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RESEARCH ARTICLE

Identification of hypoxene metabolites in urine samples using gas chromatography–tandem mass spectrometry for anti-doping control

Pavel V. Postnikov[1,✉], Andrey V. Polosin[1], Nadezhda B. Savelieva[1], Sergey A. Kurbatkin[2], Yulia A. Efimova[2], Elena S. Mochalova[1]

[1] *National Anti-Doping Laboratory (Institute), M.V. Lomonosov Moscow State University (NADL MSU), Moscow, 105005 Russia*

[2] *MIREA – Russian Technological University (M.V. Lomonosov Institute of Fine Chemical Technologies), Moscow, 119571 Russia*

✉ *Corresponding author, e-mail: pletneva@mirea.ru*

Abstract

Objectives. Hypoxen is a drug which possesses antioxidant and antihypoxic effects. It achieves this by increasing the utilization of oxygen by mitochondria, intensifying oxidative phosphorylation, and as a result, improving tissue respiration. Athletes take it during prolonged exercise, in order to increase efficiency and reduce physical overwork. Since 2023, the World Anti-Doping Agency has included the drug in the monitoring program, in the belief that it can be used to gain a competitive advantage. It is thus a candidate for inclusion in the Prohibited List as a potential regulator of the human metabolism. Currently, there are no studies or scientific publications focusing on the identification of hypoxene in biofluids for the purpose of anti-doping control. The aim of this study is to determine the possible metabolites of the drug and their chromato-mass spectrometric characteristics in urine samples using gas chromatography–tandem mass spectrometry (GC–MS/MS) for doping control screening purposes.

Methods. Sample preparation of urine samples was carried out using enzymatic hydrolysis, liquid–liquid extraction and derivatization. The GC–MS/MS method was used for analysis. Screening of hypoxene metabolites was carried out in the mode of total ion current after fragmentation of selected parent ions.

Results. Three specific metabolites of hypoxene (*m*/*z* 342, 300, and 346, including trimethylsilyl derivatives) were identified in urine samples of volunteers ($n = 3$). They can act as markers for taking the target antihypoxant, and their possible structural formulas are given. The excretion curves of two metabolites with an *m*/*z* of 300 and 346 respectively in urine were studied. The maximum concentration is reached after 8–14 and 1.5–6 h, respectively. It was established, that these metabolites are reliably identified in urine 90 h or more after a single dose of the drug.

Conclusions. Possible structures of hypoxene metabolites in urine samples from volunteers were determined for the first time and their chromato-mass spectrometric characteristics were established. The approach developed in this study can be used for screening analysis of hypoxene for the purpose of anti-doping control.

Keywords

hypoxen, metabolites, antihypoxant, antidoping control,
gas chromatography–tandem mass-spectrometry (GC–MS/MS)

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НАУЧНАЯ СТАТЬЯ

Идентификация метаболитов гипоксена в образцах мочи методом газовой хроматографии – тандемной масс-спектрометрии с целью антидопингового контроля

П.В. Постников[1, ✉], А.В. Полосин[1], Н.Б. Савельева[1], С.А. Курбаткин[2], Ю.А. Ефимова[2], Е.С. Мочалова[1]

[1] *Национальная антидопинговая лаборатория (Институт), Московский государственный университет им. М.В. Ломоносова (НАДЛ МГУ), Москва, 105005 Россия*

[2] *МИРЭА – Российский технологических университет (Институт тонких химических технологий им. М.В. Ломоносова), Москва, 119571 Россия*

✉ *Автор для переписки, e-mail: drpavelpostnikov@gmail.com*

Аннотация

Цели. Гипоксен — лекарственный препарат, обладающий антиоксидантным и антигипоксическим эффектами за счет увеличения утилизации митохондриями кислорода, интенсификации окислительного фосфорилирования и, как следствие, улучшения тканевого дыхания. Спортсмены принимают его при длительных нагрузках для увеличения работоспособности и уменьшения физического переутомления. Всемирное антидопинговое агентство с 2023 г. внесло препарат в мониторинговую программу на основании того, что он является потенциальным регулятором метаболизма, т.е. может использоваться для получения конкурентного преимущества и быть претендентом на включение в Запрещенный список. В настоящее время отсутствуют какие-либо исследования и научные публикации по идентификации гипоксена в биожидкостях для антидопингового контроля. В связи с этим, целью работы было определение возможных метаболитов препарата и их хромато-масс спектрометрических характеристик в образцах мочи методом газовой хроматографии – тандемной масс-спектрометрии (ГХ–МС/МС) для скрининг-процедуры допинг-контроля.

Методы. Пробоподготовку образцов мочи проводили с применением ферментативного гидролиза, жидкость-жидкостной экстракции и дериватизации. Для анализа использовали метод ГХ–МС/МС. Скрининг метаболитов гипоксена осуществлялся в режиме полного ионного тока после фрагментации выбранных парент-ионов.

Результаты. В образцах мочи добровольцев ($n = 3$) идентифицированы три специфичных метаболита гипоксена (m/z 342, 300 и 346, включая триметилсилил-производные), которые могут выступать в качестве маркеров приема целевого антигипоксанта; приведены их возможные структурные формулы. Изучены кривые выведения двух метаболитов с m/z 300 и m/z 346 с мочой, максимальная концентрация которых достигается спустя 8–14 и 1.5–6 ч соответственно. Установлено, что данные метаболиты надежно идентифицируются в моче спустя 90 ч и более после однократного приема препарата.

Выводы. Впервые определены возможные структуры метаболитов гипоксена в образцах мочи добровольцев и установлены их хромато-масс-спектрометрические характеристики. Разработанный подход может быть применен для скринингового анализа с целью антидопингового контроля.

Ключевые слова

гипоксен, метаболиты, антигипоксант, допинг-контроль, газовая хроматография/тандемная масс-спектрометрия

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INTRODUCTION

Hypoxen (sodium polydihydroxyphenylene thiosulfonate) is a group of synthetic quinone derivatives with a pronounced antihypoxic effect. The drug was created by Soviet scientists in the 1970s, and has been authorized for medical use since 1997 under the name *Olyphenum* [1]. The mechanism of its action is not fully understood, but an important role in this mechanism may be played by the following factors: a reduction of the damaging effect of reactive oxygen species on biomembranes; a reduction of lactate acidosis concentration in hypoxia conditions due to correction of the disturbed electrotransport function of mitochondrial enzyme complex (MEC-1) [1] and a reduction of calcium load in mitochondria. Hypoxene is able to lay an additional channel bypassing the damaged first and second complexes of the mitochondrial respiratory chain, which contributes to the restoration of the formation of adenosine triphosphate and creatine phosphate, disturbed by a variety of factors [1]. In addition to the antihypoxic effect, the drug has pronounced pro- and antioxidant properties [2–3]. Thus, it can activate adrenaline autooxidation by 213%[1] and also suppresses the formation of superoxide anion O^{2-}. Its antioxidant properties are due to the fact that hydroxyl groups of polyhydrophenylene structure easily give up the hydrogen atom and can bind free radicals.

The clinical efficacy of the drug has been demonstrated in many scientific studies [4–8]. It is used in complex treatment and in the recovery period after pneumonias and acute bronchitis. It can also be used in alcohol intoxication [7], for the prevention of heart attacks, angina pectoris and strokes [4], in increased physical exertion and hypoxia, and it has a gastroprotective effect [8]. Hypoxene has been used in pharmacological training of athletes for more than a decade as a means of increasing the body's resistance to hypoxia and increasing performance by restoring the disturbed processes of adenosine triphosphoric acid formation [9]. Nevertheless, certain aspects of its effect on a number of body systems of athletes remain unexplored.

As of 2023, the World Anti-Doping Agency (WADA) has placed hypoxene on the monitoring program as a potential metabolic regulator with the wording "to assess inappropriate in- and out-of-competition use in sport"[2]. The drug may end up on the Prohibited List[3] as early as 2025. In this regard, the anti-doping community is faced with the task of its identification in biological fluids. There is no information in scientific literature regarding the identification of the drug for the purpose of anti-doping control in human biofluids, or on the study of its metabolism and pharmacokinetics. The only information given concerns the confirmation of the synthesized structure by matrix activated laser desorption/ionization (MALDI) [10], electron paramagnetic resonance and IR spectroscopy [10, 11]. For the first time, our article proposes an analytical approach based on the gas chromatography-triple quadrupole tandem mass spectrometry (GC–MS/MS) method for the detection of possible metabolites of hypoxene in urine samples of volunteers for the purpose of anti-doping control.

EXPERIMENTAL

Reagents and objects of analysis

For the experiments we used hypoxene, purchased in the pharmacy network, produced by the *Olifen Corporation* (Moscow, Russia), and urine samples of several volunteers (n = 3) who had not previously taken hypoxene, quinone derivatives or any dietary supplements at the age of 35 ± 7 years. The volunteers' gender was not taken into account. The drug is sold through pharmacy chains and is authorized for use as an over-the-counter product. In order to search for possible metabolites, volunteers took the drug according to the following scheme: Day 1 — 5 capsules, Day 2 — 6 capsules, Day 3 — 7 capsules, at the same time in the afternoon, having previously passed a blank urine before the course of intake. Urine samples were collected in sterile 80 mL urine containers, labeled, stored at +4°C and analyzed the next day.

To study the excretion of hypoxene, three other volunteers took a single therapeutic dose of the drug (8 capsules), after which urine samples were collected daily for a period of 7 days according to the following scheme: on the first two days every 3–5 h, the following days—once in the morning on an empty stomach. Samples with the date and time of collection were also stored at +4°C or frozen at −20°C until sample

[1] Study of the mechanism of antihypoxic action of artificial quinone derivatives. Grant No. 04-04-97279. 2004. URL: https://www.elibrary.ru/item.asp?edn=cwevtp. Accessed January 21, 2024.

[2] The 2024 Monitoring Program. URL: https://www.wada-ama.org/sites/default/files/2023-09/2024_list_monitoring_program_en_final_22_september_2023.pdf. Accessed January 29, 2024.

[3] The 2024 List of prohibited substances. URL: http://rusada.ru/upload/iblock/836/drtkaf3eckdo1jrnjxacdwqkbm054m1n/Запрещенный%20список%202024%20(1).pdf. Accessed January 29, 2024.

preparation. The work was performed in accordance with the WADA Code of Ethics (WADA Code of Ethics)[4]: written permission was obtained from volunteers to use their biological material for research.

As an internal standard, 17α-methyltestosterone (certified standard, 1 mg/mL solution, *NMI*, Australia) was used. Also used in the experiment were diethyl ether and *n*-pentane from *JT Baker* (Netherlands); anhydrous sodium sulfate, carbonate, potassium hydrogen carbonate, DL-dithiothreitol, ammonium iodide, potassium dihydrophosphate, sodium phosphate bivalent dihydrate, sodium azide from *Sigma-Aldrich* (USA); compressed argon 5.0 with a purity of at least 99.999% and helium compressed 6.0 with a purity of at least 99.999% (Russia). *N*-methyl-*N*-(trimethyl)trifluoroacetamide from *Macherey-Nagel* (Germany) was used for derivatization. For hydrolysis, β-glucuronidase from *E. Coli* K12 (*Roche Diagnostics*, Germany). Deionized water, with a specific resistance of 18.2 MOhm·cm, was used for preparation of buffer solutions.

Auxiliary equipment

Solid-state heater with programmable temperature (*Thermo*, USA); crimper, decapper, polypropylene vials with silanized inserts of 0.2 mL (*Macherey-Nagel*, Duren, Germany); automatic variable volume pipettes 500–5000 µL, 10–200 µL (*Eppendorf*, Germany) and tips for them; tabletop centrifuge with horizontal rotor Rotixa 50 RS (*Hettich*, Germany); automatic shaker; OHaus Discovery DV215CD analytical scales (5 digit accuracy) (*OHaus Corp*, Switzerland); glass tubes with screw caps 16 × 125 mm; Vortex liquid shaker (*Scientific industries Inc.*, USA); low-temperature liquid thermostat (−30 ± 5°C) (*Grant Instruments*, United Kingdom); incubator thermostat (55 ± 3°C) (*Binder*, Germany); HP Ultra-1 gas chromatography column 17 m × 0.2 mm, 0.11 µm (*Agilent*, USA).

Sample preparation

For the sample preparation of urine samples of the volunteers before (negative control) and after drug administration, 16 mL glass tubes were used and 3 mL urine samples were taken for analysis [10]. A buffer mixture of 1 mL for hydrolysis was added to each tube. The buffer mixture for hydrolysis was prepared as follows: 54 g Na_2HPO_4-$2H_2O$, 68 g K_2HPO_4 and 1 g sodium azide were brought to 1000 mL with deionized water (pH 6.2–6.5). Next, the contents of 2 vials of β-glucuronidase (2 × 15 mL) were transferred into a 1000 mL measuring flask, 150 µL of 1-mg/mL methyltestosterone solution (internal standard) was added. Then a freshly prepared phosphate buffer solution was brought to the mark. The tubes were then shaken on a Vortex type apparatus and incubated for 60 ± 10 min in a thermostat at 55 ± 3°C. After that, 1–2 g of anhydrous sodium sulfate was added to each tube, shaken for 10 s, 1 mL of carbonate buffer solution was added, and shaken again for 5–10 s. The carbonate buffer solution was added to each tube (carbonate buffer solution was prepared as follows: 60 g each of K_2CO_3 and $KHCO_3$ were weighed and the solution was brought to 800 mL with deionized water (pH 9.6–9.9). The resulting solution was stored in a dark glass bottle). After cooling, 5 mL of diethyl ether (or *n*-pentane) was added and stirred for 20 ± 5 min on a rotary shaker for liquid–liquid extraction. The solution was centrifuged for 3–4 min at 3000 rpm and the tubes were placed in a low-temperature liquid thermostat. The organic solvent was then transferred to derivatization tubes and evaporated to dryness at 70 ± 5°C for 20–30 min. Derivatization reagent of 50 µL was added to each tube and heated at 70 ± 5°C for 25 min. Subsequently, the reaction mixture was cooled and transferred to vials with silanized glass inserts, tightly closed and analyzed.

Parameters of instrumental analysis by gas chromatography–triple quadrupole tandem mass spectrometry

A GC–MS/MS analysis was performed using a Trace 1310 gas chromatograph coupled to a TSQ Quantum XLS triple quadrupole mass spectrometer and a TriPlus RSH autosampler (*Thermo Fisher Scientific*, USA). The chromatographic column was Agilent HP Ultra 1 (20 m × 0.18 mm, film thickness 0.18 µm). The carrier gas was helium (purity 6.0), flow rate 1.1 mL/min. The injector temperature and at the interface line device was 280°C, and the ion source (Trace GC Ultra) temperature was 270°C. The interface temperature is 300°C. Sample injection was carried out in flow division mode (1 : 20), and the volume of injected sample was 2 µL. Temperature program: the initial starting temperature is 179°C; next rise to 235°C at a rate of 4°C/min and rise to 310°C at a rate of 20°C/min; delay time is 4.25 min. The signal-to-noise (S/N) ratio was considered to be 3 : 1. Mass spectrometer: onset of ion current registration is 2.9 min; emission current 35–100 µA; scan rate 3.3 scans/min; and target gas—argon with

[4] WADA Code of Ethics. URL: https://www.wada-ama.org/sites/default/files/2022-01/wada_code_of_ethics_nov_2021_final.pdf. Accessed February 02, 2024.

the pressure 1 mTorr. Ion transmission width at the first and third quadrupoles is 0.7. Metabolites were detected in the scanning mode of total ion current from specific precursor ions.

RESULTS AND DISCUSSION

According to the information given in [13], hypoxene (*Olyphenum*) may consist of a polymer chain of hydroquinone links (2 to 6 links) linked covalently with each other in meta-positions, and is the sodium salt of poly-(2,5-dihydroxyphenylene)-4-thiosulfonic acid. It has been previously shown [10] that there may be more hydroquinone links in such compounds, and its molecular mass ranges from 352 to 784 g/mol. The structural formula of the active substance of the medicinal product is presented in Fig. 1.

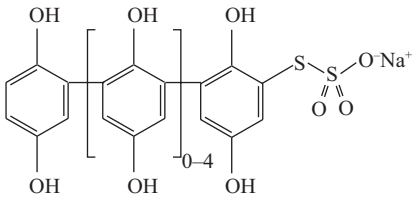

Fig. 1. Structural formula of hypoxene

As stated earlier, there is no information or scientific publications in the public domain about possible metabolites of the drug and their identification in human biological fluids. We compared for the first time chromatograms of urine samples of volunteers before and after administration of maximum daily doses of hypoxen. As a result, 4 compounds were detected (Figs. 2–5), three of which are absent in blank urine and may be specific metabolites of sodium hypoxene (polydihydroxyphenylene thiosulfonate) ingestion (indicated by red arrows in Figs. 3–5). The intensity of the peaks of these substances correlates with the doses of the drug taken.

The two substances found with retention times of 5.27 min (*m*/*z* 254) and 5.44 min (*m*/*z* 182) correspond to trimethylsilyl derivatives (TMS-derivatives) of hydroquinone. However, they cannot be used for doping control purposes, as hydroquinone is naturally present in the body and is used in food production as an antioxidant.

After appropriate sample preparation, TMS-derivatives of hydroquinone were detected both in the blank urine of volunteers and in the aqueous solution of the drug and urine samples after its administration. In addition, hydroquinone itself can presumably also act as a byproduct in the synthesis of the drug.

Three other substances with retention times of 8.99, 9.55, and 13.41 min were not detected in blank urine, although they were present in the aqueous solution of the drug and samples after administration of different doses of the drug. Thus, they can be deemed substances of exogenous origin. Figures 3–5 compares the chromatogram sections of blank urine samples and samples after hypoxene administration. They show the mass spectra of possible substances-metabolites of hypoxene which can be used as markers of antihypoxant administration. No interfering peaks were observed when comparing the chromatograms of urine samples from volunteers before and after drug administration. In order to confirm the exogenous origin of each of these substances, a sample preparation of a solution of hypoxene in water was performed. We assumed that three ions determined in the full ion current scan mode from specific precursor ions, an *m*/*z* of 300, 342, and 346, could be characteristic for the determination of drug metabolites (Figs. 3c, 4c, and 5c). The inferred fragmentation patterns of these ions are shown in Fig. 6. Thus, the substance with an *m*/*z* of 342 may contain three hydroquinone fragments in its structure, and the thiosulfonate group may be replaced by –OH (Fig. 6a), corresponding to the information given [10]. In addition, the reason for this substitution may be the instability of the latter group to heating and the use of alkaline carbonate buffer with pH 9.6–9.9 during sample preparation. Also, this substance, presumably, can be formed as a byproduct during the synthesis of the drug.

Closest in structure to the metabolite of hypoxene is a substance with an *m*/*z* of 346 (Fig. 5). It is a silylated derivative of sodium dihydroxyphenylene thiosulfonate consisting of two hydroquinone links.

Also, one of the metabolites (*m*/*z* 300) may contain a mercapto group instead of thiosulfonate (Fig. 6b).

In general, the presence of closely related structures in urine samples of volunteers after administration of the drug with or without any of the –SH, –OH, –S–SO_2–ONa, or –CH_3 functional groups cannot be ruled out (Fig. 6).

When studying the excretion of the drug from the body, it was found that hypoxene is a mixture of homologs with a different number of hydroquinone links in their structure. The composition of the drug is heterogeneous and may differ from series to series. Thus, the metabolite with an *m*/*z* of 342 was present in urine samples of all volunteers taking the drug of series 170522 (Serial number Y9aYRMYBoBe6c), but was absent after taking series 220622 (Serial number ApfKe3z2WOmo3). The presence of metabolites with an *m*/*z* of 300 and 346 respectively was confirmed in urine samples after ingestion of both the above mentioned hypoxene series.

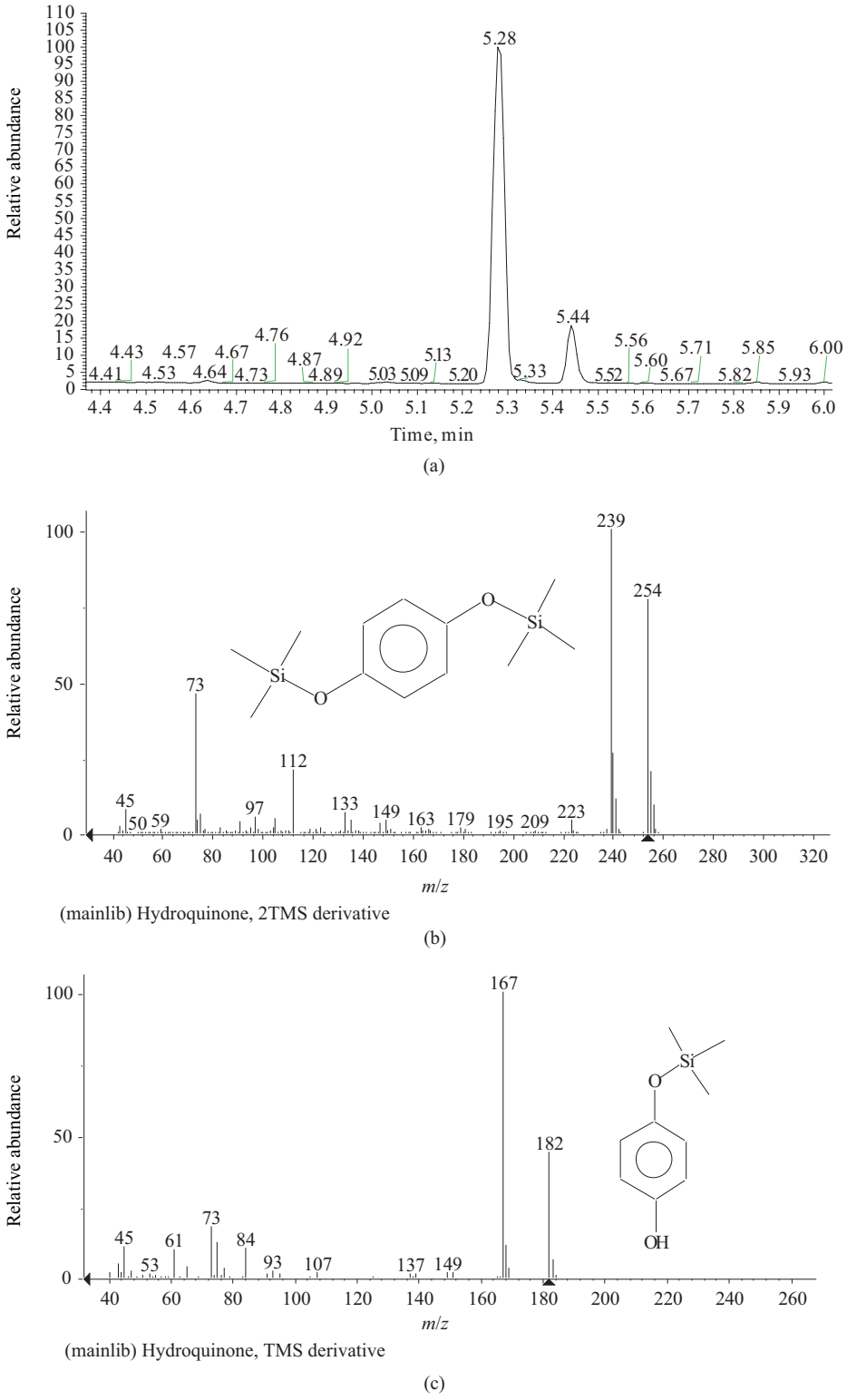

Fig. 2. Chromatogram (a) and mass spectra (b, c) of TMS derivatives of hydroquinone

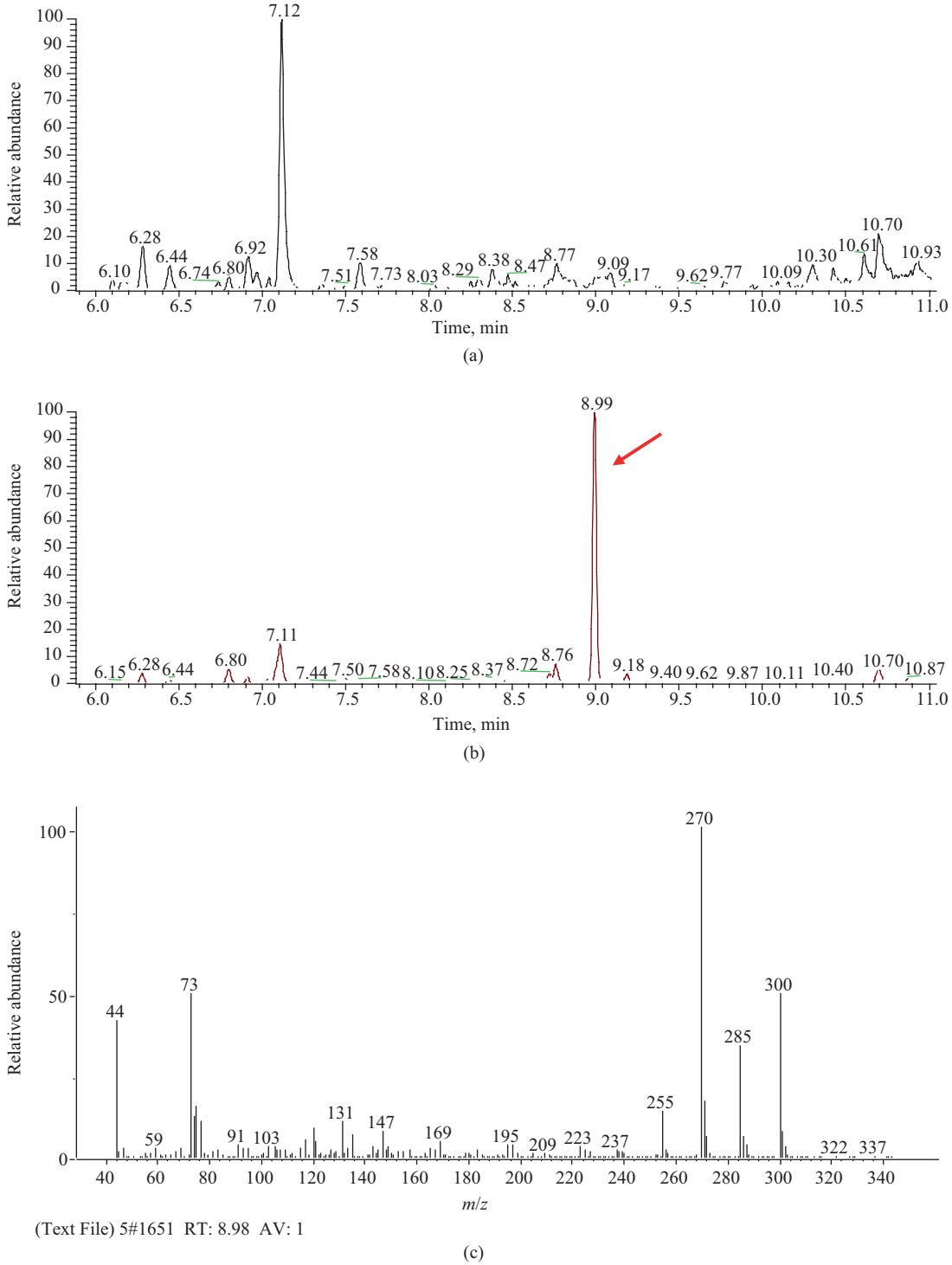

Fig. 3. Chromatogram of a urine sample of a volunteer before (a), after (b) drug administration and mass spectrum (c) of the detected substance with a retention time of 8.99 min. The red arrow indicates the peak of hypoxene metabolite.

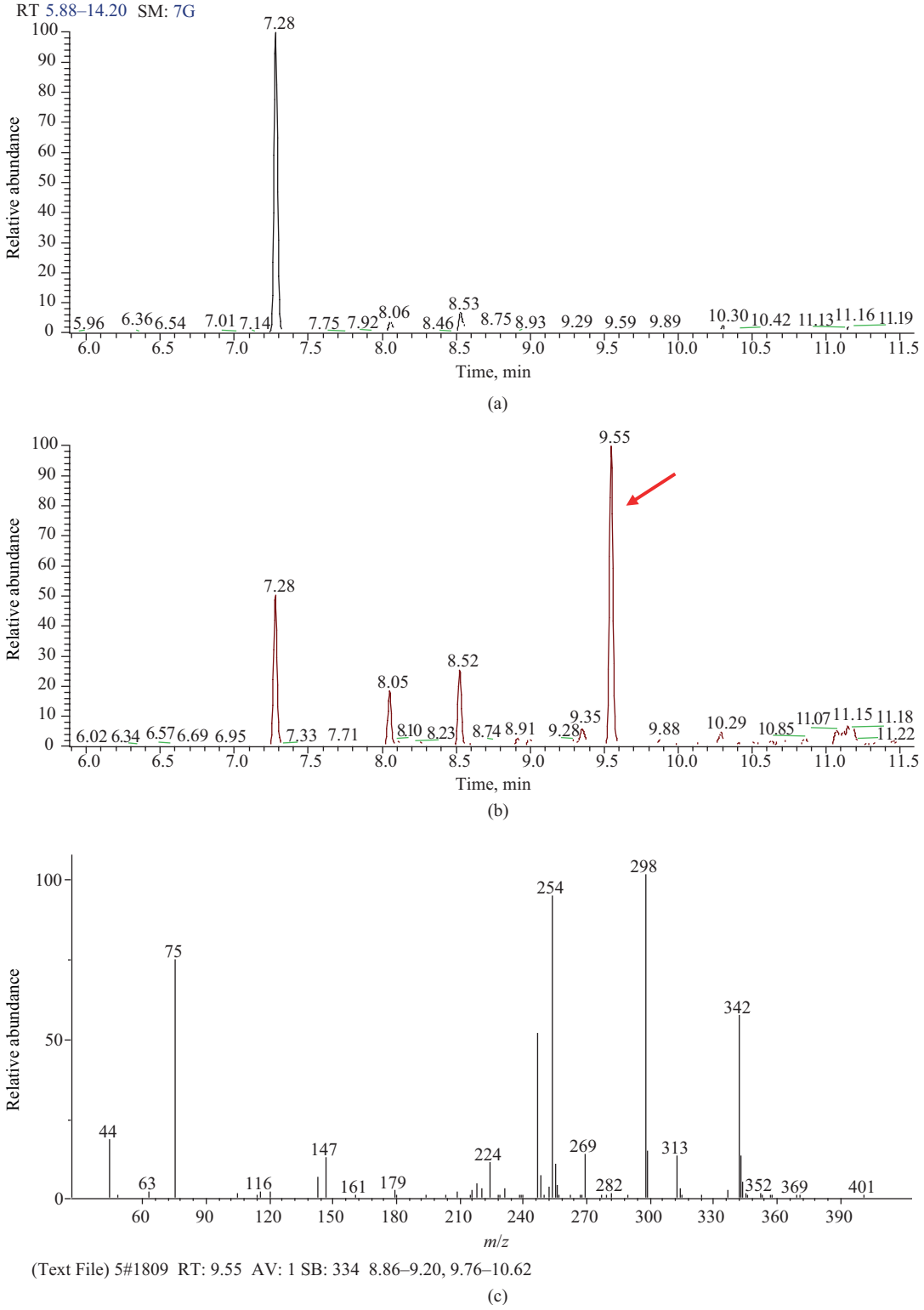

Fig. 4. Chromatogram of a urine sample of a volunteer before (a), after (b) drug administration and mass spectrum (c) of the detected substance with a retention time of 9.55 min. The red arrow indicates the peak of hypoxene metabolite

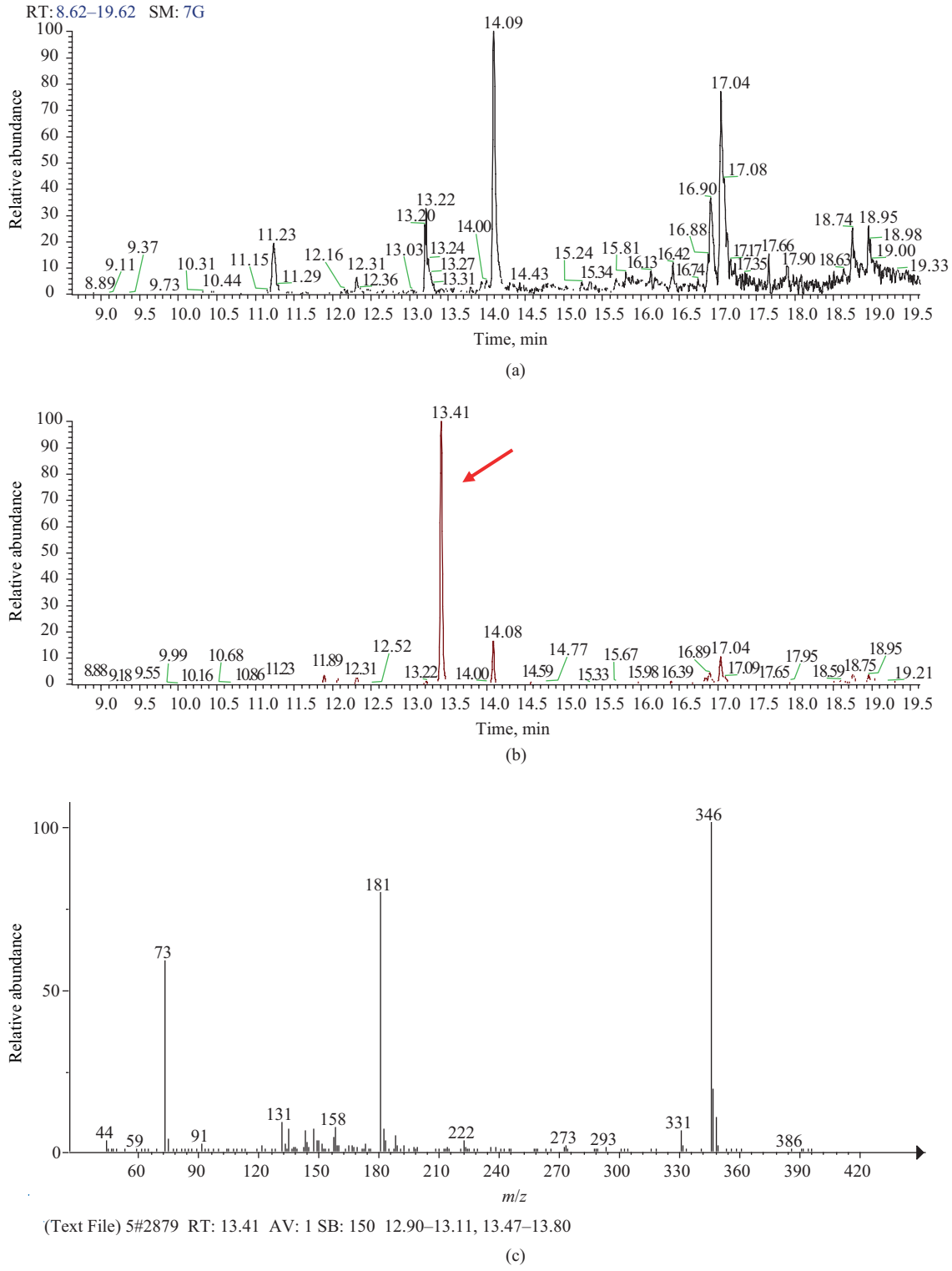

Fig. 5. Chromatogram of a urine sample of a volunteer before (a), after (b) drug administration and mass spectrum (c) of the detected substance with a retention time 13.41 min. The red arrow indicates the peak of hypoxene metabolite

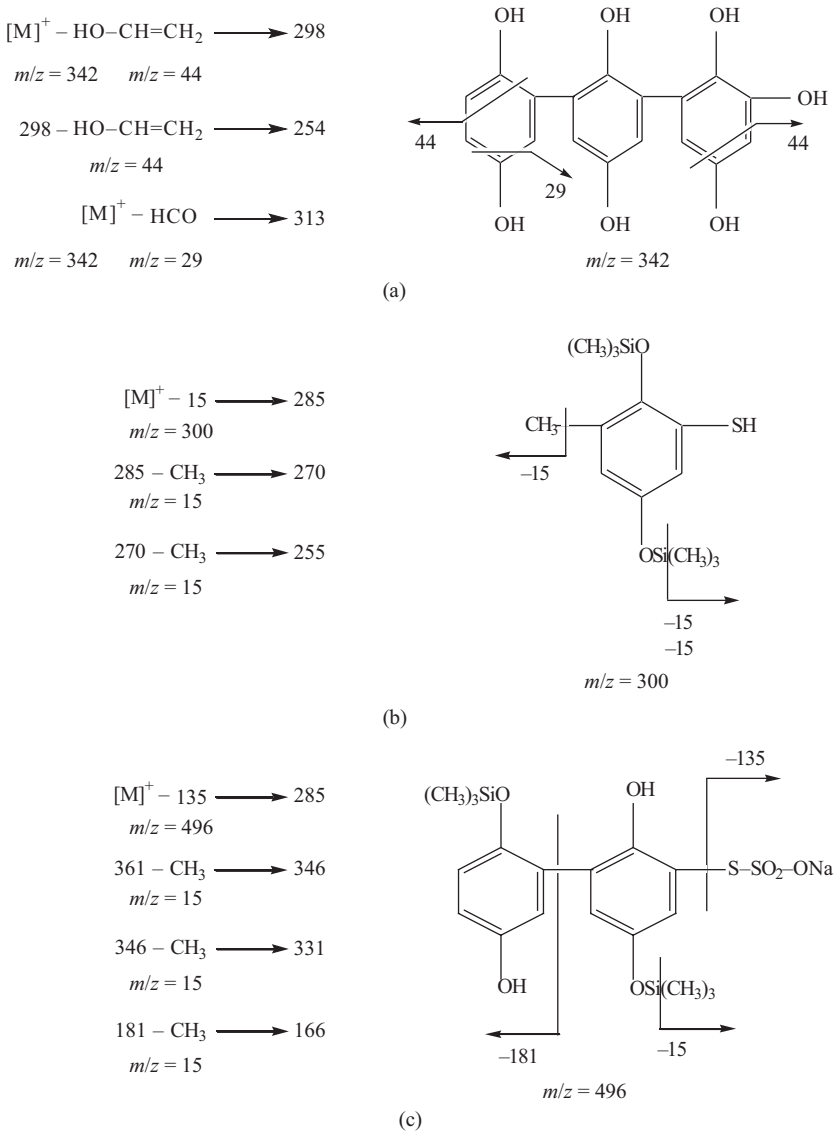

Fig. 6. Proposed fragmentation patterns of ions with *m/z* of 342 (a), 300 (b), and 346 (c) and proposed structural formulas of hypoxene metabolites (including TMS derivatives)

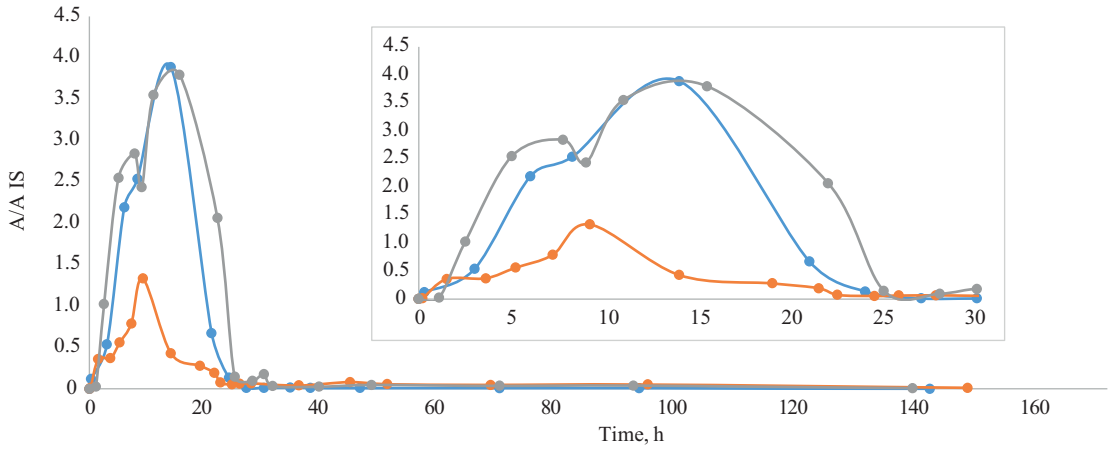

Fig. 7. Urinary excretion curves of hypoxene metabolite with an *m/z* of 300 for three volunteers. The first 30 h after a single administration of hypoxene (2 g, 8 capsules) are plotted separately. A/A IS is the ratio of the substance peak area to the internal standard peak area

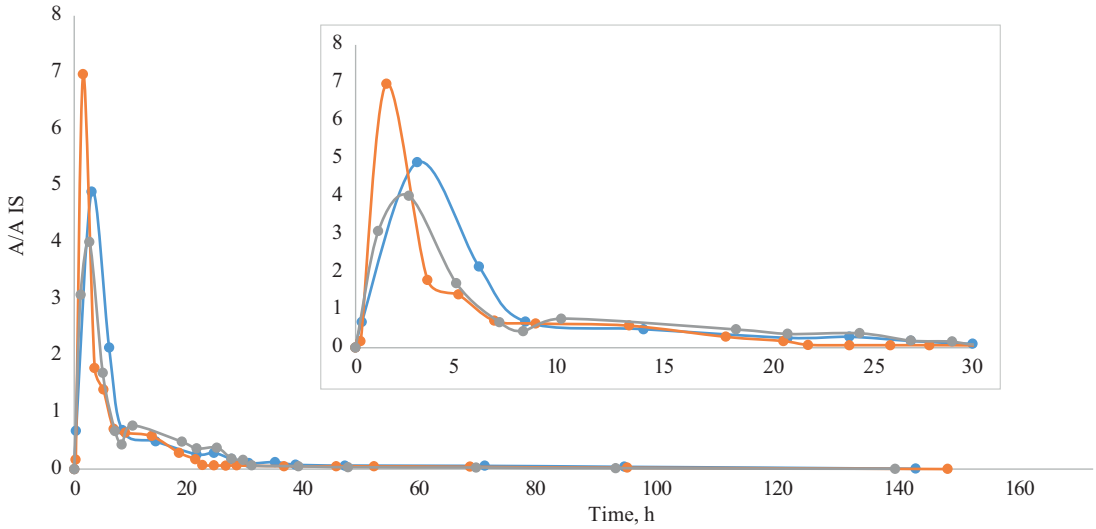

Fig. 8. Urinary excretion curves of hypoxene metabolite with *m*/*z* of 346 in three volunteers. The first 30 h after a single administration of hypoxene (2 g, 8 capsules) are plotted separately. A/A IS is the ratio of the peak area of the substance to the peak area of the internal standard

Figures 7 and 8 show the excretion curves of metabolites with an *m*/*z* of 300 and 346 of hypoxene.

The above data shows that for the metabolite with an *m*/*z* of 300, the maximum concentration in the urine of volunteers is reached 8–14 h after the drug administration (8 capsules once). Moreover, even after 90 h or more, this metabolite can be reliably detected. However, in a urine sample taken 2 weeks after a single dose of hypoxene, it is no longer present (data not shown). For the metabolite with an *m*/*z* of 346, the maximum concentration in urine can be determined as early as 1.5–6.0 h after administration, and it is reliably detected in urine more than 90 h after administration. However, it too cannot be identified after 2 weeks or more (data not shown). It is possible that hypoxene has a cumulative effect, and if multiple doses or courses of administration are administered, its excretion time may be significantly increased. However, this is an area for further investigation. When taking a single dose, the volunteers did not feel any sudden onset of strength, vigor or other effect. One experienced slight dizziness which passed after a few hours.

The main candidates for inclusion in the WADA banned list are primarily substances from the monitoring program. Hypoxene, which has been included in this program since 2023, may be banned as early as 2025. This is similar to meldonium which was previously included in the training regimens of Russian and CIS[5] athletes. This sulfur-containing oligoquinone can be classified under Item 4 “Metabolic Modulators” of Article S4 “Hormones and Metabolic Modulators,” including AMP[6]-activated protein kinase activators, mildronate and trimetazidine, which are antihypoxants of direct energizing action. Post-load reoxygenation due to increasing oxidative stress is a major component of post-hypoxic cell damage [11, 12]. In view of this, various antioxidants, including hypoxene, are actively used for antihypoxic therapy by activating oxidative phosphorylation and reducing the formation of reactive oxygen species. As mentioned earlier, hypoxene is used in sports medicine to restore the body after prolonged physical exertion and increase performance capacity.

Murzaeva *et al.* [13] conducted experiments on the effect of hypoxene on the bioenergetic processes in rat liver and heart mitochondria. They concluded that in the concentration of 0.05–10 μg/mL the drug increases the conjugation of respiratory chain work, stimulates respiration and partially reduces the accumulation of H_2O_2. Ignatiev *et al.* [14] studied the effect of this polyquinone on gas exchange parameters, exercise tolerance, and dyspnea severity in chronic obstructive pulmonary disease of moderate and severe course. Clinically significant changes were obtained in the assessment of dyspnea severity. The number of patients with clinically significant improvement reached 46% after a 6-month course of hypoxene administration. The authors also stress that on-demand salbutamol use decreased by 0.5 doses per patient during hypoxene when

5 CIS— Commonwealth of Independent States.

6 AMP—adenosine monophosphate.

compared with data at the start of therapy. Improvement of patients' exercise tolerance was also noted: saturation increased and recovery period was shortened.

CONCLUSIONS

During the first pilot studies on the identification of possible metabolites of hypoxene by GC–MS/MS method, we identified 5 substances, 3 of which are specific for the drug administration and can indicate its presence in biological fluids of volunteers (*m*/*z* 300, 342, and 346). It was found that hypoxene is a mixture of homologues with a differing number of hydroquinone links in its structure. The composition of the drug is heterogeneous and may differ from series to series. The article presents structural formulas and fragmentation schemes of metabolites, two of which (with an *m*/*z* of 300 and 346, respectively) can be unambiguously identified from series to series. Their excretion curves with urine are given. It was found that the maximum concentration of these metabolites in urine is reached after 8–14 h and 1.5–6 h, respectively. It was also observed that they were reliably detected by GC–MS/MS for a period of more than 90 h after a single dose of 2 g (8 capsules). Hydroquinone derivatives are present in the urine of almost every human being. Therefore, for the purposes of doping control, the monitored substance needs to be distinguished from substances with a similar structure, possibly ingested with food. At the moment, the selection of optimal selective reaction monitoring transitions is underway to determine hypoxene metabolites and obtain more accurate data on the time of their elimination from the body.

The search for markers of the presence of new substances from the WADA monitoring program has undoubted practical significance for the purposes of modern anti-doping control, since hypoxene is a potential candidate for inclusion in the Prohibited List in 2025, and the methods of its determination and identification criteria have not yet been developed.

Authors' contributions

P.V. Postnikov—writing the text of the article, formulation of aims and objectives, development of a plan for conducting experiments, conducting experimental research, discussion of experiments and results, editing the manuscript, editing the final version of the article, and preparing materials for publication.

A.V. Polosin—conducting experimental research, discussion of experiments and results.

N.B. Savelieva—discussion of experiments and results, preparing materials for publication.

S.A. Kurbatkin—preparing materials for publication.

Yu.A. Efimova—editing the manuscript and preparing materials for publication.

E.S. Mochalova—preparing materials for publication.

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REFERENCES

1. Levchenkova O.S., Novikov V.E., Pozhilova E.V. Pharmacodynamics of antihypoxants and their clinical use. *Obzory po klinicheskoi farmakologii i lekarstvennoi terapii = Reviews on Clinical Pharmacology and Drug Therapy.* 2012;10(3):3–12 (in Russ.). https://doi.org/10.17816/RCF1033-12
2. Shatalin Yu.V., Naumov A.A., Potselueva M.M. A comparison of antioxidant properties of hypoxen and duroquinone by the method of chemiluminescence. *Biofizika.* 2008;53(1):100–106 (in Russ.).
3. Murzaeva S.V., Belova S.P., Mironova G.D. Determination of the antioxidant properties of activators of mitochondrial ATP-dependent potassium channels with the Amplex Red fluorescent indicator. *Appl. Biochem. Microbiol*. 2013;49(4):333–340. https://doi.org/10.1134/S0003683813040108 [Original Russian Text: Murzaeva S.V., Belova S.P., Mironova G.D. Determination of the antioxidant properties of activators of mitochondrial ATP-dependent potassium channels with the Amplex Red fluorescent indicator. *Prikladnaya biokhimiya i mikrobiologiya.* 2013;49(4):345–352 (in Russ.). https://doi.org/10.7868/s0555109913040107]
4. Semigolovskii N.Yu. Use of antihypoxants in the acute period of myocardial infarction. *Anesteziol. Reanimatol.* 1998;(2):56–59 (in Russ.).

СПИСОК ЛИТЕРАТУРЫ

1. Левченкова О.С., Новиков В.Е., Пожилова Е.В. Фармакодинамика и клиническое применение антигипоксантов. *Обзоры по клинической фармакологии и лекарственной терапии.* 2012;10(3):3–12. https://doi.org/10.17816/RCF1033-12
2. Шаталин Ю.В., Наумов А.А., Поцелуева М.М. Сравнительная характеристика антиоксидантных свойств гипоксена и дурохинона методом хемилюминисценции. *Биофизика*. 2008;53(1):100–106.
3. Мурзаева С.В., Белова С.П., Миронова Г.Д. Определение антиоксидантных свойств активаторов митохондриального АТФ-зависимого калиевого канала с помощью флуоресцентного индикатора Amplex Red. *Прикладная биохимия и микробиология*. 2013;49(4):345–352. https://doi.org/10.7868/s0555109913040107
4. Семиголовский Н.Ю. Применение антигипоксантов в остром периоде инфаркта миокарда. *Анестезиология и реаниматология*. 1998;(2):56–59.
5. Иванова Л.А., Павлова М.В., Арчакова Л.И. Антиоксиданты в комбинированной терапии больных хроническим деструктивным туберкулезом легких. *Терапевтический архив*. 1994;66(11):54–56.
6. Ганапольский В.П., Шабанов П.Д. Метеоадаптогенные свойства антигипоксантов. *Экспериментальная и клиническая фармакология*. 2009;72(6):36–41.

5. Ivanova L.A., Pavlova M.V., Archakova L.I. Antioxidants in the combined therapy of patients with chronic destructive pulmonary tuberculosis. *Therapeutic Archive.* 1994;66(11):54–56 (in Russ.).
6. Ganapolskii V.P., Shabanov P.D. Meteoadaptogenic properties of antihypoxic drugs. *Eksperimental'naya i Klinicheskaya Farmakologiya.* 2009;72(6):36–41 (in Russ.).
7. Dolgareva S.A., Sorokin A.V., Konoplya N.A., Bushmina O.N., Bystrova N.A., Ovod A.I. The use of immunomodulators, antioxidants and hepatoprotectors for the correction of the liver, erythrocites and the immune system disorders in chronic ethanol intoxication. *Biomed. Khim.* 2018;64(4):360–367 (in Russ.). https://doi.org/10.18097/pbmc20186404360
8. Novikov V.E., Kriukova N.O., Novikov A.S. Gastroprotective properties of mexidol and hypoxen. *Eksperimental'naya i Klinicheskaya Farmakologiya.* 2010;73(5):15–18 (in Russ.).
9. Churganov O.A., Gavrilova E.A. Influence of gipoksen supplement on some values of psychological, immune, biochemical and functional status of athletes. *Vestnik sportivnoi nauki = Sports Science Bulletin.* 2009;(1):36–38 (in Russ.).
10. Zagorsky A.L., Kalninsh K.K., Toropov D.K. *Mixture of poly-(1,4-dihydroxy)-phenylenes (polyhydroquinones)*: RF Pat. 2294918. Publ. 10.03.2007 URL: https://yandex.ru/patents/doc/RU2294918C1_20070310. Accessed January 29, 2024.
11. Zagorsky A.L., Kalninsh K.K., Toropov D.K. *Mixtures of poly(1,4-dihydroxy)-phenylenes (polyhydroquinones)*: Pat. WO2007073236. Publ. 28.06.2007. https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2007073236. Accessed February 02, 2024.
12. Savelieva N.B., Ishutenko G.V., Polosin A.V., Radus F.V., Polyansky D.S., Kurbatkin S.A., Efimova Yu.A., Postnikov P.V. Validation of a method for the quantitative determination of narcotic and psychotropic substances in urine by UHPLC–MS/MS. *Fine Chem. Technol.* 2022;17(3):253–267. https://doi.org/10.32362/2410-6593-2022-17-3-253-267
13. Shoibonov B.B., Ontoboev A.N., Zinchenko A.A., Aleshkin V.A. *Hypoxen is an Inhibitor of the c1q Subcomponent and c1 Component of the Human Complement System*: RF Pat. 2205002. Publ. 27.05.2003. https://patents.google.com/patent/RU2205002C1/ru. Accessed February 05, 2024.
14. James A.M., Cochemé H.M., Smith R.A.J., Murphy M.P. Interactions of mitochondria-targeted and untargeted ubiquinones with the mitochondrial respiratory chain and reactive oxygen species. Implications for the use of exogenous ubiquinones as therapies and experimental tools. *J. Biol. Chem.* 2005;280(22):21295–21312. https://doi.org/10.1074/jbc.M501527200
15. Grishina E.V., Khaustova Ya.V., Pogorelova V.G., *et al.* Accelerated utilization of lactate under the effect of hypoxen after intensive exercise. *Bull. Exp. Biol. Med.* 2008;145(2):198–201. https://doi.org/10.1007/s10517-008-0049-y [Original Russian Text: Grishina E.V., Khaustova Ya.V., Pogorelova V.G., Pogorelov A.G., Kuz'mich M.K., Maevskii E.I. Accelerated utilization of lactate under the effect of hypoxen after intensive exercise. *Zhurnal eksperimental'noi biologii i meditsiny.* 2008;145(2):158–161 (in Russ.).]
16. Murzaeva S.V., Abramova M.B., Popova I.I., *et al.* Effect of hypoxenum on bioenergetic processes in mitochondria and the activity of ATP-sensitive potassium channel. BIOPHYSICS. 2010;55(5):727–732. https://doi.org/10.1134/S0006350910050076
7. Долгарева С.А., Сорокин А.В., Конопля Н.А., Бушмина О.Н., Быстрова Н.А., Овод А.И. Использование иммуномодуляторов, антиоксидантов и гепатопротекторов для коррекции нарушений в печени, эритроцитах и иммунной системе при хронической интоксикации этанолом. *Биомедицинская химия.* 2018;64(4):360–367. http://dx.doi.org/10.18097/PBMC20186404360
8. Новиков В.Е., Крюкова Н.О., Новиков А.С. Гастропротекторные свойства мексидола и гипоксена. *Экспериментальная и клиническая фармакология*. 2010;73(5):15–18.
9. Чурганов О.А., Гаврилова Е.А. Влияние препарата гипоксен на некоторые показатели психологического, иммунного, биохимического и функционального статуса спортсменов. *Вестник спортивной науки.* 2009;(1):36–38.
10. Загорский А.Л., Калниньш К.К., Торопов Д.К. *Смесь поли (1,4-дигидрокси)-фениленов (полигидрохинонов)*: пат. 2294918 РФ. Заявка № 2005139599/04; заявл. 20.12.2005; опубл. 10.03.2007. https://yandex.ru/patents/doc/RU2294918C1_20070310. Дата обращения 29.01.2024 г.
11. Zagorsky A.L., Kalninsh K.K., Toropov D.K. *Mixtures of poly(1,4-dihydroxy)-phenylenes (polyhydroquinones)*: Pat. WO2007073236. Publ. 28.06.2007. https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2007073236. Дата обращения 02.02.2024 г.
12. Савельева Н.Б., Ишутенко Г.В., Полосин А.В., Радус Ф.В., Полянский Д.С., Курбаткин С.А., Ефимова Ю.А., Постников П.В. Валидация методики количественного определения наркотических и психотропных веществ в моче методом СВЭЖХ-МС/МС. *Тонкие химические технологии.* 2022;17(3):253–267. https://doi.org/10.32362/2410-6593-2022-17-3-253-267
13. Шойбонов Б.Б., Онтобоев А.Н., Зинченко А.А., Алёшкин В.А. *Гипоксен – ингибитор c1q субкомпонента и c1 компонента системы комплемента человека*: пат. 2205002 РФ. Заявка № 2002118468/14; заявл. 11.07.2002; опубл. 27.05.2003. https://patents.google.com/patent/RU2205002C1/ru. Дата обращения 05.02.2024 г.
14. James A.M., Cochemé H.M., Smith R.A.J., Murphy M.P. Interactions of mitochondria-targeted and untargeted ubiquinones with the mitochondrial respiratory chain and reactive oxygen species. Implications for the use of exogenous ubiquinones as therapies and experimental tools. *J. Biol. Chem.* 2005;280(22):21295–21312. https://doi.org/10.1074/jbc.M501527200
15. Гришина Е.В., Хаустова Я.В., Погорелова В.Г., Погорелов А.Г., Кузьмич М.К., Маевский Е.И. Ускорение утилизации лактата под влиянием гипоксена после напряженной мышечной работы. *Журнал экспериментальной биологии и медицины*. 2008;145(2):158–161.
16. Мурзаева С.В., Абрамова М.Б., Попова И.И., Гриценко Е.Н., Миронова Г.Д., Лежнев Э.И. Влияние гипоксена на биоэнергетические процессы в митохондриях и активность АТФ-чувствительного калиевого канала. *Биофизика*. 2010;55(5):814–821.
17. Игнатьев В.А., Петрова И.В., Цветкова Л.Н. Опыт применения гипоксена (Олифена) в лечении пациентов с хронической обструктивной болезнью легких среднетяжелого и тяжелого течения. *Terra Medica.* 2010;3(62):19–24.

[Original Russian Text: Murzaeva S.V., Abramova M.B., Popova I.I., Gritsenko E.N., Mironova G.D., Lezhnev E.I. Effect of hypoxenum on bioenergetic processes in mitochondria and the activity of ATP-sensitive potassium channel. *Biofizika.* 2010;55(5):814–821 (in Russ.).]

17. Ignat'ev V.A., Petrova I.V., Tsvetkova L.N. Experience with the use of hypoxen (*Olyphenum*) in the treatment of patients with moderate and severe chronic obstructive pulmonary disease. *Terra Medica.* 2010;3(62):19–24 (in Russ.).

About the authors

Pavel V. Postnikov, Cand. Sci. (Chem.), Head of the Doping Control Department, National Anti-Doping Laboratory (Institute), Lomonosov Moscow State University (10-1, Elizavetinskii per., Moscow, 105005, Russia). E-mail: drpavelpostnikov@gmail.com. Scopus Author ID 57021610900, RSCI SPIN-code 7251-9937, https://orcid.org/0000-0003-3424-0582

Andrey V. Polosin, Chief Specialist of the Doping Control Department, National Anti-Doping Laboratory (Institute), Lomonosov Moscow State University (10-1, Elizavetinskii per., Moscow, 105005, Russia). E-mail: polosin@dopingtest.ru. https://orcid.org/0000-0002-0009-7362

Nadezhda B. Savelieva, Chief Specialist of the Doping Control Department, National Anti-Doping Laboratory (Institute), Lomonosov Moscow State University (10-1, Elizavetinskii per., Moscow, 105005, Russia). E-mail: savelieva@dopingtest.ru. https://orcid.org/0000-0002-3988-6043

Sergey A. Kurbatkin, Assistant, I.P. Alimarin Department of Analitical Chemistry, M.V. Lomonosov Institute of Fine Chemical Technologies, MIREA – Russian Technological University (86, Vernadskogo pr., Moscow, 119571, Russia). E-mail: kurbatkins@mail.ru. https://orcid.org/0000-0002-2984-2178

Yuliya A. Efimova, Cand. Sci. (Chem.), Assistant Professor, I.P. Alimarin Department of Analitical Chemistry, M.V. Lomonosov Institute of Fine Chemical Technologies, MIREA – Russian Technological University (86, Vernadskogo pr., Moscow, 119571, Russia). E-mail: efimova_yulia@bk.ru. Scopus Author ID 25228417800, https://orcid.org/0000-0002-3582-0012

Elena S. Mochalova, Acting Director, National Anti-Doping Laboratory (Institute), Lomonosov Moscow State University (10-1, Elizavetinskii per., Moscow, 105005, Russia). E-mail: mochalova@dopingtest.ru

Об авторах

Постников Павел Викторович, к.х.н., начальник отдела допингового контроля, Национальная антидопинговая лаборатория (Институт), Московский государственный университет им. М.В. Ломоносова (Россия, 105005, Москва, Елизаветинский пер., д. 10, стр. 1). E-mail: postnikov@dopingtest.ru, drpavelpostnikov@gmail.com. Scopus Author ID 57021610900, SPIN-код РИНЦ 7251-9937, https://orcid.org/0000-0003-3424-0582

Полосин Андрей Вячеславович, главный специалист отдела допингового контроля, Национальная антидопинговая лаборатория (Институт), Московский государственный университет им. М.В. Ломоносова (105005, Россия, Москва, Елизаветинский пер., д. 10, стр. 1). E-mail: polosin@dopingtest.ru. https://orcid.org/0000-0002-0009-7362

Савельева Надежда Борисовна, главный специалист отдела допингового контроля, Национальная антидопинговая лаборатория (Институт), Московский государственный университет им. М.В. Ломоносова (105005, Россия, Москва, Елизаветинский пер., д. 10, стр. 1). E-mail: savelieva@dopingtest.ru. https://orcid.org/0000-0002-3988-6043

Курбаткин Сергей Александрович, ассистент кафедры аналитической химии им. И.П. Алимарина, Институт тонких химических технологий им. М.В. Ломоносова, ФГБОУ ВО «МИРЭА – Российский технологический университет» (119571, Россия, Москва, пр-т Вернадского, д. 86). E-mail: kurbatkins@mail.ru. https://orcid.org/0000-0002-2984-2178

Ефимова Юлия Александровна, к.х.н., доцент кафедры аналитической химии им. И.П. Алимарина, Институт тонких химических технологий им. М.В. Ломоносова, ФГБОУ ВО «МИРЭА – Российский технологический университет» (119571, Россия, Москва, пр-т Вернадского, д. 86). E-mail: efimova_yulia@bk.ru. Scopus Author ID 25228417800, https://orcid.org/0000-0002-3582-0012

Мочалова Елена Сергеевна, исполняющий обязанности директора Национальной антидопинговой лаборатории (Института), Московский государственный университет им. М.В. Ломоносова (105005, Россия, Москва, Елизаветинский переулок, д. 10, стр. 1). E-mail: mochalova@dopingtest.ru

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