

Development and validation of an LC-PDA method for detection of the natural β_2 agonist higenamine

Vanya Rangelov Kozhuharov^{1,2}, Stanislava Ivanova^{1,2}, Vanya Nalbantova^{1,2}, Niko Benbassat^{1,2}, Kalin Ivanov^{1,2}

¹ Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Plovdiv, 4002 Plovdiv, Bulgaria

² Research Institute, Medical University of Plovdiv, 4002, Plovdiv, Bulgaria

Corresponding author: Vanya Rangelov Kozhuharov (vanya.kozhuharov@mu-plovdiv.bg)

Received 23 February 2025 ♦ Accepted 24 April 2025 ♦ Published 21 May 2025

Citation: Rangelov Kozhuharov V, Ivanova S, Nalbantova V, Benbassat N, Ivanov K (2025) Development and validation of an LC-PDA method for detection of the natural β_2 agonist higenamine. Pharmacia 72: 1–7. <https://doi.org/10.3897/pharmacia.72.e151153>

Abstract

Higenamine is a natural β_2 agonist that can be found in the extracts of many plants, including *Nelumbo nucifera* Gaertn., *Tinospora crispa* L., *Nandina domestica* Thunb., *Gnetum parvifolium* Warb., *Asarum sieboldii* Miq., *Asarum heterotropoides* F. Schmidt, *Aconitum carmichaelii* Debeaux, and *Aristolochia brasiliensis* Mart. & Zucc. Since 2017, the compound has been included in WADA's prohibited list, and its detection in the biological samples of professional athletes is regarded as a violation of the anti-doping rules. Currently, many dietary supplements (DSs) contain plants that are rich in higenamine, posing a risk of inadvertent intake by athletes. In general, the presence of higenamine in DSs containing extracts from *Nelumbo nucifera* Gaertn. and other plant species that are a source of this β_2 agonist is undisclosed. Most of these plants are involved in the treatment of various medical conditions in Eastern folk medicine, and their intake is associated with many benefits. The objective of this study was to establish an LC-PDA method for detecting higenamine in plant extracts and dietary supplements (DSs). The method development utilized the Shimadzu LCMS-2050 system (Shimadzu Co., Kyoto, Japan) along with a PDA detector. Detection was performed using gradient elution. The mobile phase consisted of formic acid in water and acetonitrile. The PDA detector was set at 282 nm. The total run time was 16 min. The method exhibited a good correlation coefficient of 0.999, a limit of detection (LOD) of 0.643 $\mu\text{g/mL}$, and a limit of quantification (LOQ) of 1.949 $\mu\text{g/mL}$. The method was validated in accordance with ICH guidelines, providing a fast and reliable detection process.

Keywords

dietary supplements, higenamine, LC-PDA, lotus plumule, *Nelumbo nucifera*, traditional medicine

Introduction

Despite significant advances in the synthesis of new molecules, plant species continue to be recognized as one of the most important sources of biologically active compounds (Zhang et al. 2017). At present, many essential drugs used in medical practice are derived from plants.

Furthermore, extracts from medicinal plants are commonly included in the formulation of numerous dietary supplements (DSs), which are intended to enrich the diet and promote overall well-being.

Nowadays, the global DSs market is experiencing significant growth, driven by increasing health awareness, lifestyle changes, and a rising demand for plant-based

and personalized nutrition solutions (Djaoudene et al. 2023). It has been reported that the consumption of DSs by athletes has increased significantly in the last 10 years (Garthe and Maughan 2018). In general, plant-based DSs contain extracts derived from the whole plant or extracts from seeds, roots, leaves, or fruits. These extracts usually consist of many compounds, such as polyphenols, carotenoids, alkaloids, flavonoids, saponins, lignans, etc., that can potentially improve the performance or recovery of athletes (Herbold et al. 2004; Williams 2006).

Although DSs are considered an important part of professional athletes' diets, many studies have reported that DSs are the main reason for unintentional doping—numerous undeclared pharmacologically active compounds were detected in various DSs. In most cases, the undeclared compounds found in DSs are the result of poor manufacturing practices. Many contaminated DSs are labeled as vitamins, minerals, proteins, and other healthy-lifestyle products (Parr et al. 2011; Woo et al. 2013; Reeuwijk et al. 2014; Venhuis et al. 2014; Russo et al. 2016; Cooper et al. 2018; Kerpel dos Santos et al. 2018).

The detection of prohibited substances in dietary supplements leads to the enforcement of specific administrative measures and sanctions designed to protect consumer health, in accordance with the regulatory framework (Zlatanova 2025).

However, unintentional doping is not limited to the consumption of contaminated DSs. The intake of certain plant extracts may also pose a risk for professional athletes. Many of these extracts contain naturally occurring compounds with stimulant properties, and athletes are often unaware that such substances may be prohibited under anti-doping regulations. A notable example is methylhexanamine (Geranamine), a stimulant listed on the World Anti-Doping Agency's (WADA) Prohibited List, which can be isolated from species of the *Geranium* and *Pelargonium* genera (Geraniaceae) (Austin et al. 2014). Higenamine is another example of a natural compound that is banned. It is a β_2 agonist that can be found in the extracts of many different plants, including *Nelumbo nucifera* Gaertn., *Tinospora crispa* L., *Nandina domestica* Thunb., *Gnetum parvifolium* Warb., *Asarum sieboldii* Miq., *Asarum heterotropoides* F., *Aconitum camichaelii* Debeaux., and *Aristolochia brasiliensis* Mart. & Zucc. (Rangelov Kozhuharov et al. 2022). Since 2017, the compound has been included in WADA's prohibited list, and detection of higenamine in the biological specimens of professional athletes is regarded as a violation of the anti-doping rules (World Anti-Doping Agency 2024).

Higenamine was isolated for the first time in 1976 from the roots of *Aconitum japonicum* (Ranunculaceae) by Kosuge (Kosuge and Yokota 1976). In Chinese and Japanese folk medicine, *Aconitum japonicum* has a significant role. Its extracts are used to treat fever, collapse, pain, gastroenteritis, diarrhea, edema, bronchial asthma, and other conditions (Singhuber et al. 2009).

Nowadays, higenamine is a common ingredient in many DSs. Most of these supplements are used in obesity/

overweight management. The intake of higenamine is associated with performance-enhancing effects (Lee et al. 2013).

It is considered that *Nelumbo nucifera* Gaertn. (Nymphaeaceae) contains significantly higher levels of higenamine compared to other plant species. The plant is also known as "Indian lotus," "Chinese water lily," and sacred lotus (Mehta et al. 2013). It is considered an aquatic species, requiring full sun to thrive. Its rhizomes are yellow, stout, and creeping. The fruits are green, and the leaves are large (20–90 cm in diameter) and aerial as well as floating orbicular (Mehta et al. 2013). The flowers are approximately 10–25 cm in diameter, have many stamens, and have a variety of colors ranging from pink to white (Moro et al. 2013; Paudel and Panth 2015). The plant is well-established in traditional medicine and is still used for the management and treatment of a great variety of conditions (Rangelov Kozhuharov et al. 2022).

Materials and methods

Standards, reagents, and test samples

- Higenamine hydrochloride HPLC $\geq$ 95% (Merck KGaA, Darmstadt, Germany).
- Acetonitrile, methanol, and formic acid LC-MS analytical grade (Merck KGaA, Darmstadt, Germany). Before use, all solvents were filtered through 0.45 μ m membrane filters (Millipore, Milford, MA, USA) and degassed in an ultrasonic bath (BANDELIN, Berlin, Germany).
- *Nelumbo nucifera* Gaertn. seed (purchased online). The identity of the plant material was confirmed by Assoc. Prof. Niko Benbassat at the Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Plovdiv.

Standard solutions preparation

A standard solution of higenamine hydrochloride was prepared in methanol at a concentration of 1 mg/mL. Ultrasonication was used for better dissolution. The prepared solution was stored at 4 °C until use.

Working standard solutions, used for the construction of calibration curves, were freshly prepared by diluting the stock solutions in methanol Merck KGaA (Darmstadt, Germany) immediately before analyses.

Sample preparation

The plant material lotus plumule (*Nelumbo nucifera* Gaertn.) was subjected to primary processing to remove foreign impurities and crushed to a particle size of less than 1.40 mm. The plant material (30.0 g) was subjected to extraction using a Soxhlet apparatus and 95% ethanol as a solvent for 6 h. The resulting extract was concentrated under a vacuum and lyophilized using Lgj-18 lyophilizer. The dissolution of the extract was carried out in methanol

using an ultrasonic bath (Bandelin, Berlin, Germany) for 30 minutes, after which the dissolved dry extract was filtered through a 0.45 μm syringe filter. The sample was diluted with methanol to fall within the calibration range. Triplicates of the sample were made.

Instrumentation

The instrumentation used in this study included a Shimadzu LCMS-2050 system (Shimadzu Co., Kyoto, Japan) equipped with a photodiode array (PDA) detector. For chromatographic separation, a Shim-pack GIST C18 column (4.6 $\times$ 150 mm, 3 μm ; Shimadzu Co., Kyoto, Japan) was employed. A Lyophilizer LGJ-18, a Soxhlet extraction apparatus, and a rotary vacuum evaporator with a water bath (Buchi R-100) were also used.

Chromatographic conditions

The HPLC analyses were performed using a Shimadzu LCMS-2050 system (Shimadzu Co, Kyoto, Japan) with a PDA detector. The system was equipped with a Shim-pack GIST C18 (4.6 $\times$ 150 mm, 3 μm) column (Shimadzu Co., Kyoto, Japan) at 45 $^{\circ}\text{C}$. After achieving optimal chromatographic conditions, the method was validated according to ICH guidelines.

The injection volume was 10 μL , the duration of the method was 16 min, and the wavelength was set at $\lambda = 282$. The method was based on a gradient elution mode (Table 1).

Table 1. Mobile-phase composition.

Time (min)	Water (0.1% Formic Acid)	Acetonitrile	Flow rate (mL/min)
Initial (0)	95	5	0.2
5	30	70	0.5
10	20	80	0.5
15	95	5	0.5

Results and discussion

Development and validation of the method

In order to establish the optimal conditions for the simultaneous determination of higenamine in DSs and extracts, several gradient methods were tested using different mobile phases and solvent ratios: methanol/water, acetonitrile/water, and acetonitrile with acidified water. It was established that the mobile phase composed of acetonitrile/0.1% formic acid in water provided better selectivity than the other options. By optimizing the mobile phase to include 0.1% formic acid, better selectivity and peak resolution were achieved. The column temperature was varied by 5 $^{\circ}\text{C}$, and different flow rates were also tested. Chromatograms illustrating the various mobile phases and flow rates tested in the method development process are shown in Fig. 1. The selected gradient is presented in the section Materials and Methods. The method was performed within 16 minutes. The retention time of higenamine was 7.2 minutes.

Method validation

The method was validated according to ICH guidelines for linearity, accuracy, precision, limit of detection, limit of quantification, and robustness (Tietje and Brouder 2010).

Linearity

The linearity and range of the method were established with a standard solution of higenamine at six different concentrations. The working solutions were injected three times for each concentration, and the corresponding peak area was observed. The concentration ranged from 1.25 $\mu\text{g/mL}$ to 50 $\mu\text{g/mL}$. The linear regression of higenamine was $y = 16726x + 16634$, with a correlation coefficient of $R^2 = 0.9997$ (Fig. 2). This indicates that the

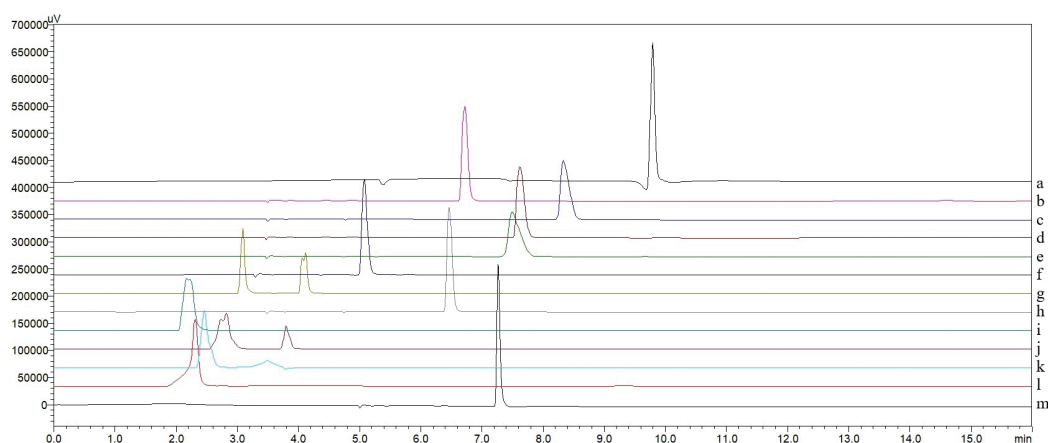


Figure 1. Chromatograms of higenamine under various chromatographic conditions using a mobile phase consisting of 0.1% formic acid in water (A) and acetonitrile (B), with a flow rate of 0.5 mL/min, except where stated: (a) 95:5 $\rightarrow$ 30:70 $\rightarrow$ 20:80 $\rightarrow$ 95:5 with reduced flow rate (0.3 mL/min); (b) 95:5 $\rightarrow$ 5:95 $\rightarrow$ 95:5; (c) 95:5 $\rightarrow$ 60:40 $\rightarrow$ 95:5; (d) 95:5 $\rightarrow$ 70:30 $\rightarrow$ 50:50 $\rightarrow$ 95:5; (e) 90:10 $\rightarrow$ 80:20 $\rightarrow$ 60:40 $\rightarrow$ 40:60 $\rightarrow$ 90:10; (f) 80:20 $\rightarrow$ 60:40 $\rightarrow$ 40:60 $\rightarrow$ 80:20; (g) 80:20; (h) 95:5 $\rightarrow$ 40:60 $\rightarrow$ 30:70 $\rightarrow$ 95:5; (i) 40:60; (j) 70:30; (k) 70:30 (water/acetonitrile); (l) 20:80 (water/acetonitrile); (m) Current method: 95:5 $\rightarrow$ 30:70 $\rightarrow$ 20:80 $\rightarrow$ 95:5, with flow rate from 0.2 to 0.5 mL/min.

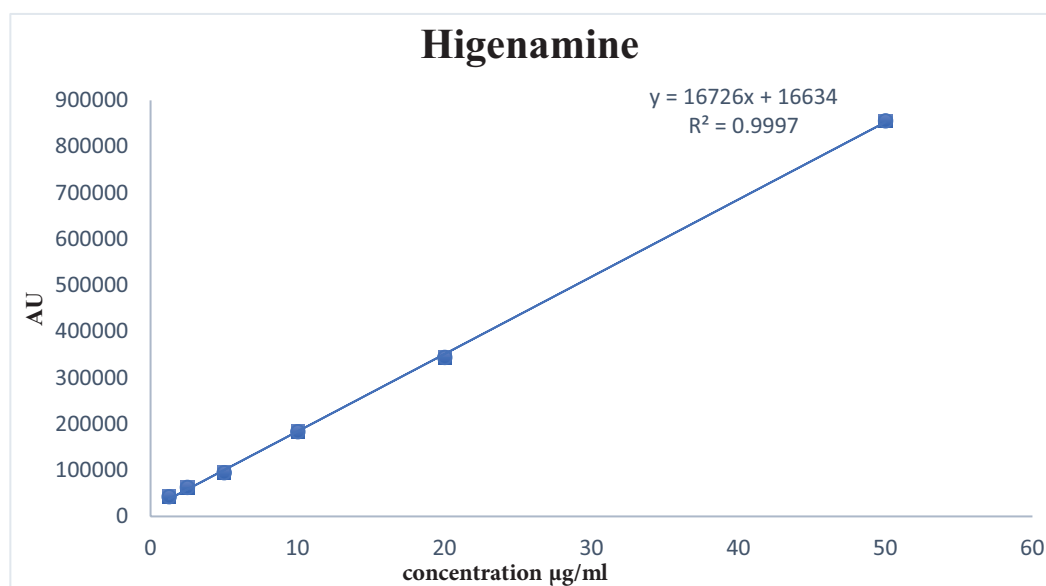


Figure 2. Calibration curve of higenamine.

method demonstrates a high degree of linearity over the chosen concentration range.

An example chromatogram of a standard solution of higenamine is given in Fig. 3.

Accuracy and precision

To verify the accuracy of the developed method, a recovery test was conducted at three known concentration levels. The following concentrations were used: high (20 µg/mL), medium (10 µg/mL), and low (5 µg/mL). The accuracy for the following points was as follows: for high value, 98.30%; for medium, 100.58%; and for low, 96%. By calculating the coefficient of variation for the three known concentrations, each with three replicates, the precision of the method was calculated as intra-day and inter-day precision. The peaks were observed, and %CVs were calculated. The results are presented in Table 2.

Table 2. Precision and accuracy results of the developed method.

	Concentration (µg/mL)	Accuracy (%)	Precision CV (%)	
			Intra-day precision	Inter-day precision
Higenamine	5	96	0.66	1.36
	10	100.58	0.45	1.17
	20	98.30	1.13	1.42

Limit of detection and limit of quantification

The detection limit (LOD) and quantification limit (LOQ) were calculated using the standard deviation of the response (σ) and the slope of the calibration curve (S). We used the following formulas: $LOD = 3.3 \sigma/S$ and $LOQ = 10 \sigma/S$. The LOD and LOQ for higenamine were 0.643 µg/mL and 1.949 µg/mL, respectively. The LC-PDA method for higenamine analysis showed exceptional linearity with a correlation coefficient of

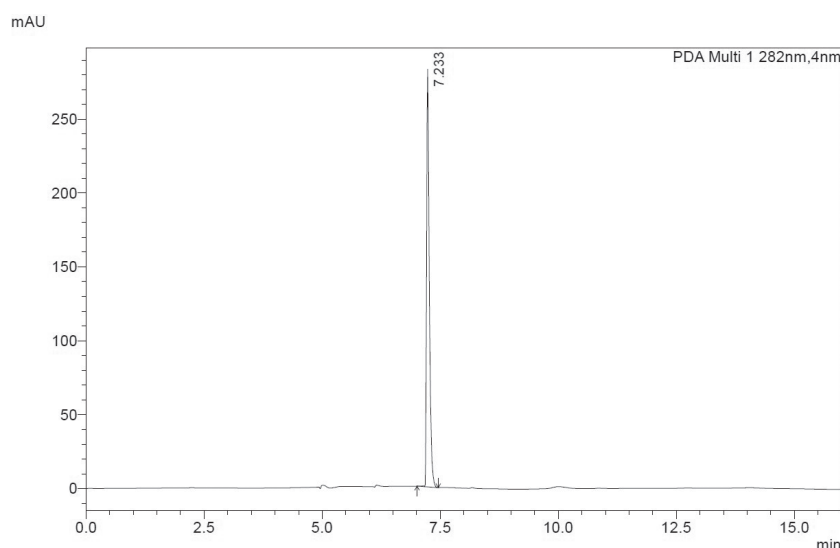


Figure 3. Chromatogram of a standard solution of higenamine hydrochloride obtained by LC-PDA.

0.9997. The accuracy results show that the mean percentage deviation varies within a narrow range from 96% to 100.58%. The LOD of 0.643 µg/mL and LOQ of 1.949 µg/mL provide sensitivity and accuracy of the method for low concentrations of higenamine.

Robustness

Robustness is a key indicator of an analytical method's reliability when subjected to minor, intentional variations in chromatographic parameters. In this study, robustness was evaluated by altering the column temperature within a controlled range of 42–46 °C. The impact of these temperature changes on the retention time of the compound was examined. The results demonstrated that the resolution remained consistent despite temperature fluctuations, confirming that small variations in temperature do not compromise chromatographic separation.

Method application

The developed and validated method was successfully applied for the quantification of higenamine levels in Lotus Plumule (*Nelumbo nucifera* Gaertn.) dry extract. The identification of higenamine in the extract was performed by comparing its retention time with that of the reference standard. The assay was carried out in triplicate, and the mean value of the analyzed extract was 276.011 µg/g. The LC chromatogram is shown in Fig. 4.

According to the data collected in the literature review, the concentration of higenamine in Lotus plumule is higher compared to other plants. Yen et al. investigated the total amount of alkaloids in six herbal extraction products from Lotus Plumule and five extracts obtained using different extraction schemes. The concentration of higenamine ranged from 263.9 to 969.5 µg/g (Yen et al. 2020).

Analyses of the *Nelumbo nucifera* Gaertn. extract using the developed method revealed a higenamine content of 276.011 µg/g. These findings support the recommendation that athletes should avoid the consumption of foods or supplements containing lotus (*Plumula nelumbinis*),

both during and outside of competition, to prevent potential violations of anti-doping regulations.

Despite its rich history of use, randomized human trials on lotus extract remain limited. There are some studies that have examined the pharmacokinetics of higenamine after lotus plumule intake. Yen et al. conducted a study in which six volunteers consumed 0.8 g of lotus plumule extract powder, corresponding to 679.6 µg of higenamine, three times daily for three days. HPLC-MS/MS was used to determine the amount of higenamine (Yen et al. 2020). In another study, volunteers took capsules of *Plumula nelumbinis* at a concentration of 0.34 g/capsule six times daily for seven days. Urine samples were analyzed by UPLC-MS/MS (Yan et al. 2019). The higenamine concentration in the urine of most sample groups from both studies was higher than the limit specified in the WADA regulations (World Anti-Doping Agency (WADA) 2019, Technical Document). Stojanovic et al. demonstrated the detection of higenamine above permissible levels up to 20 hours after its ingestion. They developed and validated a rapid and highly sensitive online SPE LC HRMS method (Stojanovic et al. 2024). These findings clearly indicate that the intake of *Lotus plumule* herbal extracts poses a significant risk for anti-doping rule violations. Therefore, it is strongly recommended that professional athletes avoid the consumption of herbal products containing *lotus plumule*, as it may result in elevated urinary concentrations of higenamine and consequent breaches of anti-doping regulations.

Currently, few reliable methods are available for detecting higenamine in dietary supplements. All of them are aimed at identifying higenamine, but their implementation in daily practice remains challenging. The UHPLC-MS/MS method of A. Stajić et al., the LC-MS/MS method of Lin et al., and the UHPLC-QTOF-MS method of Cohen et al. have high accuracy, precision, and low detection limits (Stajić et al. 2017; Cohen et al. 2021; Lin and Hsu 2024).

For its significance in the rapid and accurate detection of higenamine in tablets, syrups, etc., Qiu et al. developed an indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) (Qiu et al. 2025).

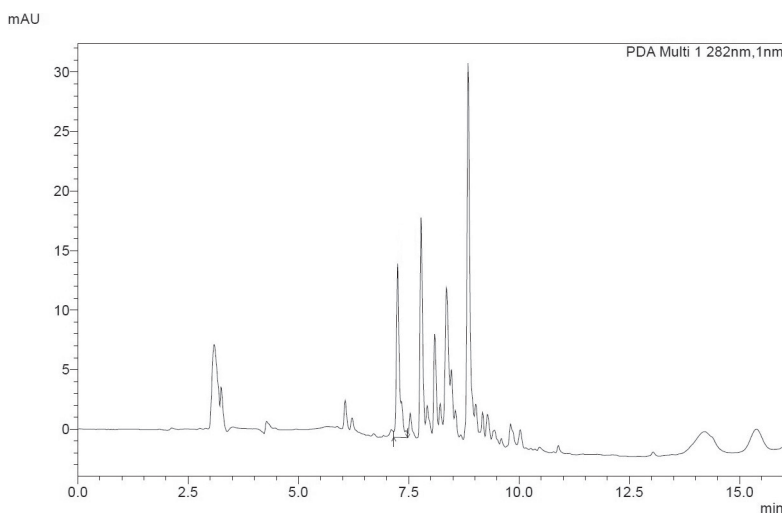


Figure 4. LC-PDA chromatogram of the lotus plumule dry extract in methanol showing the presence of higenamine.s

Despite their outstanding performance, these methods are associated with significant difficulties and costs in their application for routine doping control. Although mass spectrometry-based methods are highly sensitive, they are resource-intensive. Many smaller laboratories may not have the necessary equipment or expertise, limiting the widespread use of these advanced techniques. The developed LC-PDA method achieves acceptable detection limits, making it suitable for preliminary screening of higenamine in dietary supplements and plant extracts. In cases where further confirmation is required, positive samples can be subsequently analyzed using more advanced techniques such as LC-MS/MS. The implementation of this method has the potential to significantly reduce testing costs without substantial compromises in sensitivity, thereby offering a more practical and accessible approach for routine higenamine detection in plant extracts and dietary supplements.

Conclusion

The developed LC-PDA method provides a reliable, cost-effective, and practical approach for the detection of higenamine in plant extracts and dietary supplements. The method demonstrates high accuracy, precision, and sensitivity, positioning it as a valuable alternative to mass spectrometry-based techniques, which require more advanced instrumentation. The results confirm that lotus plumule (*Nelumbo nucifera* Gaertn.) contains significant levels of higenamine, posing a risk to professional athletes under anti-doping regulations. Due to the widespread presence of higenamine in herbal supplements and the potential for unintentional doping, routine screening using accessible methodologies is essential. The study highlights the need for increased awareness among athletes and regulatory bodies regarding the risks of higenamine-containing supplements. Continued research and regulatory oversight should prioritize the development of improved detection methods and the implementation of clear, accurate labeling standards to minimize the risk of inadvertent anti-doping rule violations.

Acknowledgements

We would like to express our gratitude to the program “Research, Innovation and Digitalisation for Smart

Transformation” 2021–2027, funded by the European Union, Project BG16RFPR002-1.014-0007 Center for Competence “PERIMED-2” for supporting this article.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors’ representatives participating in the study.

The authors declared that no experiments on animals were performed for the present study.

The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

Funding

No funding was reported.

Author contributions

Conceptualization, V.R.K., V.N., S.I., and K.I.; methodology, V.R.K., V.N., K.I., and S.I.; validation, V.R.K. and S.I.; investigation, V.R.K., V.N., K.I. and S.I.; resources, V.R.K. and V.N.; data curation, V.R.K. and S.I.; writing—original draft preparation, V.R.K., V.N., K.I. and S.I.; writing—review and editing, K.I., S.I. and N.B.; supervision, K.I., S.I. and N.B. All authors have read and agreed to the published version of the manuscript.

Author ORCIDs

Vanya Rangelov Kozhuharov  <https://orcid.org/0000-0001-7071-4867>

Stanislava Ivanova  <https://orcid.org/0000-0003-1282-7868>

Vanya Nalbantova  <https://orcid.org/0000-0001-8276-7210>

Niko Benbassat  <https://orcid.org/0000-0001-8876-2728>

Kalin Ivanov  <https://orcid.org/0000-0002-5689-2920>

Data availability

All of the data that support the findings of this study are available in the main text.

References

- Austin KG, Travis J, Pace G, Lieberman HR (2014) Analysis of 1,3-dimethylamylamine concentrations in Geraniaceae, geranium oil and dietary supplements: 1,3 dimethylamylamine, dietary supplements, geranium oil and Geraniaceae. *Drug Testing and Analysis* 6: 797–804. <https://doi.org/10.1002/dta.1491>
- Cohen PA, Travis JC, Vanhee C, Ohana D, Venhuis BJ (2021) Nine prohibited stimulants found in sports and weight loss supplements: deterenol, phenpromethamine (Vonedrine), oxilofrine, octodrine, beta-methylphenylethylamine (BMPEA), 1,3-dimethylamylamine (1,3-DMAA), 1,4-dimethylamylamine (1,4-DMAA), 1,3-dimethylbutylamine (1,3-DMBA) and higenamine. *Clinical Toxicology* 59(11): 975–981. <https://doi.org/10.1080/15563650.2021.1894333>
- Cooper ER, McGrath KCY, Li X, Heather AK (2018) Androgen bioassay for the detection of nonlabeled androgenic compounds in nutritional supplements. *International Journal of Sport Nutrition and Exercise Metabolism* 28: 10–18. <https://doi.org/10.1123/ijnsnem.2017-0018>

- Djaoudene O, Romano A, Bradai YD, Zebiri F, Ouchene A, Yousfi Y, Amrane-Abider M, Sahraoui-Remini Y, Madani K (2023) A global overview of dietary supplements: Regulation, market trends, usage during the COVID-19 pandemic, and health effects. *Nutrients* 15: 3320. <https://doi.org/10.3390/nu15153320>
- Garthe I, Maughan RJ (2018) Athletes and supplements: Prevalence and perspectives. *International Journal of Sport Nutrition and Exercise Metabolism* 28: 126–138. <https://doi.org/10.1123/ijsnem.2017-0429>
- Herbold NH, Visconti BK, Frates S, Bandini L (2004) Traditional and nontraditional supplement use by collegiate female varsity athletes. *International Journal of Sport Nutrition and Exercise Metabolism* 14: 586–593. <https://doi.org/10.1123/ijsnem.14.5.586>
- Kerpel dos Santos M, Gleco E, Davidson JT, Jackson GP, Pereira Limberger R, Arroyo LE (2018) DART-MS/MS screening for the determination of 1,3-dimethylamylamine and undeclared stimulants in seized dietary supplements from Brazil. *Forensic Chemistry* 8: 134–145. <https://doi.org/10.1016/j.forc.2018.03.005>
- Kosuge T, Yokota M (1976) Studies on cardiac principle of aconite root. *Chemical and Pharmaceutical Bulletin* 24: 176–178. <https://doi.org/10.1248/cpb.24.176>
- Lee S-R, Schrieffer JM, Gunnels TA, Harvey IC, Bloomer RJ (2013) Acute oral intake of a higenamine-based dietary supplement increases circulating free fatty acids and energy expenditure in human subjects. *Lipids in Health and Disease* 12: 148. <https://doi.org/10.1186/1476-511X-12-148>
- Lin Y-A, Hsu M-C (2024) Determination of doping higenamine in Chinese herbal medicines and their concentrated preparations by LC-MS/MS. *Journal of Pharmaceutical and Biomedical Analysis* 246: 116188. <https://doi.org/10.1016/j.jpba.2024.116188>
- Mehta NR, Patel EP, Patani PV, Shah B (2013) *Nelumbo nucifera* (Lotus): A review on ethanobotany, phytochemistry and pharmacology. *Indian Journal of Pharmaceutical and Biological Research* 1: 152–167. <https://doi.org/10.30750/ijpbr.1.4.26>
- Moro CF, Yonekura M, Kouzuma Y, Agrawal GK, Rakwal R (2013) Lotus – A Source of Food and Medicine: Current Status and Future Perspectives in Context of the Seed Proteomics. *International Journal of Life Sciences* 7: 1–5. <https://doi.org/10.3126/ijls.v7i1.6394>
- Parr M, Pokrywka A, Kwiatkowska D, Schänzer W (2011) Ingestion of designer supplements produced positive doping cases unexpected by the athletes. *Biology of sport / Institute of Sport* 28: 153–157. <https://doi.org/10.5604/959289>
- Paudel KR, Panth N (2015) Phytochemical profile and biological activity of *Nelumbo nucifera*. *Evidence-Based Complementary and Alternative Medicine* 2015: 1–16. <https://doi.org/10.1155/2015/789124>
- Qiu J, Zhang R, Liu W, Lu Y, Liu J (2025) Immunomagnetic bead-based sample preparation method and immunoassay for detecting higenamine in herbal products. *Journal of Pharmaceutical and Biomedical Analysis* 257: 116712. <https://doi.org/10.1016/j.jpba.2025.116712>
- Rangelov Kozuharov V, Ivanov K, Ivanova S (2022) Higenamine in plants as a source of unintentional doping. *Plants* 11: 354. <https://doi.org/10.3390/plants11030354>
- Reeuwijk NM, Venhuis BJ, de Kaste D, Hoogenboom RLAP, Rietjens IMCM, Martena MJ (2014) Active pharmaceutical ingredients detected in herbal food supplements for weight loss sampled on the Dutch market. *Food Additives & Contaminants: Part A* 31: 1783–1793. <https://doi.org/10.1080/19440049.2014.958574>
- Russo G, Barbato F, Grumetto L (2016) A validated LC/UV method for the determination of four adulterating drugs in herbal slimming capsules. *Journal of Pharmaceutical and Biomedical Analysis* 117: 436–445. <https://doi.org/10.1016/j.jpba.2015.09.027>
- Singhuber J, Zhu M, Prinz S, Kopp B (2009) Aconitum in traditional chinese medicine—A valuable drug or an unpredictable risk? *Journal of Ethnopharmacology* 126: 18–30. <https://doi.org/10.1016/j.jep.2009.07.031>
- Stajić A, Anđelković M, Dikić N, Rašić J, Vukašinović-Vesić M, Ivanović D, Jančić-Stojanović B (2017) Determination of higenamine in dietary supplements by UHPLC/MS/MS method. *Journal of Pharmaceutical and Biomedical Analysis* 146: 48–52. <https://doi.org/10.1016/j.jpba.2017.08.017>
- Stojanovic B, Rasic J, Andjelkovic M, Dikic N, Dragicevic N, Djordjevic B, Forsdahl G, Gmeiner G (2024) Urinary excretion profile of higenamine in females after oral administration of supplements – Doping scenario. *Journal of Chromatography B* 1235: 124047. <https://doi.org/10.1016/j.jchromb.2024.124047>
- Tietje C, Brouder A [Eds] (2010) International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. In: *Handbook of Transnational Economic Governance Regimes*. Brill | Nijhoff, 1041–1053. <https://doi.org/10.1163/ej.9789004163300.i-1081.897>
- Venhuis B, Keizers P, van Riel A, de Kaste D (2014) A cocktail of synthetic stimulants found in a dietary supplement associated with serious adverse events: A cocktail of synthetic stimulants found in a dietary supplement associated with serious adverse events. *Drug Testing and Analysis* 6: 578–581. <https://doi.org/10.1002/dta.1664>
- Williams M (2006) Dietary Supplements and Sports Performance: Herbs. *Journal of the International Society of Sports Nutrition* 3: 1. <https://doi.org/10.1186/1550-2783-3-1-1>
- Woo H, Kim JW, Han KM, Lee JH, Hwang IS, Lee JH, Kim J, Kweon SJ, Cho S, Chae KR, Han SY, Kim J (2013) Simultaneous analysis of 17 diuretics in dietary supplements by HPLC and LC-MS/MS. *Food Additives & Contaminants: Part A* 30: 209–217. <https://doi.org/10.1080/19440049.2012.738939>
- World Anti Doping Agency (2024) WADA The Prohibited List. World Anti Doping Agency. <https://www.wada-ama.org/en/prohibited-list> [October 25, 2024]
- World Anti-Doping Agency [WADA] (2019) WADA technical document TD2019MRPL—minimum required performance levels for detection and identification of non-threshold substances. https://www.wada-ama.org/sites/default/files/resources/files/td2019mrpl_eng.pdf [November 17, 2021]
- Yan K, Wang X, Wang Z, Wang Y, Luan Z, Gao X, Wang R (2019) The risk of higenamine adverse analytical findings following oral administration of *plumula nelumbinis* capsules. *Drug Testing and Analysis* 11: 1731–1736. <https://doi.org/10.1002/dta.2701>
- Yen C-C, Tung C-W, Chang C-W, Tsai C-C, Hsu M-C, Wu Y-T (2020) Potential Risk of Higenamine Misuse in Sports: Evaluation of Lotus Plumule Extract Products and a Human Study. *Nutrients* 12: 285. <https://doi.org/10.3390/nu12020285>
- Zhang N, Lian Z, Peng X, Li Z, Zhu H (2017) Applications of Higenamine in pharmacology and medicine. *Journal of Ethnopharmacology* 196: 242–252. <https://doi.org/10.1016/j.jep.2016.12.033>
- Zlatanova M (2025) Administrative regulation of food supplements – coercive administrative measures and administrative penalties. In: *Medical Law and Healthcare*. Ciela Norma AD, 15–41.