2 lists compound classes from the WADA Prohibited List that were included in the targeted method by each vendor. Because of the time allocated for the project, manufacturers B and C have mainly focused on the QC compounds, which in our view was still sufficient to demonstrate the robustness and the applicability of SFC-MS in the doping control field. Manufacturer A's method is the most comprehensive and, apart from our QC compounds, includes many other compounds from the WADA Prohibited List.

Time frames for different arms of our current study were necessarily varied and mainly dependent on the instrument availability at each vendor's site. Samples were grouped in batches to accommodate the autosampler tray size and instrument time availability. Our findings for retention time stability were similar to those of Losacco's study.[25] Even incorporating additional variables, especially the different instruments, the different methods used and the large number of different urine samples better representing the variety of samples encountered routinely by the anti-doping laboratory, a good retention time stability was demonstrated in our study (Tables 3–5). The modern SFC-MS back pressure regulators enable the retention time

TABLE 2 Compound classes and number of target analytes by each vendor.

WADA compound class	Vendor A	Vendor B	Vendor C
Anabolic steroids	24	4	5
β_2-Agonists	7	3	3
β_2-Blockers	23	2	2
Cannabimimetics	1	1	1
Diuretics	26	3	3
Glucocorticoids	17	6	8
Hypoxia inducible factors	1		
Internal standard	5	4	4
Modulators	17	3	3
Monitoring programme	5	2	2
Narcotics	5	1	1
Opioids	6	3	3
Stimulants	60	11	15
Total	**197**	**43**	**50**

Note: WADA, World Anti-Doping Agency.

precision reported. They also keep the delta pressure between the system and the analytical column stable as changes on the column pressure are influencing the retention time. Apart from retention time stability, we also investigated the variability of additional chromatographic variables such as peak symmetry. Results for vendors A, B and C peak symmetry are presented in Tables 3–5 and discussed in more detail in the subsequent section. In general, peaks are Gaussian with clear peak starts and ends. Vendors A and B's mean peak symmetry at 10% peak height show similar values, although this measure should be compared with care because the calculations are performed utilising different commercial integration algorithms.

Losacco et al. undertook an SFC-MS interlaboratory study[39] where four laboratories all equipped with the same Waters SFC system and using the same columns, but different Waters triple quadrupole mass spectrometers, took part. Our study was performed on three different instruments from three different manufacturers. We did not aim to compare the vendors among themselves but to determine whether current SFC-MS instruments provided the necessary performance and robustness for routine application in the anti-doping field. To illustrate the difficulty of comparison, in

TABLE 3 Vendor A data—summary statistics of mean retention time and mean peak symmetry measures over whole study.

Internal standard	Number of results	Mean t_R (min)	t_R %CV	Mean peak symmetry 10%	Peak symmetry %CV
d_3-ephedrine	1418	6.16	0.49	1.08	8.9
d_3-salbutamol	1425	8.22	0.40	1.21	6.9
Mefruside	1411	5.29	0.40	1.01	9.3
d_3-testosterone	1422	3.41	0.96	1.17	7.1

Note: Number of results–total number of injections performed where the internal standard component was detected to include in the statistical analysis; t_R, retention time; CV, coefficient of variation.

TABLE 4 Vendor B data – summary statistics of mean retention time and mean peak symmetry measures over the whole study period.

Internal standard	Number of results	Mean t_R (min)	t_R %CV	Mean peak symmetry 10%	Mean peak symmetry 10% %CV
d_3-ephedrine	1214	2.65	0.18	0.94	6.9
d_3-salbutamol	1215	3.43	0.14	1.04	14.5
Mefruside	1215	2.22	0.34	1.06	9.2

Note: Number of results–total number of injections performed where the internal standard component was detected to include in the statistical analysis; t_R, retention time; CV, coefficient of variation.

TABLE 5 Vendor C data – summary statistics of mean retention time and mean peak symmetry measures over whole study.

Internal standard	Number of results	Mean t_R (min)	t_R % CV	Number of results	Mean tailing factor 10%	Mean tailing factor 10% %CV
d_3-ephedrine	900	3.39	2.0	896	1.07	3.7
d_3-salbutamol	900	4.81	1.7	541	1.84	20
Mefruside	900	3.01	1.2	897	1.02	2.8
d_3-testosterone glucuronide	892	5.54	0.9	815	2.74	24
d_6-DHEA sulphate	900	4.12	1.2	899	1.18	7.2

Note: Number of results–total number of injections performed where the internal standard component was detected to include in the statistical analysis; t_R, retention time; CV, coefficient of variation.

Losacco's interlaboratory study, nikethamide was an outlier in terms of retention time stability. They considered that this was because of the low modifier percentage (2–4%) in the mobile phase and poor retention.[39] In our study, nikethamide gave good performance on all three instruments. Although the instrument conditions and stationary phases were different among the three vendors, nikethamide was an early eluting compound under all our conditions (e.g. manufacturer A – $t_R = 1.48$ min, B – $t_R = 0.66$ min, C – $t_R = 1.50$ min). An endogenous peak appearing at the same retention time was observed in vendor B's data but not with vendor A's or C's data.

3.3 | Statistical analysis

3.3.1 | Statistical analysis – vendor A

All samples were divided into 19 batches. The number of measurements, mean retention time and %CV, as well as peak symmetry data are presented in Table 3. Vendor A provided data for the internal standards d_3-ephedrine, d_3-salbutamol, mefruside and d_3-testosterone (hydrolysed glucuronide).

The retention time stability was good (t_R %CV ≤ 0.5% for d_3-ephedrine, d_3-salbutamol and mefruside, apart from d_3-testosterone). Vendor A calculates peak symmetry at 10% peak height by dividing the area of the integrated peak after the peak maximum with that before (Figure 1). This represents the US Pharmacopoeia (USP), European Pharmacopoeia (Ph. Eur.) and Japanese Pharmacopoeia (JP) adopted approaches to calculating peak symmetry. Vendor A's processing software uses a non-parametric integrator which finds peak start and end reliably, independent of noise and signal height. Generally, peak symmetry for the internal standards were very good and stable (peak symmetry %CV ≤ 10%). We have found that proper system equilibration was required before use, which mainly affected the retention time and not the peak shape. In particular, the modifier and make-up solutions, which influence the retention time, were adjusted and the system needed to stabilise to the modified conditions. After the analysis of batch 5, there was a gap in the analysis of 3 to 4 weeks before running batch 6. Although a number of checks were made after the mass spectrometer had been turned back on and the solvent lines carefully flushed, it subsequently became apparent that around 10 h after tuning and flushing was needed for the system to become fully stabilised.

3.3.2 | Statistical analysis – vendor B

Vendor B included data for the internal standards d_3-ephedrine, d_3-salbutamol and mefruside. Samples were divided into 23 batches. The number of measurements, mean retention time, peak symmetry and %CV are presented in Table 4. Retention time CVs were very good (%CV ≤ 0.5) for all internal standards tested, particularly for d_3-ephedrine (mean peak symmetry %CV ≤ 10%). Because of its hydrophilicity and basicity, ephedrine may give poor performance under standard C_{18} reversed-phase LC conditions (e.g. asymmetric peak). As already mentioned, one way suggested to overcome this issue is the use of HILIC columns.[21,22] In our study, the combination of polar column (Torus Diol) and basic modifier (methanol with 0.1% ammonia) resulted in good retention and symmetrical peak shape for d_3-ephedrine/ephedrine.

Mean peak symmetry 10% is *b*/*a* ratio based on 10% peak height threshold rather than peak start and end similar to the procedure applied in the US Pharmacopoeia, European Pharmacopoeia and Japanese Pharmacopoeia (JP) (Figure 1). The *b*/*a* variable was influenced by peak tailing and is obtained by dividing the ratio of peak areas after and before a vertical drop line from the peak top to the integrated peak baseline.

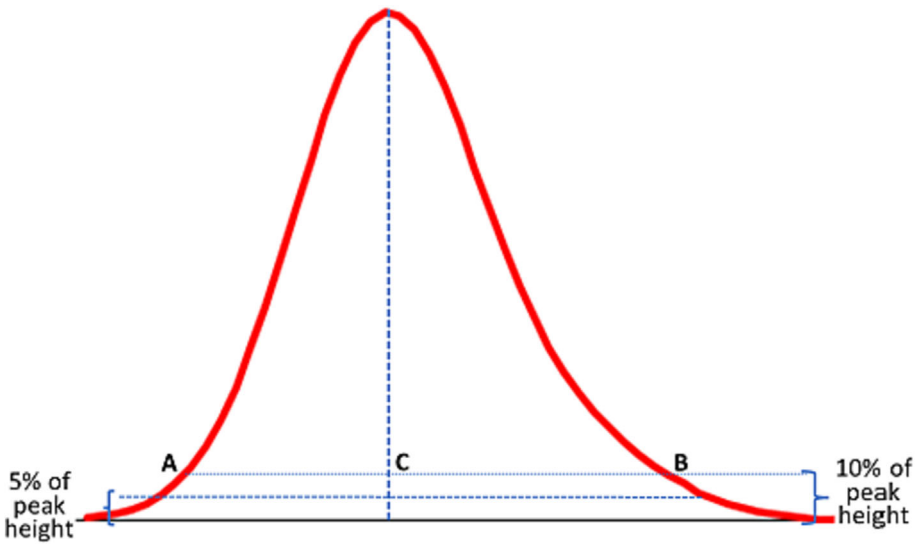

FIGURE 1 Figure 1 illustrates how chromatographic peak asymmetry is typically calculated based on the peak width to the right of the central line (C–B) and to the left of that line (A–C) based on 5% or 10% of the peak height. Asymmetry: As = BC/AC. Tailing factor: Tf = BC/(2 × AC) (USP/JP/EU Pharmacopoeia).

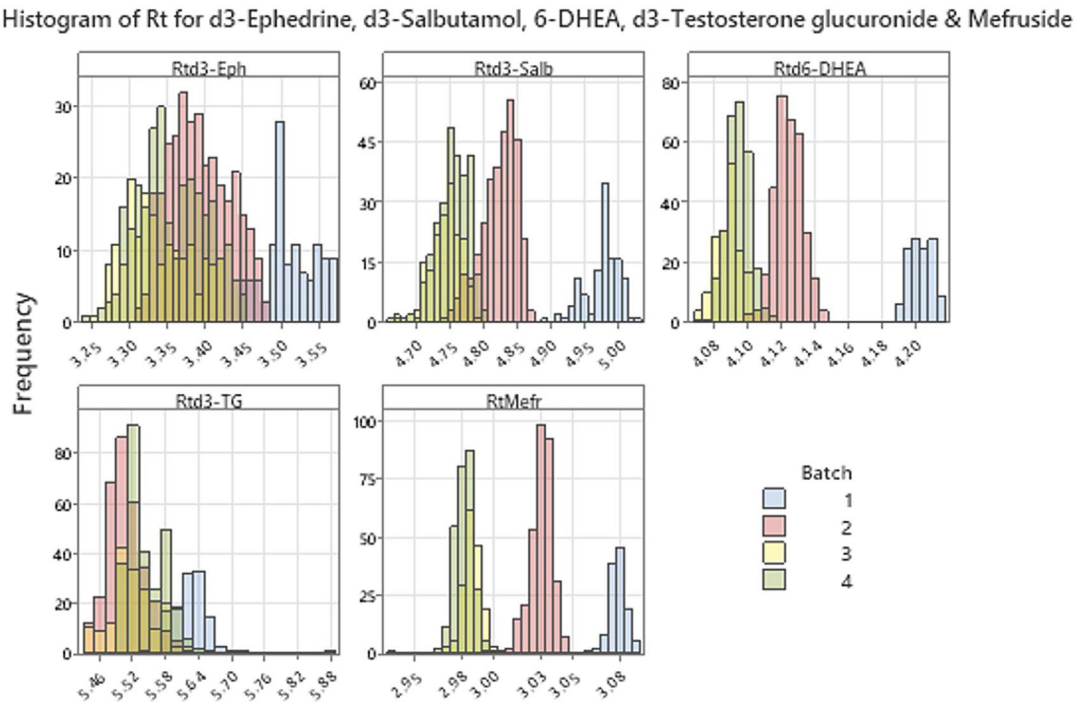

FIGURE 2 Histograms of vendor C internal standard compounds retention times showing intra- and inter-batch variability.

3.3.3 | Statistical analysis – vendor C

Vendor C ran d_3-ephedrine, d_3-salbutamol, mefruside, d_6-DHEA sulphate and d_3-testosterone glucuronide as internal standards. The samples were divided into four batches each of 200 samples, which although less than 1000 was sufficient to provide a good idea of instrument robustness. The number of measurements, mean retention time and %CV are presented in Table 5. CVs are good within batches (%CV ≤ 0.5) but not between batches (Figure 2). These changes between batches were tight and small enough and within WADA identification criteria.

4 | CONCLUSIONS

Our study clearly demonstrated the applicability of SFC-MS for routine anti-doping analysis. We analysed approximately 3 × 1000 common anti-doping samples in total using SFC-MS equipment from three different vendors. Despite all differences (e.g. three different instruments, columns, methods of sample preparation and overall study time), we believe our results are very useful for WADA accredited laboratories to consider implementing SFC-MS for initial testing procedures. Each analytical column sustained more than 1000 injections, the pumping systems performed consistently and there was no need for any further MS cleaning in addition to the routine/regular cleaning procedures as explained by each vendor. We proved that the technology was mature, independent of the vendor and could be used on a daily routine basis in any anti-doping laboratory, fulfilling WADA requirements.

Our work confirmed SFC-MS to be a robust analytical technique. A large number of anti-doping samples and QCs were delivered to each manufacturer. We worked closely with each manufacturer in agreeing the method of operation and helped with the analysis of the samples and data review and undertook the independent statistical analysis of the data. All three instruments appeared to meet the requirement for robustness needed for routine use. Most of our compounds showed excellent chromatography (e.g. Gaussian peak shape). There were only a few examples of co-elution because of interference with endogenous compounds that could benefit from modification of the sample preparation or chromatographic conditions. Retention times were generally remarkably stable over the analysis time (%CV ≤ 0.5% for most internal standard compounds analysed by vendors A and B and within batches for vendor C). Despite using a 'dilute and inject' approach for sample preparation, the SFC columns were found to be stable over more than 1000 injections of samples, standards and QCs on three different systems. In general, SFC-MS was found to be similar to LC–MS in terms of performance for routine use but with the advantage of orthogonality providing additional information of the chromatographic properties of the analyte, thereby further confirming the identity of a doping agent. SFC-MS may be considered as a complementary technique to LC–MS, which is readily available in anti-doping laboratories. Our study is impartial, and anti-doping laboratories should have freedom of choice regarding the SFC-MS equipment according to their preference, availability and discussions with manufacturers.

The majority of compounds demonstrated the suitability of the SFC-MS methods for anti-doping analysis. Robust and stable conditions were shown through the duration of the study using

one single analytical column even if only very limited sample pre-purification was applied. Just like any other analytical technique, SFC-MS requires optimisation of various variables such as finding an adequate combination of solvents for mobile phase and to prevent carryover or sample preparation. Proper system equilibration prior to use as well as optimising mass spectrometry conditions are, of course, also required.

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CONFLICT OF INTEREST STATEMENT

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REFERENCES

1. Van Eenoo P, Van Gansbeke W, De Brabanter N, Deventer K, Delbeke FT. A fast, comprehensive screening method for doping agents in urine by gas chromatography–triple quadrupole mass spectrometry. *J Chromatogr A*. 2011;1218(21):3306-3316. doi:10.1016/j.chroma.2010.09.082
2. Musenga A, Cowan DA. Use of ultra-high pressure liquid chromatography coupled to high resolution mass spectrometry for fast screening in high throughput doping control. *J Chromatogr A*. 2013;1288:82-95. doi:10.1016/j.chroma.2013.03.006
3. Parr MK, Bokland M, Liebetrau F, Schmidt A, Schänzer W, Sterk SS. Enantiomeric separation of clenbuterol as analytical strategy to distinguish abuse from meat contamination. In: Schänzer W, Geyer H, Gotzmann A, Mareck U, eds. *Recent advances in doping analysis (21)*. Sportverlag Strauss; 2013:26.
4. Nováková L, Desfontaine V, Ponzetto F, 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*. 2016;915:102-110. doi:10.1016/j.aca.2016.02.010
5. Nováková L, Grand-Guillaume Perrenoud A, Nicoli R, Saugy M, Veuthey J-L, Guillarme D. Ultra high performance supercritical fluid chromatography coupled with tandem mass spectrometry for screening of doping agents. I: investigation of mobile phase and MS conditions. *Anal Chim Acta*. 2015;853:637-646. doi:10.1016/j.aca.2014.10.004
6. Nováková L, Rentsch M, Grand-Guillaume Perrenoud A, et al. Ultra high performance supercritical fluid chromatography coupled with tandem mass spectrometry for screening of doping agents. II: analysis of biological samples. *Anal Chim Acta*. 2015;853:647-659. doi:10.1016/j.aca.2014.10.007
7. Desfontaine V, Nováková L, Ponzetto F, et al. Liquid chromatography and supercritical fluid chromatography as alternative techniques to gas chromatography for the rapid screening of anabolic agents in urine. *J Chromatogr A*. 2016;1451:145-155. doi:10.1016/j.chroma.2016.05.004
8. Parr MK, Wuest B, Naegele E, et al. SFC-MS/MS as an orthogonal technique for improved screening of polar analytes in anti-doping control. *Anal Bioanal Chem*. 2016;408(24):6789-6797. doi:10.1007/s00216-016-9805-4
9. Parr MK, Blokland MH, Liebetrau F, et al. Distinction of clenbuterol intake from drug or contaminated food of animal origin in a controlled administration trial—the potential of enantiomeric separation for doping control analysis. *Food Addit Contam A*. 2017;34(4):525-535. doi:10.1080/19440049.2016.1242169
10. Xhaferaj M, Naegele E, Parr MK. Ion exchange in supercritical fluid chromatography tandem mass spectrometry (SFC-MS/MS): application for polar and ionic drugs and metabolites in forensic and anti-doping analysis. *J Chromatogr A*. 2020;1614:460726. doi:10.1016/j.chroma.2019.460726
11. Guillarme D, Desfontaine V, Heinisch S, Veuthey J-L. What are the current solutions for interfacing supercritical fluid chromatography and mass spectrometry? *J Chromatogr B*. 2018;1083:160-170. doi:10.1016/j.jchromb.2018.03.010
12. West C, Lemasson E, Bertin S, Hennig P, Lesellier E. An improved classification of stationary phases for ultra-high performance supercritical fluid chromatography. *J Chromatogr A*. 2016;1440:212-228. doi:10.1016/j.chroma.2016.02.052
13. Ishibashi M, Ando T, Sakai M, et al. High-throughput simultaneous analysis of pesticides by supercritical fluid chromatography/tandem mass spectrometry. *J Chromatogr A*. 2012;1266:143-148. doi:10.1016/j.chroma.2012.09.067
14. Desfontaine V, Guillarme D, Francotte E, Nováková L. Supercritical fluid chromatography in pharmaceutical analysis. *J Pharm Biomed Anal*. 2015;113:56-71. doi:10.1016/j.jpba.2015.03.007
15. Desfontaine V, Losacco GL, Gagnebin Y, et al. Applicability of supercritical fluid chromatography – mass spectrometry to metabolomics. I – optimization of separation conditions for the simultaneous analysis of hydrophilic and lipophilic substances. *J Chromatogr A*. 2018;1562:96-107. doi:10.1016/j.chroma.2018.05.055
16. Losacco GL, Ismail O, Pezzatti J, et al. Applicability of supercritical fluid chromatography–mass spectrometry to metabolomics. II–assessment of a comprehensive library of metabolites and evaluation of biological matrices. *J Chromatogr A*. 2020;1620:461021. doi:10.1016/j.chroma.2020.461021
17. Joseph JF, Parr MK. Application of SFC for bioanalysis. In: Ma S, Chowdhury SK, eds. *Identification and quantification of drugs, metabolites, drug metabolizing enzymes, and transporters*. Elsevier; 2020:151-183. doi:10.1016/B978-0-12-820018-6.00005-3
18. Teubel J, Wüst B, Schipke CG, Peters O, Parr MK. Methods in endogenous steroid profiling—a comparison of gas chromatography mass spectrometry (GC–MS) with supercritical fluid chromatography tandem mass spectrometry (SFC-MS/MS). *J Chromatogr A*. 2018;1554:101-116. doi:10.1016/j.chroma.2018.04.035
19. Wolrab D, Peterka O, Chocholoušková M, Holčapek M. Ultrahigh-performance supercritical fluid chromatography/mass spectrometry in the lipidomic analysis. *TrAC Trends Anal Chem*. 2022;149:116546. doi:10.1016/j.trac.2022.116546
20. Aboushady D, Hanafi RS, Parr MK. Quality by design approach for enantioseparation of terbutaline and its sulfate conjugate metabolite for bioanalytical application using supercritical fluid chromatography. *J Chromatogr A*. 2022;1676:463285. doi:10.1016/j.chroma.2022.463285

21. Gray N, Heaton J, Musenga A, Cowan DA, Plumb RS, Smith NW. Comparison of reversed-phase and hydrophilic interaction liquid chromatography for the quantification of ephedrines using medium-resolution accurate mass spectrometry. *J Chromatogr A*. 2013;1289:37-46. doi:10.1016/j.chroma.2013.03.021
22. Heaton J, Gray N, Cowan DA, Plumb RS, Legido-Quigley C, Smith NW. Comparison of reversed-phase and hydrophilic interaction liquid chromatography for the separation of ephedrines. *J Chromatogr A*. 2012;1228:329-337. doi:10.1016/j.chroma.2011.09.026
23. Görgens C, Guddat S, Orlovius A-K, et al. "dilute-and-inject" multi-target screening assay for highly polar doping agents using hydrophilic interaction liquid chromatography high resolution/high accuracy mass spectrometry for sports drug testing. *Anal Bioanal Chem*. 2015;407(18):5365-5379. doi:10.1007/s00216-015-8699-x
24. Wuest B, Gavrilović I, Cowan D, de la Torre X, Botrè F, Parr MK. Analysis of doping control samples using supercritical fluid chromatography-tandem mass spectrometry: ready for routine use. *J Sep Sci*. 2023;46(7):2200880. doi:10.1002/jssc.202200880
25. Losacco GL, Marconetto E, Nicoli R, et al. Supercritical fluid chromatography–mass spectrometry in routine anti-doping analyses: estimation of retention time variability under reproducible conditions. *J Chromatogr A*. 2020;1616:460780. doi:10.1016/j.chroma.2019.460780
26. Parr MK, Botrè F. Supercritical fluid chromatography mass spectrometry as an emerging technique in doping control analysis. *TrAC Trends Anal Chem*. 2022;147:116517. doi:10.1016/j.trac.2021.116517
27. WADA TD2019MRPL. https://www.wada-ama.org/sites/default/files/resources/files/td2019mrpl_eng.pdf. Accessed 29 June, 2023.
28. WADA TD2017DL. https://www.wada-ama.org/sites/default/files/resources/files/2016-12-13_td2017dl.pdf. Accessed 29 June, 2023.
29. WADA International Standard for Laboratories. *verison 11* 2021; https://www.wada-ama.org/sites/default/files/resources/files/isl_2021.pdf. Accessed 29 June, 2023.
30. WADA 2019 Prohibited List. https://www.wada-ama.org/sites/default/files/wada_2019_english_prohibited_list.pdf. Accessed 29 June, 2023.
31. WADA TD2022MRPL. https://www.wada-ama.org/sites/default/files/resources/files/td2022mrpl_v1.0_final_eng.pdf. Accessed 29 June, 2023.
32. Beck O, Ericsson M. Methods for urine drug testing using one-step dilution and direct injection in combination with LC-MS/MS and LC-HRMS. *Bioanalysis*. 2014;6(17):2229-2244. doi:10.4155/bio.14.192
33. Doué M, Dervilly-Pinel G, Pouponneau K, Monteau F, Le Bizec B. Analysis of glucuronide and sulfate steroids in urine by ultra-high-performance supercritical-fluid chromatography hyphenated tandem mass spectrometry. *Anal Bioanal Chem*. 2015;407(15):4473-4484. doi:10.1007/s00216-015-8573-x
34. Romand S, Rudaz S, Guillarme D. Separation of substrates and closely related glucuronide metabolites using various chromatographic modes. *J Chromatogr A*. 2016;1435:54-65. doi:10.1016/j.chroma.2016.01.033
35. Regalado EL, Helmy R, Green MD, Welch CJ. Chromatographic resolution of closely related species: drug metabolites and analogs. *J Sep Sci*. 2014;37(9–10):1094-1102. doi:10.1002/jssc.201400038
36. WADA TD2015IDCR. https://www.wada-ama.org/sites/default/files/resources/files/td2015idcr_-_eng.pdf. Accessed 29 June, 2023.
37. WADA TD2021IDCR. https://www.wada-ama.org/sites/default/files/resources/files/td2021idcr_final_eng_0.pdf. Accessed 29 June, 2023.
38. WADA TD2022DL. https://www.wada-ama.org/sites/default/files/2022-01/td2022dl_v1.0_final_eng_0.pdf. Accessed 29 June, 2023.
39. Losacco GL, Rentsch M, Plachká K, et al. Ultra-high performance supercritical fluid chromatography coupled to tandem mass spectrometry for antidoping analyses: assessment of the inter-laboratory reproducibility with urine samples. *Anal Sci Adv*. 2020;2(1-2):1-8. doi:10.1002/ansa.202000131

SUPPORTING INFORMATION

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