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Doping control analysis of trimetazidine and characterization of major metabolites using mass spectrometric approaches

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Since January 2014, the anti-anginal drug trimetazidine [1-(2,3,4-trimethoxybenzyl)-piperazine] has been classified as prohibited substance by the World Anti-Doping Agency (WADA), necessitating specific and robust detection methods in sports drug testing laboratories. In the present study, the implementation of the intact therapeutic agent into two different initial testing procedures based on gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) is reported, along with the characterization of urinary metabolites by electrospray ionization-high resolution/high accuracy (tandem) mass spectrometry. For GC-MS analyses, urine samples were subjected to liquid-liquid extraction sample preparation, while LC-MS/MS analyses were conducted by established 'dilute-and-inject' approaches. Both screening methods were validated for trimetazidine concerning specificity, limits of detection (0.5–50 ng/mL), intra-day and inter-day imprecision (<20%), and recovery (41%) in case of the GC-MS-based method. In addition, major metabolites such as the desmethylated trimetazidine and the corresponding sulfoconjugate, oxo-trimetazidine, and trimetazidine-*N*-oxide as identified in doping control samples were used to complement the LC-MS/MS-based assay, although intact trimetazidine was found at highest abundance of the relevant trimetazidine-related analytes in all tested sports drug testing samples. Retrospective data mining regarding doping control analyses conducted between 1999 and 2013 at the Cologne Doping Control Laboratory concerning trimetazidine revealed a considerable prevalence of the drug particularly in endurance and strength sports accounting for up to 39 findings per year.

Keywords: sport; doping; mass spectrometry; angina pectoris; metabolism

Introduction

Following its patent application in 1962,[1] the therapeutic agent trimetazidine [1-(2,3,4-trimethoxybenzyl)-piperazine] (Figure 1) was introduced in 1963 under the trade name *Vastarel* in France for the treatment of (stable) angina pectoris.[2] Ever since, the coronary vasodilator trimetazidine underwent numerous studies concerning its mechanism(s) of action and potential additional fields of medical application,[3] demonstrating among other findings a decrease in coronary vascular resistance, an increased coronary blood flow, the inhibition of 3-ketoacyl coenzyme A thiolase (being a key enzyme in the β-oxidative metabolic pathway of the myocardium), and cytoprotective/anti-ischemic effects in other organs.[4] As a consequence of the 3-ketoacyl coenzyme A thiolase inhibition, improvements in left ventricle dysfunction patients were observed when being treated with trimetazidine due to the lowered demand for oxygen when glucose is utilized instead of free fatty acids. Although being considered a well-tolerated drug,[5] an increasing number of case reports concerning drug-induced parkinsonism, gait disorder, and tremor caused by trimetazidine have been published,[6–8] resulting in a recommendation for a careful cost-benefit assessment of the drug. In the course of drug development and follow-up studies, analytical approaches for trimetazidine have been developed based on thin-layer chromatography,[9] liquid chromatography with fluorescence detection,[10] gas chromatography-mass spectrometry (GC-MS),[11] and more recently liquid chromatography-(tandem) mass spectrometry (LC-MS/MS),[12–17] one of which focusing particularly on the characterization of trimetazidine metabolites in human plasma and urine.[18]

Since January 2014, trimetazidine belongs to the list of prohibited substances established by the World Anti-Doping Agency (WADA)[19] and a first adverse analytical finding was recently reported during the XXII. Olympic Winter Games in Sochi,[20] outlining the importance of validated detection assays allowing for robust, fast, and cost-effective analyses. Commonly, existing initial test methods are assessed concerning their capability to cover the newly added analyte and/or its main metabolite(s) in routine doping controls. In the present study, two analytical procedures were used to probe for the presence or absence of trimetazidine in urine samples collected for sports drug testing purposes with one employing GC-MS and the other being based on LC-MS/MS. Moreover, urinary metabolites of trimetazidine as detected in routine doping control samples were

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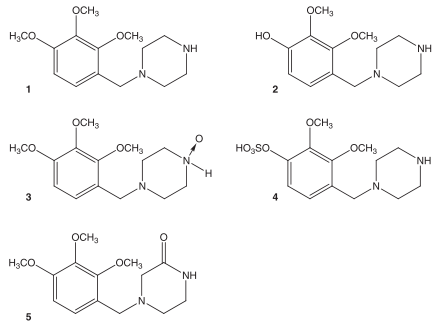


Figure 1. Chemical structures of trimetazidine (**1**, mol wt = 266 Da) and its major metabolites desmethyl-trimetazidine (**2**, here 4-desmethyl-trimetazidine is shown as an example, mol wt = 252 Da), trimetazidine-*N*-oxide (**3**, mol wt = 282 Da), desmethyl-trimetazidine sulphate (**4**, here the sulfoconjugate of 4-desmethyl-trimetazidine is shown as an example, mol wt = 332 Da), and oxo-trimetazidine (**5**, mol wt = 280 Da)

characterized by means of high resolution/high accuracy mass spectrometry and compared to literature data. Eventually, retrospective data mining was conducted, providing insights into the prevalence of the anti-anginal therapeutic in doping control samples analyzed in the WADA-accredited laboratory in Cologne, Germany, since 1999.

Experimental

Chemicals and reference compounds

Ammonium acetate (p.a.), sodium sulphate, and acetic acid (glacial) were bought from Merck (Darmstadt, Germany). Acetonitrile (HPLC grade) was supplied by J.T. Baker (Deventer, the Netherlands) and deionized water was obtained from a Millipore MilliQ water purification system (Schwalbach, Germany). *Tert*. butyl methyl ether (distilled before use) was from KMF (Sankt Augustin, Germany), trimetazidine dihydrochloride from Sigma (Schnelldorf, Germany), the internal standard (ISTD) for LC-MS/MS analyses, $^{13}C_1^2H_2$-hydrochlorothiazide, was from LGC Promochem (Wesel, Germany), and the ISTD dodecyl diisopropylamine (DIPA-12) for GC-MS measurements was synthesized in our laboratory.[21]

Structure characterization by liquid chromatography-high resolution/high accuracy (tandem) mass spectrometry

Accurate mass measurements of trimetazidine and its urinary metabolites were performed on a Thermo LTQ Orbitrap (Bremen, Germany) mass spectrometer. The instrument was operated in positive electrospray ionization mode and mass accuracy (mass error < 5 ppm as calculated from 30 averaged spectra) was ensured by external calibration using the manufacturer's calibration mixture (including caffeine, Met-Arg-Phe-Ala, and Ultramark). The ionization voltage was 4000 V, the capillary temperature was set to 275°C, and respective protonated precursor ions were isolated using a width of 1.5 Da. The precursor ion species in both MS^2 and MS^3 experiments were dissociated at normalized collision energies between 10 and 20 (arbitrary units, Xcalibur Software version 2.1). Damping gas in the linear ion trap was helium (purity grade 5.0), and gas supplied to the curved linear ion trap (CLT) was nitrogen obtained from a CMC nitrogen generator (CMC Instruments, Eschborn, Germany). Samples were introduced into the MS via an Open Accela liquid chromatograph (Thermo, Dreieich, Germany) equipped with a Thermo Accucore C-8 XL column (3 × 100 mm, 4 μm) with a flow rate of 400 μL/min. The gradient decreased from a composition of 99% 0.1% formic acid (A) and 1% acetonitrile (B) to 70% A within 10 min, followed by re-equilibration to starting conditions (2 min).

Liquid chromatography-tandem mass spectrometry in routine doping controls

An existing initial testing setup was expanded to cover trimetazidine and major metabolites identified in the present work using diagnostic precursor-product ion pairs as outlined in Table 1. The instrumentation included an Agilent 1260 Series liquid chromatograph (Waldbronn, Germany) coupled to an Applied Biosystems API5500 QTrap mass spectrometer (Darmstadt, Germany) utilizing electrospray ionization (ESI) and multiple reaction monitoring (MRM). For further details, the interested reader is referred to the publication by Guddat *et al.*[22]

GC-MS/NPD analysis

GC-MS analyses were accomplished by means of an Agilent 6890/5973 GC-MS system (Waldbronn, Germany) equipped with an additional nitrogen-phosphorus detector (NPD) as commonly used for sports drug testing purposes with regard to nitrogen-containing, volatile compounds.[23] The set-up allowed for a single injection providing both MS and NPD data with two separate Agilent HP5MS columns (inner diameter: 0.25 mm, film thickness: 0.25 μm) being installed and operated simultaneously. In order to obtain matching retention times, the column directed to the mass selective detector (MSD) was longer (28 m) than the one column connected to the NPD (24 m) to account for different pressure conditions in the MSD and the NPD. The injection volume was 5 μL (split ratio 1:3), the GC carrier gas was helium (constant pressure at 1.24 bar (18 psi)), and a temperature gradient was employed starting at 98°C for 0.4 min increasing to 340°C with 30°C/min. The final temperature was kept for 3.4 min. The mass spectrometer was operated with electron ionization (EI, 70 eV) and full scan analysis (*m/z* 40-400, 2 scans/s).

Sample preparation – LC-MS/MS

The sample preparation consisted of the addition of 50 ng of the ISTD ($^{13}C_1^2H_2$-hydrochlorothiazide) to a urine aliquot of 1 mL, which was vortexed for 15 s and subsequently centrifuged at 10 000 *g* for one min. One hundred μL were then transferred to autosampler vials, and 2 μL were injected into the LC-MS/MS system.

Sample preparation – GC-MS

The analysis of trimetazidine using GC-MS was conducted according to an established routine doping control method.[24,25] In brief, 5 mL of urine are enriched with the internal standard (DIPA-12), the pH is adjusted to 14 with aqueous potassium hydroxide, and the mixture extracted with *tert*.-butyl methyl ether. The organic layer is subsequently analyzed by GC-MS.

Assay validation

Method validation for both GC-MS- and LC-MS/MS-based test methods was performed in conformity with the guidelines of the International Conference on Harmonisation (ICH).[26] Since trimetazidine is categorized under the section S6 of the WADA

Table 1. Elemental compositions of protonated molecules of trimetazidine and major metabolites and resulting product ions using high resolution/high accuracy MS^n experiments. Product ions for routine doping control measurements are shown in **bold**.

Compound	Precursor ion (*m/z*)		elemental comp. (exp.)	error (ppm)	collision energy (arb. units)	Product ion (*m/z*)	elemental comp. (exp.)	error (ppm)	cleaved species
	MS^2	MS^3							
trimetazidine	267.1700		$C_{14}H_{23}O_3N_2$	-1.0	15	**181.0859**	$C_{10}H_{13}O_3$	-0.3	$C_4H_{10}N_2$
						166.0619	$C_9H_{10}O_3$	-3.5	$C_4H_{10}N_2$, CH_3•
						136.0516	$C_8H_8O_2$	-2.2	$C_4H_{10}N_2$, CH_3•, CH_2O
		181.0859	$C_{10}H_{13}O_3$	-0.3	25	166.0619	$C_9H_{10}O_3$	-3.5	CH_3•
M1	253.1545		$C_{13}H_{21}O_3N_2$	-0.8	15	**167.0703**	$C_9H_{11}O_3$	0.5	$C_4H_{10}N_2$
						139.0754	$C_8H_{11}O_2$	0.6	$C_4H_{10}N_2$, CO
						87.0920	$C_4H_{11}N_2$	3.4	$C_9H_{10}O_3$
		167.0703	$C_9H_{11}O_3$	0.5	25	139.0754	$C_8H_{11}O_2$	0.6	CO
						107.0496	C_7H_7O	4.2	CO, CH_3OH
M2	283.1649		$C_{14}H_{23}O_4N_2$	-1.3	15	**181.0858**	$C_{10}H_{13}O_3$	-0.4	$C_4H_{10}N_2O$
						166.0617	$C_9H_{10}O_3$	-4.5	$C_4H_{10}N_2O$, CO
M3	333.1109		$C_{13}H_{21}O_6N_2S$	-1.2	15	**253.1543**	$C_{13}H_{21}O_3N_2$	-1.1	SO_3
						167.0702	$C_9H_{11}O_3$	-0.2	SO_3, $C_4H_{10}N_2$
						87.0921	$C_4H_{11}N_2$	4.6	SO_3, $C_9H_{10}O_3$
M4	281.1502		$C_{14}H_{23}O_4N_2$	2.2	15	**181.0859**	$C_{10}H_{13}O_3$	-0.3	$C_4H_8N_2O$
						166.0620	$C_9H_{10}O_3$	-2.8	$C_4H_8N_2O$, CH_3•
						113.0713	$C_5H_9N_2O$	3.0	$C_9H_{14}O_3$
						85.0762	$C_4H_9N_2$	2.4	$C_9H_{14}O_3$, CO

Prohibited List,[19] a minimum required performance limit (MRPL) of 100 ng/mL applies.[27]

Lower limit of detection

The 'lowest content that can be measured with reasonable statistical certainty'[28] as suggested by ICH to be fulfilled when a signal-to-noise ratio ≥ 3 is obtained for the target analyte was considered in our study for the methods' lower limit of detection (LLOD). Therefore, ten different blank urine samples spiked with the ISTDs only, and further six different blank urine specimens fortified with 1 ng/mL and 100 ng/mL of trimetazidine for LC-MS/MS and GC-MS analyses, respectively, were prepared and measured according to the established protocols providing the data necessary to estimate the LLOD.

Intra-day and inter-day precision

Thirty urine samples were spiked at low, medium, and high concentrations with trimetazidine, the urinary levels of which varied as outlined in Table 2. The samples were prepared and analyzed on three different days, and the intra-day as well as inter-day precision was calculated for each concentration level.

Recovery (GC-MS only)

Six blank urine specimens were spiked to 1000 ng/mL of trimetazidine prior to sample preparation and another set of six blank samples was processed followed by the addition of a total of 5 μg of trimetazidine into the organic layer prior to GC-MS analysis. Peak area ratios were computed to provide the information for calculating the method's recovery.

Specificity

Ten different blank urine specimens obtained from three female and seven male healthy volunteers were prepared and analyzed as described above in order to probe for interfering peaks in the selected ion chromatograms at expected retention times of trimetazidine.

Table 2. Summary of assay validation results for trimetazidine

LC-MS/MS approach						GC-MS approach						
	Intraday precision (n=30)		Interday precision (n=30+30+30)					Intraday precision (n=30)		Interday precision (n=30+30+30)		
LOD [ng/mL]	Concentration [ng/mL]	CV (%)	Concentration [ng/mL]	CV (%)	Calibration curve	LOD [ng/mL]	Recovery (%)	Concentration [ng/mL]	CV (%)	Concentration [ng/mL]	CV (%)	Calibration curve
0.5	10	4.3	10	5.6	linear (r = 0.996)	50	41	100	11.4	100	14.7	linear (r = 0.989)
	100	3.6	100	3.8				1000	9.0	1000	15.1	
	200	2.7	200	4.6				2000	10.0	2000	15.3	

CV = coefficient of variance

Linearity

Calibration curves were prepared in urinary matrix for trimetazidine in respective working ranges, i.e. for GC-MS between 50 and 2000 ng/mL (50, 100, 250, 500, 750, 1000, 1250, 1500, 1750, and 2000 ng/mL) and for LC-MS/MS between 5 and 200 ng/mL (5, 10, 25, 50, 75, 100, 125, 150, 175, and 200 ng/mL).

Test for ion suppression/enhancement effects (LC-MS/MS only)

In order to estimate ion suppression effects possibly caused by matrix interference on the LC-MS/MS apparatus, a pool of 10 different blank urine samples was analyzed (n = 6) and compared to solvent only (n = 6), both specimens spiked to 100 ng/mL of trimetazidine. Peak area ratios (trimetazidine/ISTD) were compared in both sets of measurements and compared to allow assessing a potential influence of ion suppression/enhancement effects.[29,30]

Doping control urine samples – retrospective data mining

Between 1999 and 2013, more than 140 000 doping control urine samples were analyzed by the aforementioned GC-MS full scan-based screening method, enabling the detection of trimetazidine and allowing for the retrospective extraction of relevant information from the electronic data in accordance to reference databases.[31]

Four authentic doping control urine samples from 2013, which showed the athletes' approval for using the sample for research purposes, were found to contain trimetazidine and were used for metabolite identification purposes. The samples were subjected to both routine doping control assays as well as the above mentioned liquid chromatography-high resolution/high accuracy (tandem) mass spectrometry analysis to obtain elemental composition and structure information on main trimetazidine metabolites.

Results and discussion

The use of trimetazidine in elite sport has been prohibited according to WADA regulations being enforced since January 2014.[19] In order to provide fast and robust analytical results for sports drug testing purposes, the therapeutic agent as well as its main metabolites were characterized by mass spectrometric approaches, and two analytical options commonly available in accredited doping control laboratories were evaluated concerning their capability to determine the administration of the banned substance by means of urine analysis.

Structure characterization by liquid chromatography-high resolution/high accuracy (tandem) mass spectrometry

In order to appropriately characterize the mass spectrometric behaviour of trimetazidine and its metabolites under conditions of electrospray ionization and collision-induced dissociation (CID), product ion mass spectra of trimetazidine and four major metabolites were recorded (Figure 2) using high resolution/high accuracy mass spectrometry, and dissociation routes were suggested based on MS^n experiments. The elemental compositions determined for the most abundant product ions of each compound are listed in Table 1. The dissociation of the intact drug, which was the only analyte available as certified reference material, predominantly yielded the product ion at *m/z* 181 (Figure 2a) after protonation and collisional activation. It is suggested that this ion at *m/z* 181 results from the elimination of piperazine (86 Da) as supported by the determination of the accurate mass of the charge-retaining moiety and, thus, its elemental composition. The loss of piperazine is postulated to produce the 2,3,4-trimethoxycyclohepta-2,4,6-trien-1-ylium ion (Scheme 1a), which consecutively eliminates a methyl radical (15 Da) and formaldehyde to generate the low-abundant but diagnostic product ions at *m/z* 166 and 136, respectively, as corroborated by MS^n experiments (Table 1). Due to the preferred cleavage of the molecule between the piperazine and the benzoylic residue, the location of metabolic alterations on either of the moieties is possible. A major metabolic reaction is the demethylation of trimetazidine leading to 2-desmethyl- and 3/4-desmethyl-trimetazidine with 2-desmethyl-trimetazidine being a minor metabolite.[18] In Figure 2b, the product ion mass spectrum of the protonated species $[M+H]^+$ of M1 is presented, which was assigned as 4-desmethyl-trimetazidine. Supporting evidence for this assumption was obtained by similar relative retention times as observed by Jackson *et al.* in 1996[18]; moreover, the product ion at *m/z* 87, attributed to protonated piperazine (Table 1), is facilitated by the formation of a stable leaving group presumably consisting of 2,3-dimethoxy-4-methylenecyclohexa-2,5-dienone (Scheme 1b). A comparable rearrangement is possible in case of the ortho-demethylated (2-desmethyl) trimetazidine but not with 3-desmethyl-trimetazidine, suggesting that the metabolite presented is indeed 4-desmethyl-trimetazidine as the 2-dealkylated analog is separated by LC.[18] In analogy to the intact trimetazidine, the product ion at *m/z* 167 (corresponding to *m/z* 181) is generated, which releases carbon monoxide to form *m/z* 139 as shown in MS^3 experiments (Table 1). At *m/z* 283, two species (M2) were observed that were assigned to oxygenated trimetazidine as corroborated by its elemental composition (Table 1). The collision-induced dissociation of the protonated molecule yielded the above reported product ions at *m/z* 181 and 166 (Figure 2c), suggesting the location of the modification at the piperazine moiety, which could either represent a hydroxyl group or an *N*-oxide. Supported by the absence of a product ion resulting from the elimination of water, the presence of two *N*-oxide isomers was assumed; however, due to the facile cleavage of the precursor ion to *m/z* 181, further measurements under atmospheric pressure chemical ionization (APCI) were conducted, which would result in the thermally deoxygenated counterpart of the trimetazidine *N*-oxide species and thus allow to verify or falsify the proposed structure.[32] The conducted experiments furnished additional information corroborating the attributed *N*-oxide structure by the elimination of both M2 isomers under APCI conditions (data not shown).

M3 was identified as the sulfoconjugate of M1, based on its elemental composition and dissociation behavior (Figure 2d) yielding the diagnostic product ions at *m/z* 253, 167, and 87 as described for M1 (*vide supra*).

Another abundant metabolite referred to as M4 was suggested to represent oxo-trimetazidine (Figure 2e) bearing the oxygen at the piperazine moiety in agreement with literature data.[18] Both elemental composition and product ion mass spectrum corroborated the attributed structure with product ions at *m/z* 181 and 166 clearly indicating the unmodified benzoyl residue, and *m/z* 113 and 85 suggesting the presence of a ketopiperazine group that further eliminates a water molecule (Scheme 1c, Table 1).

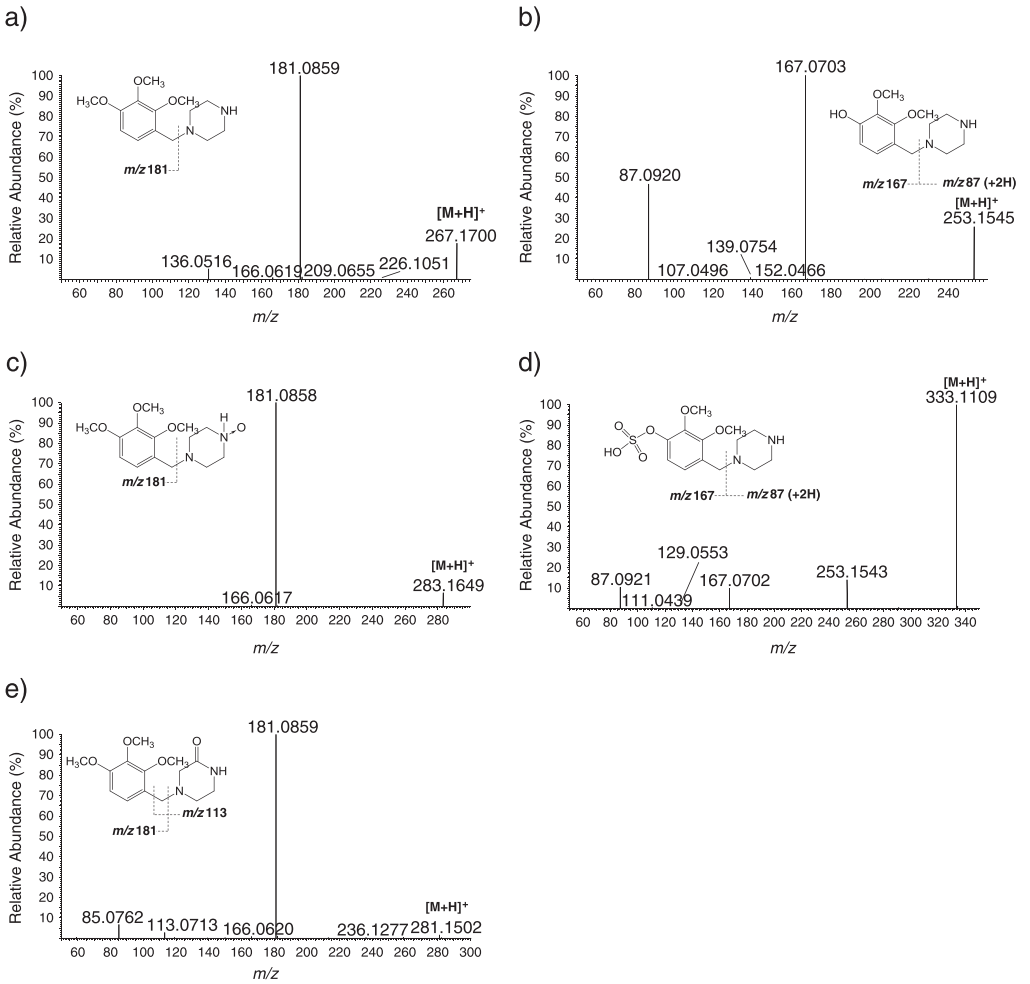
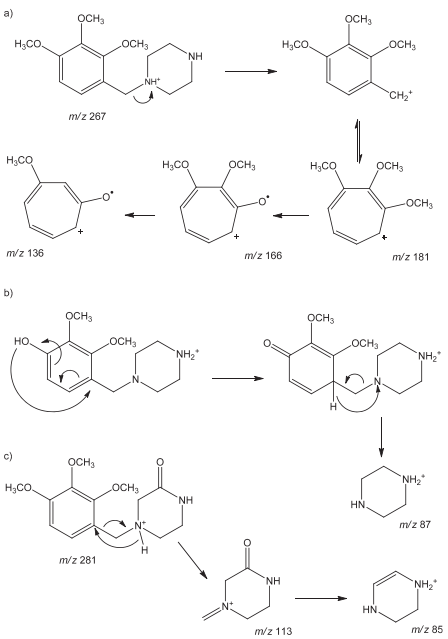


Figure 2. ESI-product ion mass spectra of protonated molecules $[M+H]^+$ of (a) trimetazidine (**1**), (b) 4-desmethyl-trimetazidine (**2**), (c) trimetazidine-*N*-oxide (**3**), (d) sulfoconjugate of 4-desmethyl-trimetazidine (**4**), and (e) oxo-trimetazidine (**5**)



Scheme 1. Proposed dissociation pathways of the protonated molecule of trimetazidine (a), 4-desmethyl-trimetazidine (b), and oxo-trimetazidine (c) under ESI-CID conditions.

Assay validation and application to doping controls – LC-MS/MS

Exploiting the above reported diagnostic product ions, an existing doping control initial test method using a triple quadrupole LC-MS/MS system was expanded to include the intact drug of trimetazidine and the metabolites M1–M4. The analytical assay was characterized concerning the validation parameters specificity, LLOD, intra-day and inter-day precision as summarized in Table 2. In Figure 3, typical extracted ion chromatograms of (a) a blank urine sample, (b) a blank urine specimen fortified with 1 ng/mL of trimetazidine, and (c) an authentic doping control specimen are illustrated. In all blank urine samples, no interfering signal was observed at expected retention times of **1–5**, and distinct signals are obtained for the selected ion transitions (Table 1) in spiked aliquots and/or post-administration samples, allowing a rapid detection of trimetazidine and the selected metabolites.

The LOD of trimetazidine was estimated at 0.5 ng/mL, and the reproducibility and repeatability of the established method yielded excellent results as shown by means of the intraday- and interday imprecisions (Table 2), which were determined to be below 10%. Calibration curves were linear according to the Mandel test.[33] Although direct urine injection might cause ion suppression effects, the influence on trimetazidine was less than 10% at the expected retention time as determined in respective experiments (Table 2).

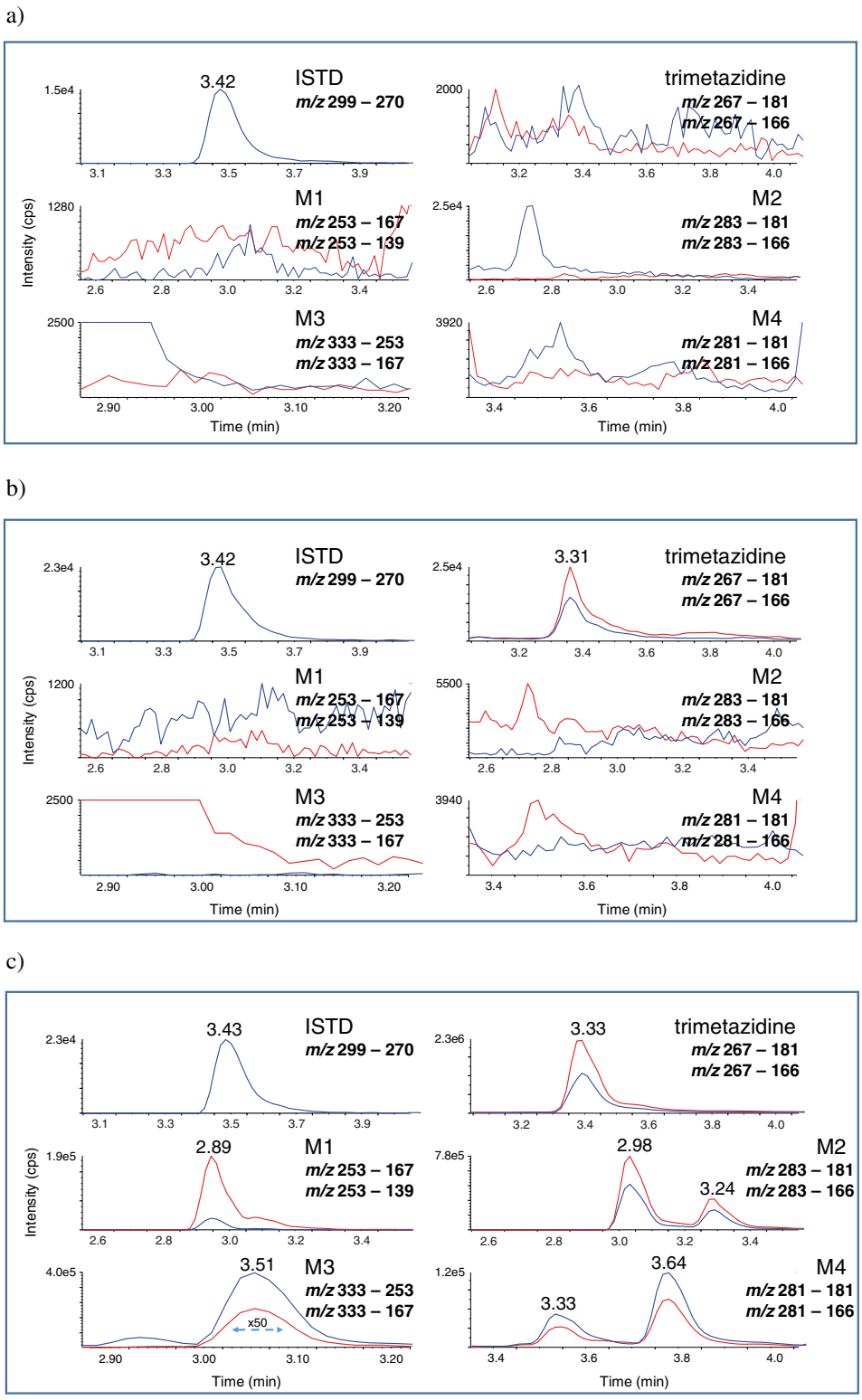


Figure 3. LC-MS/MS analyses of (a) a blank urine sample, (b) a blank urine fortified with 1 ng/mL of trimetazidine, and (c) a doping control sample of 2013 containing trimetazidine and its main metabolites M1-M4. Internal standard was $^{13}C_1^2H_2$-hydrochlorothiazide (*m/z* 299–270) in all samples

Assay validation and application to doping controls – GC-MS

Employing GC-MS with electron ionization in full scan mode, urine samples have been analyzed for a broad range of alkaline compounds in sports drug testing for decades.[34] The capability of measuring trimetazidine using this approach was assessed and also here the aforementioned parameters to characterize the methodology, i.e. specificity, LLOD, intra- day and inter-day

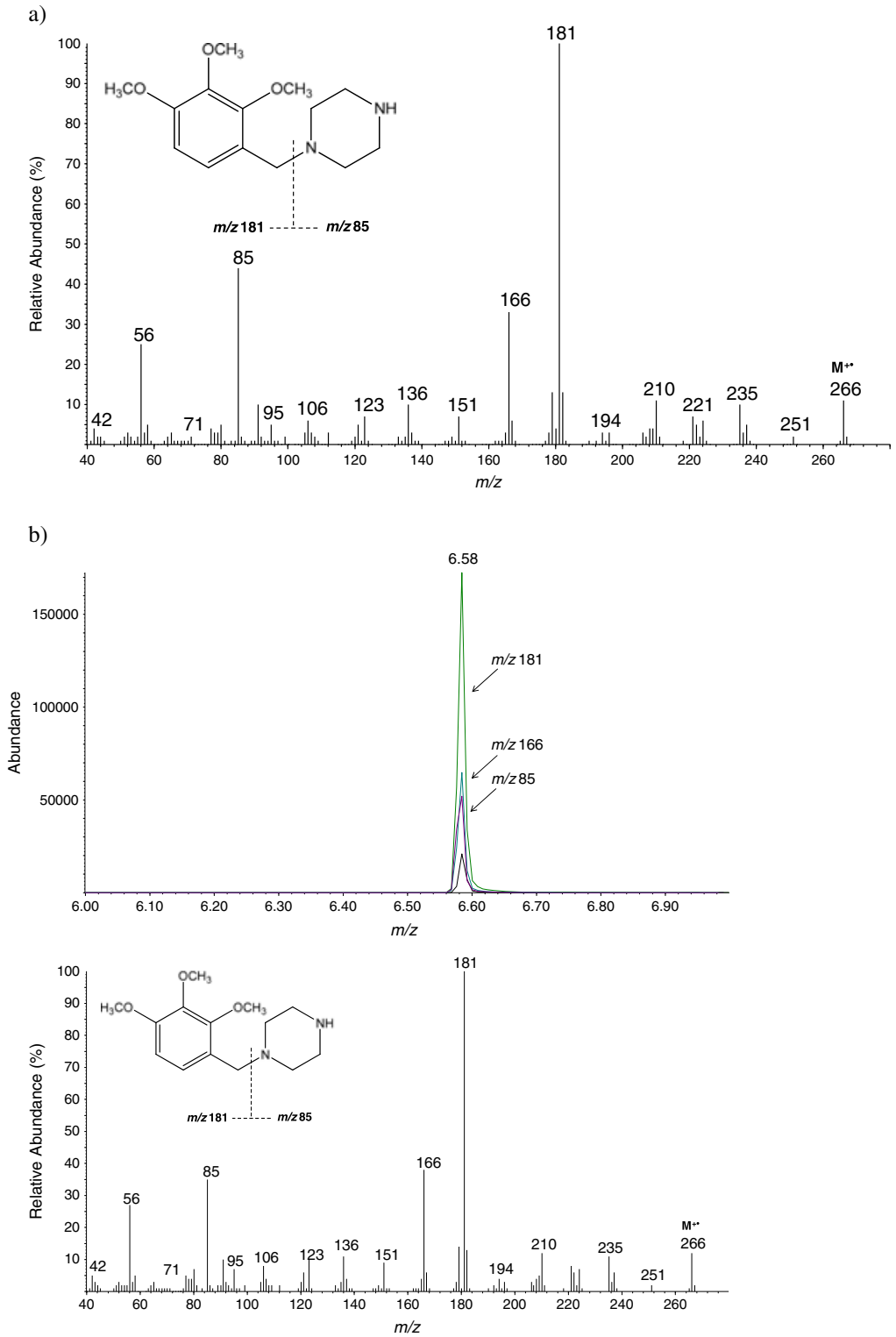


Figure 4. (a) EI mass spectrum of trimetazidine and (b) extracted ion chromatogram (*m/z* 181, *m/z* 166, and *m/z* 85) of trimetazidine found in a doping control sample in 2013 with corresponding EI mass spectrum

precision, as well as recovery were determined as compiled in Table 2.

Since the GC-MS-based procedure evidently allows to sensitively detect trimetazidine in human urine (Figure 4) down to approximately 50 ng/mL, electronic data of more than 140 000 doping control samples measured between 1999 and 2013 in the doping control laboratory of Cologne were screened, enabling the identification of 151 cases of trimetazidine use by athletes. Here, the EI mass spectra of signals attributed to trimetazidine were matched against the AORC MS Drug

Library[31] and relative retention times of the determined substance and the ISTD were considered. Among the recorded findings, endurance sport, strength sport, and other disciplines accounted for 66 (43.7%), 59 (39.1%), and 26 (17.2%) of trimetazidine 'positives', respectively. In the absence of additional data (e.g. athletes' gender, in- or out-of-competition control), no further evaluation of the findings was conducted. Nevertheless, 0.1% of all retrospectively studied samples demonstrated the use of the anti-anginal coronary vasodilator trimetazidine by elite athletes actively competing predominantly in endurance and strength sports, which might trigger questions as to the corresponding medical indications.

Conclusion

Two existing doping control analytical assays, one based on LC-MS/MS and one on GC-MS, were assessed as to their capability to detect and identify trimetazidine and its major metabolites in doping control urine samples. Both approaches enabled the analysis of the intact drug at LODs between 0.5 and 50 ng/mL, which is in agreement with current WADA requirements.[27] In addition to the detection of trimetazidine, LC-MS/MS allowed for the identification of major urinary metabolites; however, the analysis of four doping control samples containing trimetazidine revealed that the most abundant signal was generated from the administered drug rather than its metabolites. Nevertheless, in order to corroborate adverse analytical findings, products of metabolic reactions can be utilized.

The evaluation of electronic data generated over the past 15 years demonstrated a certain prevalence of trimetazidine use in elite sport. Given the limited medicinal scope of trimetazidine in clinical practice, the indication of its use in the past remains questionable and the inclusion of the therapeutic agent in the WADA Prohibited List as of January 2014 appears justified.

Acknowledgements

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