Mass spectrometry-based characterization of new drugs and methods of performance manipulation in doping control analysis

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Efficient and comprehensive sports drug testing necessitates frequent updating and proactive, preventive anti-doping research and the early implementation of new, emerging drugs into routine doping controls is an essential aspect. Several new drugs and drug candidates with potential for abuse, including so-called Rycals (ryanodine receptor calstabin complex stabilizers, for example, S-107), hypoxia-inducible factor (HIF) stabilizers and peroxisome-proliferator-activated receptor (PPAR)δ agonists (for example, GW1516), were studied using different mass spectrometry- and ion mobility-based approaches and their gas-phase dissociation behaviors were elucidated. The detailed knowledge of fragmentation routes allows a more rapid identification of metabolites and structurally related, presumably "tailor-made", analogs potentially designed for doping purposes. The utility of product ion characterization is demonstrated, in particular, with GW1516, for which oxidation products were readily identified in urine samples by means of diagnostic fragment ions, as measured using high-resolution/high-accuracy mass spectrometry and higher energy collision-induced dissociation (HCD).

Keywords: doping, sport, mass spectrometry, orbitrap, Synapt, ion mobility

Introduction

The on-going research for drugs to allow an efficient treatment of severe diseases and modern “epidemics” has continuously led to the production of compounds that are potentially misused in elite sport. Therapeutic agents such as the ryanodine-calstabin complex stabilizer (Rycal) S-107,1–7 hypoxia-inducible factor (HIF) stabilizer FG-2216,8–12 or the peroxisome proliferator-activated receptor (PPAR)δ agonist GW151613–16 are currently being developed to counteract cardiac arrhythmia, anemia, or the metabolic syndrome including type-II diabetes mellitus, respectively. The beneficial effects are suspected to tempt athletes to misuse these agents to artificially enhance performance, for example, by reducing fatigue and accelerating regeneration, increasing the amount of erythrocytes and, thus, the oxygen transport capacity, or by mimicking exercise and shifting energy consumption from carbohydrate- to lipid-based resources.17

Studies on the mass spectrometry (MS) of these agents and an early development of detection assays followed by
implementation into routine doping control procedures before drugs receive approval, represent important tools of preventive doping research and should minimize the benefits and windows of opportunity that cheating athletes might have from a surreptitious use of such emerging therapeutics. Hence, several new drug candidates, including S-107,18,19 a model substance resembling the physicochemical properties of modern isoquinoline-3-carboxamide-derived HIF-stabilizers (such as fG-2216)20 and GW151621 (Figure 1, compounds 1, 5 and 6, respectively) were characterized using state-of-the-art high resolution/high accuracy mass spectrometers and tandem mass spectrometry (MS/MS). Based on these data, approaches to identify the target analytes or metabolites in urine or blood specimens are facilitated and the utility of the knowledge of dissociation pathways and product ion generation is outlined by the elucidation of urinary metabolites formed from GW1516. Using liquid chromatography (LC) hyphenated to MS and higher energy collision-induced dissociation (HCD), two phase-I-metabolites were readily identified.

Experimental Electrospray ionization tandem mass spectrometry (ESI-MS/MS) ESI-MS/MS studies were conducted using a high-resolution/high accuracy hybrid LTO-Orbitrap mass spectrometer (Thermo, Bremen, Germany). Sample solutions at 1 µg mL\(^{-1}\) were prepared in 50% acetonitrile containing 0.1% formic acid and introduced using a syringe pump into the ESI source, which was operated in positive ionization mode at 4200 V. Damping gas in the linear ion trap was helium (purity grade 5.0) and the C-trap was operated with nitrogen obtained from a nitrogen generator (CMC Instruments, Eschborn, Germany). The precursor ion isolation widths for MS\(^{+}\) experiments was 1.5 Da and mass accuracy in MS and MS\(^{+}\) measurements was maintained below 5 ppm (calculated from 30 averaged spectra) by calibration of the instrument following the manufacturer’s recommendations.

Gas chromatography-electron ionization tandem mass spectrometry (GC-EI-MS/MS) The elucidation of fragmentation pathways of EI-generated ions was done using an Agilent 6890/5973 GC-MSD (Waldbraun, Germany), a Thermo Polaris GC ion trap (Dreieich, Germany) as well as a Finnigan MAT900ST (Bremen, Germany) mass spectrometer with E/B geometry. The low-resolution MS systems were equipped with a GC using an Agilent HP-5MS column [17 m, 0.2 mm i.d., 0.2 µm film thickness]. Target compounds were dissolved in methanol at 10 µg mL\(^{-1}\) and 2 µL were injected into the respective GC-MS systems for MS and MS/MS analyses. For the latter, the isolation widths were 1 Da and applied collision offset voltages were 1 V. For the high-resolution sector instrument, samples were introduced using a direct inlet probe and a volume of 5 µL containing approximately 5 µg of analyte was introduced in an aluminum cup into the ion source. Peak matching was utilized to determine accurate masses of target ions at a resolving power > 9500 [10% valley]. The error was less than 4 ppm for all measured elemental compositions.

Liquid chromatography electrospray ionization higher energy collision-induced dissociation (LC-ESI-HCD/MS) The analysis of an administration study urine sample for potentially formed metabolites of GW1516 was conducted using an LC-ESI-Orbitrap system (Exactive, Thermo, Bremen, Germany). The LC [Thermo Accela] was equipped with a Phenomenex Synergy column (2.1 × 50 mm, particle size 2 µm) and solvents were 0.2% formic acid (A) and methanol (B). The flow rate was set to 250 µL min\(^{-1}\) and gradient elution was conducted starting at 90% A, decreasing to 0% A in 8 min, maintaining 0% A for a further 2 min followed by re-equilibration at 90% A. The mass spectrometer was operated in positive ionization mode and calibrated using the manufacturer’s calibration mixture (consisting of caffeine, the tetrapeptide MRFA and ultramark yielding a total of seven reference masses). Mass accuracies < 5 ppm were accomplished for the period of analysis. The ionization voltage was 3.5 kV, the capillary temperature was set to 290°C and three MS settings were used throughout the analytical runs: (a) full scan MS from m/z 50–2000 at a resolution of 25,000 [FWHM], (b) full scan MS (m/z 50–2000, resolution set to 15,000) with higher energy collision-induced dissociation (HCD, employing the collision octapole positioned in-line with the curved linear ion trap (C-trap) set to 20 V and (c) full scan MS (m/z 50–2000, resolution set to 15,000) with HCD set to 50 V. Gas supplied to the C-trap was nitrogen obtained from a nitrogen generator (CMC Instruments, Eschborn, Germany). Further characterization of
putative metabolites was conducted using identical LC conditions to the above mentioned LTQ-Orbitrap MS to allow MS² experiments from selected precursor ions.

Urine samples after oral application of a single dose of 2 mg of GW1516 to a healthy male volunteer were obtained from Moscow State University (Moscow, Russia). Ethical approval for the study and written consent were obtained from the local Ethics Committee and the participant, respectively. Urine samples were prepared for LC-MS/MS analysis as described elsewhere.²²

**Electrospray ionization-ion mobility tandem mass spectrometry (ESI-IMS-MS/MS)**

In order to separate different ion species with identical mass-to-charge ratios, a Synapt HDMS quadrupole time-of-flight/ion mobility spectrometer (QToF/IMS, Waters, Milford, MA, USA) was used. The analyte S-107 was infused via nanoESI source and Au/Pd coated borosilicate glass capillaries (Proxeon Biosystems, Odense, Denmark) with flow rate of 50 nL min⁻¹ into the system at a concentration of 5 μg mL⁻¹ dissolved in 50% methanol containing 0.2% formic acid. The instrument was operated in the time-aligned parallel (t AP) fragmentation mode with wave velocities set to 40 ms⁻¹, 300 ms⁻¹ and 40 ms⁻¹ for the trap, IMS and transfer regions, respectively. Accordingly, wave heights were set to 3V, 5V and 3V and trapCE and transferCE were 6V and 13V, respectively.

**Results and discussion**

**Rycal S-107**

Potentially efficient therapeutics for the treatment of cardiac arrhythmia have been developed to prevent fatalities related to this serious health condition. A class of promising drug candidates bearing a 1,4-benzothiazepine nucleus such as S-107 (Figure 1, 1) has demonstrated efficacy to improve impaired cardiac function by stabilizing the ryanodine-receptor/calstabin complex.¹ The benefits of S-107 on cardiomyocytes was also observed in skeletal muscles;² trained laboratory rodents showed a significant reduction in fatigue, increased endurance and, thus, overall enhanced performance upon S-107 administration. Consequently, a potential benefit is also given for elite athletes. Although not yet prohibited by the World Anti-Doping Agency (WADA),²³ methods to detect benzothiazepine-derived drugs such as S-107 were established,¹⁸,¹⁹,²¹ requiring a concise understanding of the mass spectrometric behavior of the target compounds under ESI/collision-induced dissociation (CID) and EI conditions. In order to obtain detailed information on product ions with regard to elemental composition and possible mechanisms of generation, the drug candidate and stably labeled and structural analogs (Figure 1, compounds 1–4) were synthesized.¹⁹

Dissociation routes from electrospray ionization tandem mass spectrometry

The target analyte S-107 (Figure 1, 1) contains several heteroatoms which are amenable for protonation under ESI conditions. Proton affinities (PA) for related compounds, although referring to the gas phase rather than the liquid phase as present in the case of ESI, outlined the tertiary amino function of S-107 as the privileged protonation site when comparing, for example, hexahydroazepine (PA=956.7 kJ mol⁻¹) and methylthiobenzene (PA=872.6 kJ mol⁻¹),²⁴,²⁵ however, due to the mobile nature of protons under CID conditions,²⁶ the proposed dissociation pathways are considered as one of several possible routes.

![Figure 2. (a) ESI product ion mass spectrum generated from the protonated molecule of S-107 [M+H]⁺ = m/z 210 using an LTQ-Orbitrap mass spectrometer (Thermo, Bremen, Germany); (b) EI-MS spectrum of S-107 recorded on an Agilent 6890/5973 GC-MSD (Waldbronn, Germany).](image-url)
The protonated molecule at \( m/z \) 210 of the lead drug candidate yielded abundant and informative product ions upon collisional activation ([Figure 2a]). Major fragments were found at \( m/z \) 179 and \( m/z \) 153, which were attributed to the losses of methylamine (−31 Da) and 1-methyl-aziridine or azetidine (C\(_3\)H\(_7\)N\(_\bullet\), −57 Da), respectively, as confirmed by accurate mass measurements. It was suggested that the product ion at \( m/z \) 179 comprised a 1-methoxy-4-vinylsulfonyl-cycloheptane structure as supported by the analyses of the deuterated analogs 3 and 4 ([Figure 1]), which proved the initial loss of methylamine and the retention of two deuterium atoms located at C-5 [Scheme 1].

The release of azetidine from the protonated precursor ion at \( m/z \) 210 to yield the ion at \( m/z \) 153 was proposed on the basis of various measurements. First, deuteration with deuterium oxide followed by \( m/z \) selection and fragmentation of \([M+D]^+\) showed that the leaving group retains the proton that was introduced during the ionization process. Second, the analysis of the triply-deuterated S-107 (compound 3) gave rise to an abundant product ion at \( m/z \) 154 that retained one of the deuterium atoms formerly located at the \( N \)-linked methyl function. Moreover, compound 4 yielded a product ion at \( m/z \) 155 that evidently contains both deuterium atoms introduced at C-5. Consequently, the migration of a hydrogen atom from the \( N \)-linked methyl residue to C-5 was proposed, followed by the cleavage of the S-1–C-2 bond [Scheme 1]. The obtained product ion at \( m/z \) 153 subsequently eliminated a methyl radical (−15 Da), originating from both the methoxy group or C-5, to produce the ion at \( m/z \) 138.

The composition of the product ion at \( m/z \) 88 was determined as C\(_4\)H\(_7\)NS and H/D exchange experiments proved the absence of the proton introduced during the ionization process. The product ion was suggested to bear a 2-methyl-4H[1,2]-thiazete structure (Scheme 1) as supported by the analysis of the triply-deuterated analog of S-107 (Figure 1, compound 3), which yielded a corresponding ion at \( m/z \) 91 and, thus, demonstrated the presence of the intact \( N \)-linked methyl function.

**Dissociation routes from electron ionization tandem mass spectrometry**

The sulfur atom of S-107 was suggested to be the primary site of ionization using EI, yielding the molecular ion at \( m/z \) 209. From there, various different dissociation pathways were studied starting either with the loss of a hydrogen atom [−1 Da], a methyl radical [−15 Da] or ethylene [−28 Da], which yielded the secondary fragment ions at \( m/z \) 208, \( m/z \) 194 and \( m/z \) 181, respectively, in MS/MS experiments. The elimination of a hydrogen atom might be favored from C-2, which would allow the formation of a double bond conjugated to the aromatic ring system and thus delocalize the introduced charge to further rationalize the observed subsequent dissociation routes. An abundant product ion derived from \( m/z \) 208 is found at \( m/z \) 151, which is suggested to result from the loss of 1-methyl-aziridine [−57 Da] via formation of a cyclic leaving group composed of C-3, N-4 and C-5 [Scheme 2]. The elimination of methyl ethylenamine consisting of C-2, C-3 and N-4 was excluded by the analysis of the deuterated analogs 3 and 4. Both produced the same fragment ion at \( m/z \) 151 in MS/MS experiments using \([M−1]^+\) as the precursor ion and the cation of 3-methoxy-7-thia-bicyclo[4,2,0]octa-1,3,5-triene was proposed to constitute its structure. In addition to the fragment ion at \( m/z \) 151, another major product ion was observed at \( m/z \) 180, which was suggested to resemble 5-methoxy-2-methyl-2,3-dihydro-1,2-benzothiazole as a result of the loss of ethylene [−28 Da, Scheme 2].

The elimination of a methyl radical [−15 Da] from the molecular ion at \( m/z \) 209 yielded the fragment ion at \( m/z \) 194. S-107 contains two methyl functions incorporated in a methoxy and an amine residue, but both were excluded as the origin of the leaving group. The sole release of CH\(_3\)• and not CD\(_3\)• in the case of compound 3, which contained a trideuteromethyl residue at N-4, as well as an identical dissociation behavior observed in the case of the N-desmethylated analog 2, demonstrated that the methyl radical must originate from a different site of the molecule. The loss of the methyl function from the methoxy group could be rationalized by considering
the initial ionization of S-107 at S-1. The resulting conjugated electron system could form a quinone-like structure including the elimination of the methyl radical; however, the deuterated analog 3, which bears two deuterium atoms at C-5, yielded a fragment ion at m/z 195 caused by the loss of a CH$_3$D radical (–16 Da) instead of an expected ion at m/z 196 obtained by the loss of CH$_3$• (–15 Da). Hence, the release of CH$_3$• was suggested to result from the seven-membered ring structure, which presumably forms a new six-membered ring structure representing 6-methoxy-3-methyl-3,4-dihydro-2H-1,3-benzothiazine (Scheme 2). This pathway necessitated an intermediate ring opening, which rearranges to the proposed 1,3-benzothiazine nucleus due to the aligned orientation of the adjacent substituents.

In accordance to the ion at m/z 180 generated from [M–1]$^+$ (vide supra), the molecular ion, M$^+$, also eliminated ethylene (–28 Da) to give rise to m/z 181, the radical cation of 5-methoxy-2-methyl-2,3-dihydro-1,2-benzisothiazole. The carbons C-2 and C-3 were suggested to be expelled to generate the new bicyclic structure of the radical cation (Scheme 2).

### Hypoxia-inducible factor stabilizers

The most common approach to treat anemia and associated diseases has been the administration of recombinantly produced erythropoietin (EPO). With the elucidation of the mechanisms underlying the cellular oxygen sensing and aspects of the hypoxia signal pathway,\textsuperscript{8,27,28} alternatives to correct EPO deficiencies have been evaluated, for example, by means of increasing EPO gene expression. A key role in EPO gene expression is attributed to the hypoxia-inducible (transcription) factor (HIF), which was shown to be under the control of the arterial oxygen tension.\textsuperscript{29} In normoxia, the HIF-1α sub-unit is not stable and, thus, represents a limiting factor of EPO gene expression.\textsuperscript{30} Its pharmacological stabilization by means of prolyl hydroxylase inhibitors was found to mimic a reduced oxygen tension and to stimulate EPO gene expression.
Initial lead drug candidates such as FG-2216[^31,32] that reduced prolyl hydroxylase activity in non-human primate models have advanced to phase-II clinical trials and demonstrated that even patients with chronic kidney disease were able to maintain hemoglobin levels previously accomplished with recombinant human EPO.[^30] Also here, the potential for misuse in sports is given as the increased production of natural EPO would lead to an elevated level of erythrocytes but conventional EPO tests would fail to detect the pharmacologically-induced production of the peptide hormone. FG-2216 was reported to be an isoquinoline derivative,[^33] but as its full structure has not been disclosed yet, the first studies on the mass spectrometric behavior of this new class of therapeutics were conducted with model substances such as compound 5 (Figure 1).

**Dissociation routes from electrospray ionization tandem mass spectrometry**

The substance [(1-chloro-4-hydroxy-7-((2-propyl)oxy)-isoquinoline-3-carbonyl]-amino]-acetic acid (Figure 1, 5) was chosen as a model compound for ESI-MS/MS studies due to its favorable pharmacological properties as an HIF stabilizer and the structure containing a 3-substituted isoquinoline nucleus.[^33] All measurements were conducted employing identical conditions, as reported above for S-107. Due to its structural features, compound 5 was efficiently protonated using ESI, presumably at N-2, as estimated from the proton affinity of the native isoquinoline (951.7 kJ mol[^1]).[^25] However, density functional theory (DFT) calculations, with regard to the proton affinities of N-2 and the amide oxygen of compound 5, outlined negligible differences and, thus, several starting points of dissociation routes were conceivable. Few but abundant product ions were obtained after CID of the protonated precursor at m/z 339 of compound 5 (Figure 3(a)).[^20] Besides common elimination reactions, including the losses of water (−18 Da) and carbon monoxide (−28 Da) yielding the product ions at m/z 321 and m/z 293, rather unusual dissociation routes accompanied by gas-phase reactions with oxygen or water were observed in MS[^2] experiments and resulted in a nominal loss of 11 Da (Figure 3(a), inset).[^23] The isolation of the product ion at m/z 293 followed by CID produced an intense ion at m/z 282, a phenomenon which was attributed to the release of methyleneimine (−29 Da) and the addition of water (−18 Da) as supported by accurate mass measurements [Scheme 3(a)].[^20] Substantiating information for a putative dissociation pathway as well as composition and constitution of the product ion at m/z 282 as derived from the loss of 11 Da from m/z 293, was obtained by chemical syntheses including [a] an analog to compound 5 containing two deuterium atoms at the hydroxy residue between the carboxy function and the amide moiety of 5 and [b] the proposed molecule of m/z 282 (1-chloro-4-hydroxy-7-((2-propyl)oxy)-isoquinoline-3-carboxylic acid) with subsequent comparison of product ion mass spectra. As a conclusion, the elimination of methyleneimine, accompanied by the addition of water yielding the suggested structure of m/z 282, was in accordance with all experimental data. H/D exchange experiments, however, indicated the presence of two dissociation pathways yielding the same ion species at m/z 282 as well as another product ion with an identical m/z ratio but different structure. An additional and plausible fragmentation route yielding the proposed product ion at m/z 282 (1-chloro-4-hydroxy-7-((2-propyl)oxy)-isoquinoline-3-carboxylic acid) is in agreement with dissociation rearrangements observed with peptides and proteins [Scheme 3(b)]. Here, the electropositive carbonyl carbon of the amide bond is attacked by the oxygen atom of the carboxyl function followed by the release of aziridine (−57 Da). The occurrence of another ion species at m/z 282, as suggested in Scheme 3(c), was supported by ion mobility spectrometry analyses (Figure 3(b)). Using a Synapt HDMS instrument in the time-aligned parallel (TAP) fragmentation mode, two species of m/z 282 were separated and further subjected to dissociation within the transfer T-wave region, which yielded very similar product ion mass spectra of m/z 282 (Figure 3[b], bottom).

**GW1516**

The drug candidate GW1516 (Figure 1, 6, also referred to as GW501516) is being developed and tested for the treatment of dyslipidemia and metabolic syndrome in phase-II to -IV clinical trials.[^31,34,35] In animal studies, however, the potential of GW1516 to increase physical performance was observed when laboratory rodents were administered 2–5 mg kg[^−1] a day in conjunction with exercise stimuli;[^37,38] an overall improvement in endurance performance by approximately 70% was observed that was attributed to the induction of oxidative genes[^37] as well as a modified substrate preference of skeletal muscles.[^34] In January 2009, the list of prohibited substances and methods of doping was complemented by the PPARδ agonist GW1516, which has been classified as a gene doping substance by WADA.[^23] Hence, the mass spectrometric behavior of GW1516 was studied in detail using the ESI-MS/MS setup described for S-107 (vide supra) and major dissociation pathways were proposed as summarized below. Currently, very little data on the metabolism and renal elimination of GW1516 is available; however, the most frequently provided doping control samples are urine specimens and, thus, the detection of phase-I-metabolites of GW1516 has been of particular interest. Utilizing the characterized product ions of GW1516 as well as HCD combined with high-resolution/high-accuracy mass spectrometry, two phase-I metabolites were identified in an administration study urine sample.

**Dissociation routes from electrospray ionization tandem mass spectrometry**

The protonation of GW1516 (Figure 1, 6) is likely to occur at the thiazole residue of the molecule, which presumably retains the charge in most dissociation reactions. Consequently, CID of the protonated analyte m/z 454 yielded only few abundant or characteristic product ions at m/z 396, m/z 288, m/z 257, m/z 256 and m/z 188 (Figure 4(a)).[^21] The low intensity ions at m/z 396 and m/z 288 were attributed to the eliminations of oxiran-
Figure 3. (a) ESI product ion mass spectrum generated from the protonated molecule of $N$-(1-chloro-4-hydroxy-7-((2-propyl)oxy)isochinolin-3-yl)carbonylglycine (compound 5) $\text{[M + H]}^+$ = m/z 339 using an LTQ-Orbitrap mass spectrometer (Thermo, Bremen, Germany). An MS$^3$ experiment using m/z 293 as the precursor ion is shown as inset, which demonstrates the nominal loss of 11 Da to m/z 282. (b) Drift scope view of the ion mobility-TAP experiment of the ion at m/z 282, conducted on a Synapt HDMS instrument (Waters, Milford, MA, USA). Two species of ions are separated yielding two peaks (peak one: 0.65–0.90 ms, peak two: 0.90–2.75 ms) with the resulting product ion mass spectra of the two separated species of m/z 282.
2-one (–58 Da, –C₂H₃O₂), presumably originating from the acetic acid moiety and o-tolyloxy-acetic acid (–166 Da), respectively (Scheme 4). In contrast, the product ion at m/z 257 was of considerable abundance and was suggested to originate from a homolytic cleavage of the S–C bond and the loss of a (4-mercapto-2-methyl-phenoxy)-acetic acid radical (–197 Da). The conjugated π-electron system including the thiazole as well as the phenyl ring structures of GW1516 was considered to be the major driving force allowing for the generation of the radical cation at m/z 257 from an even-electron precursor ion (m/z 425). Subsequently, m/z 257 (assigned to the protonated 4,5-dimethyl-2-(4-trifluoromethyl-phenyl)-thiazole radical), released a hydrogen radical to form the ion at m/z 256 as well as a trifluoromethyl radical to yield the product ion at m/z 188 (Scheme 4).

Screening for urinary phase-I-metabolites

The characterized product ions of GW1516 were particularly useful when screening the administration study urine sample for the presence of metabolites employing HCD and high-resolution/high-accuracy MS. As illustrated in Figure 4(b), the extracted ion chromatograms [m/z 257.04–257.06, m/z 256.03–256.05, m/z 188.05–188.06] of a blank urine specimen compared with a sample collected 6 h post-administration of 2 mg of GW1516 considerably facilitated the detection of products derived from the ingested drug. Although virtually no signals were observed in the chromatograms of the blank urine, trace amounts of the intact substance were observed in post-administration samples at a chromatographic retention time of 8.8 min and potentially mono- and bis-oxygenated products were observed at 7.8 min and 8.0 min.
Figure 4. (a) ESI product ion mass spectrum generated from the protonated molecule of GW1516 (compound 6) [M + H]+ = m/z 454 using an LTQ-Orbitrap mass spectrometer (Thermo, Bremen, Germany). An MS3 experiment using m/z 257 as the precursor ion is shown as inset. (b) Extracted ion chromatograms of a blank urine specimen and a urine sample collected 6 h after oral administration of GW1516. The ion traces of m/z 257.04–257.06, m/z 256.03–256.05 and m/z 188.04–188.06 were extracted from HCD experiments at 50 V using a Thermo Exactive mass spectrometer (Bremen, Germany). (c) Product ion mass spectra of two potential metabolites of GW1516. Left: mono-oxygenated analyte, right: bis-oxygenated analyte, measured on an LTQ-ion trap (Thermo, Bremen, Germany).
respectively. The simultaneous recording of full MS data with and without CID in combination with high mass accuracy allowed the determination of elemental compositions of protonated molecules and product ions, the relations of which were further substantiated by more selective LTQ MS/MS experiments [Figure 4(c)]. The elemental compositions of the protonated mono- and bis-oxygenated metabolites were determined with \( \text{C}_{21}\text{H}_{19}\text{O}_4\text{N}_3\text{S}_2 \) (error = –2.3 ppm) and \( \text{C}_{21}\text{H}_{19}\text{O}_5\text{N}_3\text{S}_2 \) (error = –2.6 ppm), respectively and MS/MS experiments of precursor ions yielded typical dissociation pathways producing the diagnostic ion at \( m/z \) 257. Due to the presence of the ions at \( m/z \) 257 and \( m/z \) 188, the oxygenation of GW1516 is proposed to be located at the thiobenzene residue, including the privileged sulfur atom; however, detailed structures still need to be elucidated.

Conclusions

The constantly increasing number of new therapeutics continuously adds new options for cheating athletes to misuse the beneficial effects of such drugs to artificially increase their performance. The early characterization of drug candidates using mass spectrometry-based approaches and the subsequent, proactive implementation of compounds into routine doping control procedures considerably reduces the time period that these agents, if misused, might be undetected. Due to limited availability of information regarding the renal elimination and metabolism of new drug entities, diagnostic product ions resembling conserved and characteristic regions of target molecules are of great interest in sports drug testing and allow comprehensive screening analyses, even for compounds with unknown metabolism as demonstrated, for instance, with GW1516.

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