ECRONICON OPEN ACCESS

EC PHARMACOLOGY AND TOXICOLOGY
Research Article

Algorithm for the Laboratory Diagnosis of Fly Agaric Poisoning

O Yu Strelova[1]*, EA Evdokimova[1], M Yu Goncharov[1] and OL Balabanova[1,2]

[1]Federal State Budgetary Educational Institution of Higher Education, Saint Petersburg State Chemical-Pharmaceutical University of the Ministry of Health of the Russian Federation, Aptekarsky Island Municipal District, Saint Petersburg, Russian Federation

[2]I. I. Dzhanelidze Saint Petersburg Research Institute of Emergency Care, Saint Petersburg, Russian Federation

***Corresponding Author:** O Yu Strelova, Federal State Budgetary Educational Institution of Higher Education, Saint Petersburg State Chemical-Pharmaceutical University of the Ministry of Health of the Russian Federation, Aptekarsky Island Municipal District, Saint Petersburg, Russian Federation.

Received: September 29, 2026; **Published:** October 15, 2026

Abstract

Panther cap (*Amanita pantherina*) and fly agaric (*Amanita muscaria*) mushrooms contain the psychoactive compounds ibotenic acid and muscimol, which are responsible for acute poisoning. Laboratory testing, including chemical-toxicological analysis, is a mandatory component of clinical laboratory diagnostics. At present, fly agaric poisoning is diagnosed primarily on the basis of medical history. Therefore, the development of a laboratory diagnostic algorithm for biological specimens in suspected fly agaric intoxication is of considerable importance.

Keywords: *Fly Agaric Capsules; Muscimol; Ibotenic Acid; Fly Agaric (Amanita muscaria L.); Panther Cap (Amanita pantherina L.)*

Introduction

Fly agaric toxins enter the human body orally following the ingestion of capsules, tinctures, powder, dried caps, or chocolate products. The psychotropic effects of *Amanita muscaria* and *Amanita pantherina* are primarily associated with muscimol and ibotenic acid, the latter being partially metabolized to muscimol *in vivo* after ingestion [1-3].

Ibotenic acid and muscimol are water-soluble isoxazole derivatives. They exert opposing effects on the central nervous system, producing excitation and inhibition through ionotropic glutamate receptors that selectively bind N-methyl-D-aspartate and through γ-aminobutyric acid receptors, respectively. The combined action of isoxazoles and other fungal compounds, including muscarine, stizolobinic acid, stizolobic acid, and methyltetrahydrocarboline carboxylic acid (1-methyl-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid), leads to the development of mycoatropine and pantherina syndromes. Muscimol is of greatest toxicological significance because it can produce a pronounced psychodysleptic effect and depress consciousness, potentially progressing to coma. Ibotenic acid is likewise important for establishing the fact of fly agaric ingestion [4].

Interest in mushrooms of the genus Amanita has increased substantially with the growing popularity of fly agaric "microdosing." Species of particular interest within the family Amanitaceae include *Amanita pantherina* (DC.) Krombh. (panther cap) and *Amanita muscaria* (L.) (fly agaric).

Promoters of these practices, citing scientifically unsubstantiated claims, assert that "microdosing" fly agaric is harmless and, on the contrary, improves cognitive functions such as attention and memory, making these mushrooms attractive to people engaged in intellectual work. Products containing stated doses of mushroom material are sold online as dietary supplements and promoted on social media [5].

The number of acute intoxications involving *Amanita muscaria* (L.) and *Amanita pantherina* (L.) is relatively low but has remained stable over several years. However, it is impossible to determine how many people consume these mushrooms and mushroom products or capsules for recreational purposes, or how many practice microdosing [6].

In the absence of readily available commercial reference standards for the target analytes, substantial difficulties arise in the differential laboratory diagnosis of both acute poisoning with these mushrooms and mushroom-derived products and "microdosing". Botanical identification of fungal raw-material samples can provide more precise information on the composition of commercial *Amanita pantherina* (L.) and *Amanita muscaria* (L.) products and allow such materials to be used as reference samples for instrumental detection of toxic Amanita constituents in biological specimens. Based on the development of specific methods for detecting toxicants from fly agaric and panther cap mushrooms in biological specimens, an algorithm can be proposed for laboratory confirmation of the ingestion of these mushrooms. Chemical-toxicological analysis is a mandatory component of clinical laboratory diagnostics under Order No. 262n of the Ministry of Health of the Russian Federation dated 29 April 2025, "On Approval of the Procedure for Medical Examination for Intoxication (Alcohol, Narcotic, or Other Toxic Intoxication)".

Materials and Methods

For identification, samples of dried caps of *Amanita muscaria* L. and *Amanita pantherina* L. (FUNGITHERA mushroom pharmacy), as well as capsules containing powdered fruiting bodies of *A. muscaria* (Die Familie) and *A. pantherina* (ALTAI ECO), were collected. Identification was performed using a Bresser Biolux Touch 5 HDMI microscope at ×500 and ×1000 magnification.

The biological specimens studied were urine, blood, and hair obtained from patients admitted to the Acute Poisoning Treatment Center of the I. I. Dzhanelidze Saint Petersburg Research Institute of Emergency Care with a diagnosis of acute oral fly agaric poisoning based on medical history and clinical presentation. Instrumental analysis was performed using a Nexera XR liquid chromatograph coupled to an LCMS-8050 tandem mass spectrometer (Shimadzu, Japan) and a QP2020 gas chromatograph (Shimadzu, Japan) coupled to a single-quadrupole mass spectrometer.

Derivatization was performed as follows: 500 µL of water, 500 µL of 5% sodium carbonate solution, and 300 µL of 30 mM dansyl chloride in acetone were added to 1 mL of sample. The mixture was thoroughly mixed and incubated for 30 minutes at 60°C, cooled for 10 minutes at room temperature, and derivatization was stopped by adding 10 µL of concentrated sulfuric acid. Next, 2 mL of dichloromethane was added; the mixture was mixed thoroughly for 10 minutes on a MultiBioRS-24 multirotator and centrifuged at 3000 rpm for 3 minutes. The lower layer was collected, evaporated to dryness, reconstituted in 500 µL of mobile phase A, and 10 µL was injected into the chromatograph [7].

Blood proteins were precipitated with acetonitrile.

Hair: 4 mL of chymopsin in phosphate buffer (pH 7.4) was added to a 0.35g sample of washed and finely cut hair. The mixture was shaken and incubated for 3h at 37°C, then centrifuged for 10 minutes at 2000 rpm, and the supernatant was collected. A further 3 mL of enzyme solution in phosphate buffer was added to the hair; the mixture was shaken, incubated for 3h at 37°C, centrifuged at 2000 rpm, and the supernatant was collected. Acetonitrile was added at a sample-to-acetonitrile ratio of 1:0.5; the mixture was mixed for 15 minutes on a multirotator and frozen at −18°C for 25 minutes. The supernatant was then collected, the extracts were combined, and dansyl chloride derivatization was performed as described above.

Urine sample preparation by solid-phase extraction (SPE) on Oasis HLB cartridges was performed as follows: the cartridge was conditioned with 1 mL of methanol and 1 mL of purified water at a flow rate of 1 mL/min. A 1 mL urine sample was passed through the cartridge at 0.5 mL/min. The cartridge was washed with 1 mL of ethyl acetate to remove matrix components. Analytes were eluted from the sorbent with 1 mL of methanol. The eluate was evaporated to dryness, the residue was dissolved in 500 µL of ethyl acetate, and 30 µL of BSTFA with 1% TMS was added. The sample was incubated for 20 minutes at 60°C and injected into a gas chromatograph equipped with a mass-selective detector.

Results and Discussion

Samples of dried fly agaric (*A. muscaria*) caps (Figure 1) and powdered fly agaric fruiting bodies in capsules were identified.



Figure 1: *Appearance of dried fly agaric caps.*

Dried fragments of fly agaric fruiting bodies consisted of flat or slightly concave, round caps 9 - 13 cm in diameter. The upper surface of the cap was covered by a pinkish-red to red pileipellis with numerous white or grayish floccose patches up to 5 mm in diameter (Figure 1).

The gills on the underside of the cap were numerous and free, with intermediate lamellae, 3 - 7 mm wide, and white or grayish in color.

The cap flesh was dense and light gray, with a characteristic odor.

Examination of dried panther cap (*A. pantherina*) fruiting bodies (Figure 2) and powdered panther cap fruiting bodies in capsules showed that the dried fragments consisted of slightly convex, round caps 5 - 10 cm in diameter with a finely striate margin. The upper surface was covered by a light-brown pileipellis bearing numerous white floccose patches up to 3 mm in diameter.



Figure 2: *Appearance of dried panther cap mushrooms.*

The gills on the underside of the cap were numerous, 2 - 4 mm wide, and white or grayish. The cap flesh was dense, brittle, and white, with a characteristic unpleasant odor.

Microscopic examination of the fruiting-body powder revealed mycelial hyphae, basidia, and basidiospores. The mycelial hyphae were highly branched with septa (Figure 3).

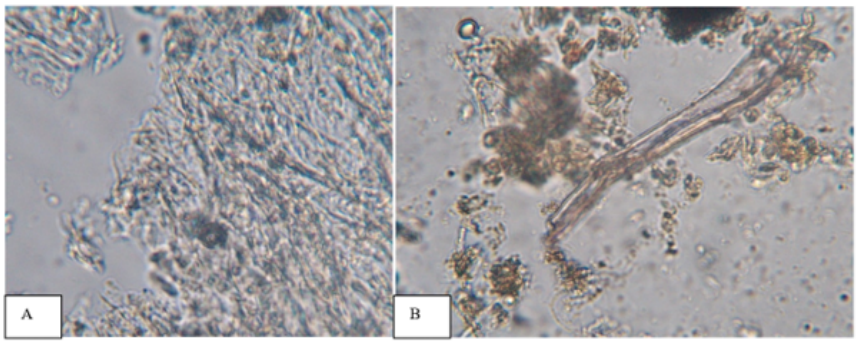


Figure 3: A: *Fragments of fly agaric mycelium; B: Fragments of panther agaric cap mycelium.*

Basidiospores were numerous, elliptical, with a reticulate surface, and up to 9 μm long (Figure 4). The powder stained blue-violet with Lugol's solution, indicating a positive reaction for starch (Figure 5).

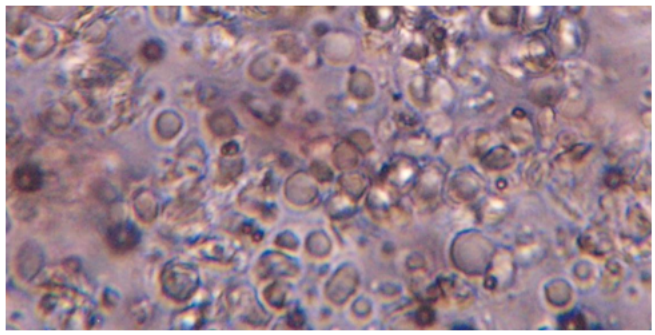


Figure 4: *Basidiospores of the panther cap.*

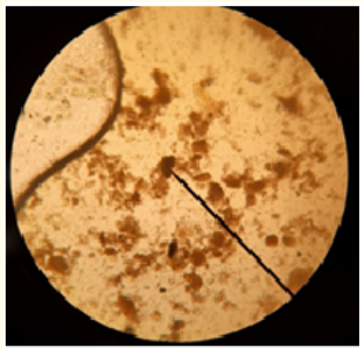


Figure 5: *Staining with Lugol's solution (Positive reaction for starch).*

Comparison of the chemical composition of capsules from different manufacturers and dried caps of panther cap and fly agaric mushrooms (Figure 6 and 7) demonstrated the presence of muscimol and ibotenic acid in all samples. This finding allows capsule extracts to be used as reference samples when developing methods for determining the target toxicants in biological specimens.

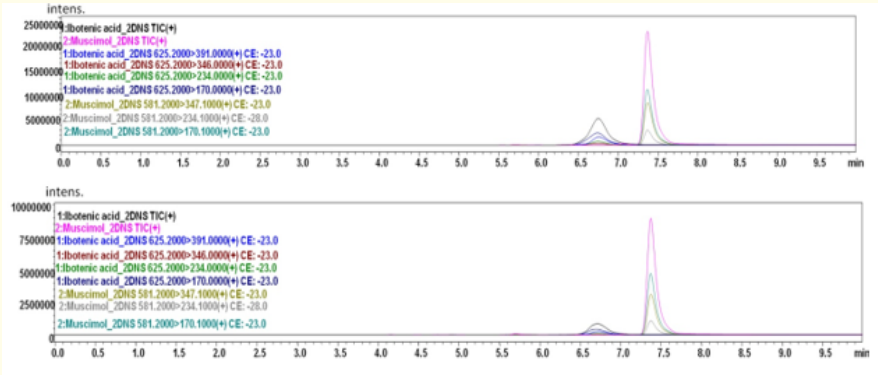


Figure 6: *Chromatogram (Shim-pack FC-ODS column) of muscimol and ibotenic acid derivatives in extracts from dried caps of Amanita muscaria and Amanita pantherina.*

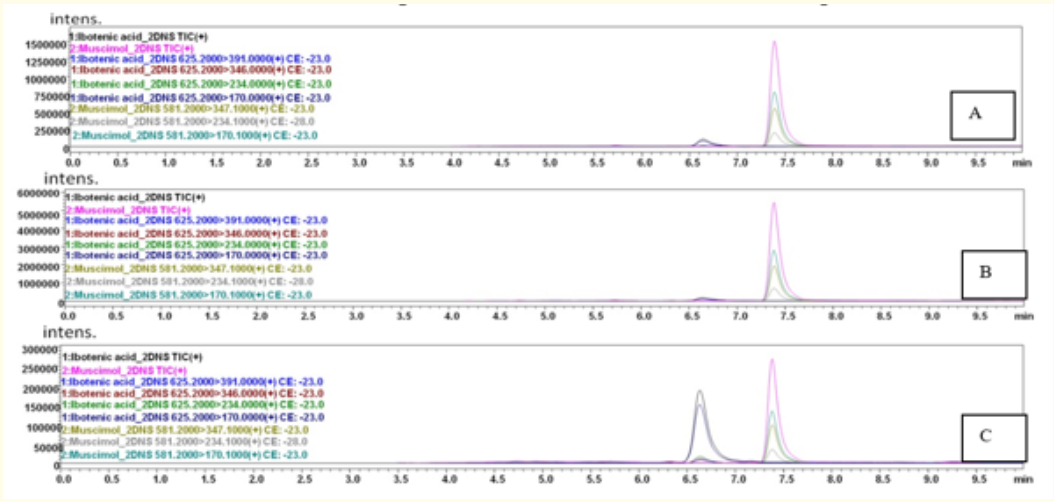


Figure 7: *Chromatogram (Shim-pack FC-ODS column) of muscimol and ibotenic acid derivatives in extracts from: (A) the contents of Amanita pantherina capsules (Fingiline, Russia); (B) the contents of Amanita pantherina capsules (ALTAI ECO, Russia); and (C) the contents of Amanita muscaria capsules (Die Familie, Russia).*

Thus, botanical identification and comparison of the chemical composition of capsules and dried caps of panther cap and fly agaric mushrooms made it possible to develop a reference material for the laboratory diagnosis of acute Amanita poisoning.

Muscimol and ibotenic acid are not routinely determined in biological specimens in chemical-toxicological practice and are not included in standard panels for narcotic and psychoactive substances.

The next stage of the study therefore focused on developing specific methods for detecting muscimol and ibotenic acid in patient urine, blood, and hair.

Experimental results showed that blood and hair do not provide reliable toxin detection because of rapid elimination and low concentrations or incorporation in these matrices. Urine is the most informative biological specimen for confirming intoxication; LC-MS/MS analysis allows both muscimol and ibotenic acid to be identified (Figure 8 and 9).

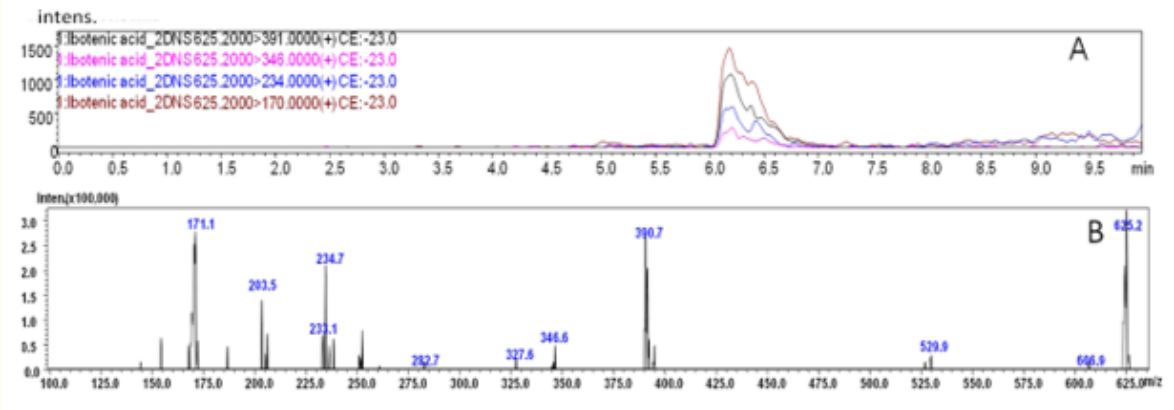


Figure 8: *MRM chromatogram (A) and product-ion spectrum of the ibotenic acid derivative (B) obtained in Product Ion Scan mode.*

Collision energy (CE): 23.

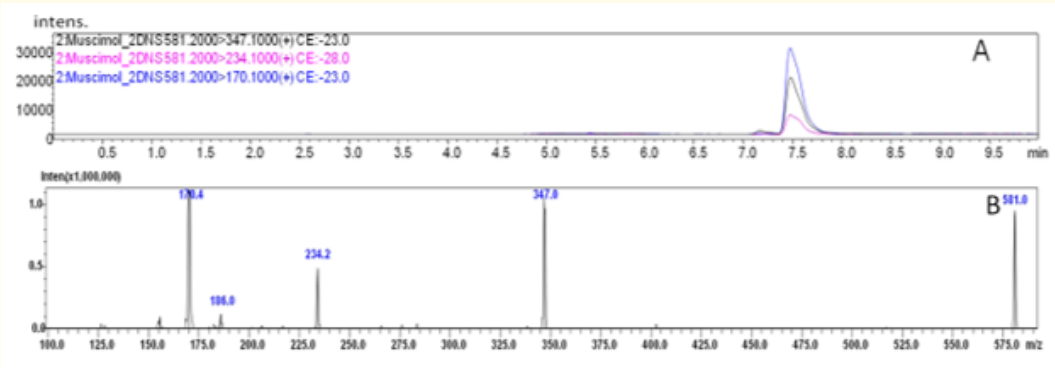


Figure 9: *MRM chromatogram (A) and product-ion spectrum of the muscimol derivative (B) obtained in Product Ion Scan mode.*

Collision energy (CE): 23.

When LC-MS/MS is unavailable, GC-MS can serve as an alternative method for diagnosing acute oral fly agaric poisoning. GC-MS offers several advantages, including high sensitivity and low detection limits, selectivity, and reliable identification supported by spectral libraries. The NIST17 mass spectral library was used in this study (Figure 10 and 11).

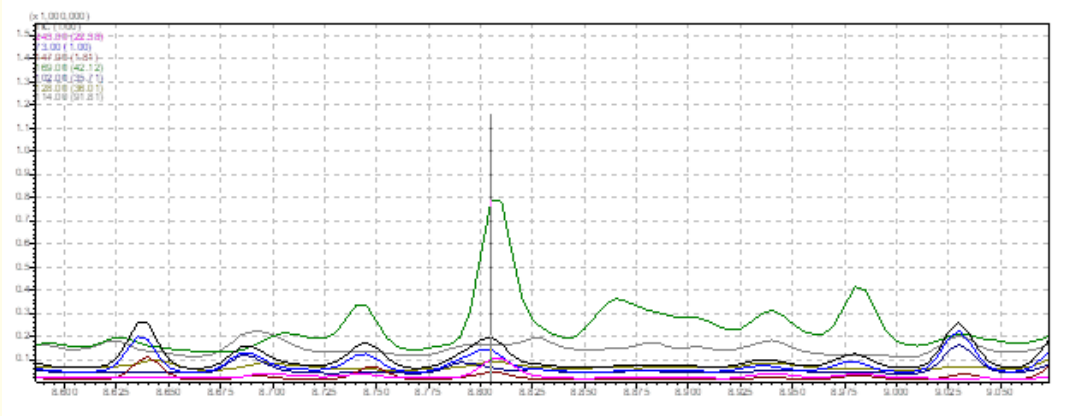


Figure 10: *GC-MS chromatogram of muscimol.*

Report View Compound Info Process Help

Hit#	Simila	Regi	Ret.	Compound Name	Mol Wt	Formula	Library
1	83	☐	960	1-(2-Methoxyethoxy)-2-methyl-2-propanol, m	162	C8H18O3	NIST17.lib
2	81	☑	1361	Muscimol, 2TMS derivative	258	C10H22N2O2	NIST17.lib
3	80	☐	1007	Acetamidoacetaldehyde	101	C4H7NO2	NIST17.lib
4	80	☐	942	(SS)- or (RR)-2,3-hexanediol	118	C6H14O2	NIST17.lib
5	80	☐	942	RS-2,3-hexanediol	118	C6H14O2	NIST17.lib
6	80	☐	734	Isoxazolidine	73	C3H7NO	NIST17.lib
7	80	☐	1141	DL-4,5-Octanediol	146	C8H18O2	NIST17.lib
8	80	☐	0	Boronic acid, ethyl-, dimethyl ester	102	C4H11BO2	NIST17.lib
9	80	☐	0	Boronic acid, ethyl-, dimethyl ester	102	C4H11BO2	NIST17.lib
10	80	☐	729	Propanamide	73	C3H7NO	NIST17.lib
11	79	☐	745	2-Butanone, 3-methoxy-3-methyl-	116	C6H12O2	NIST17.lib
12	79	☐	0	Borane, dimethoxy-	74	C2H7BO2	NIST17.lib
13	79	☐	706	N-Ethylformamide	73	C3H7NO	NIST17.lib

Figure 11: *Match of the muscimol mass spectrum with the spectral library.*

In accordance with the ICH M10 guideline "Bioanalytical Method Validation and Study Sample Analysis" (2022), the LC-MS/MS method was validated for specificity and selectivity. Chromatographic system suitability was also assessed in accordance with USP <621> Chromatography, General Pharmacopoeia Monograph 1.2.1.2.0001.15 Chromatography, and EP 2.2.46 Chromatographic Separation Techniques using the following criteria: resolution (Rs ≥ 1.5), signal-to-noise ratio (>3), retention-time agreement (deviation ±5%), efficiency (>2000 for HPLC), and symmetry (0.8-1.8). The height equivalent to a theoretical plate was additionally determined. Matrix interference and robustness were also evaluated (Table 1) [8-11].

Parameter	Observed value	Requirement according to regulatory documentation
Rs	4.2	≥1.5
Signal-to-noise ratio	Muscimol-2DNS (49.72); Ibotenic acid-2DNS (6.41)	>3
Retention time (min)	Muscimol-2DNS (7.57 ± 0.10); Ibotenic acid-2DNS (6.42 ± 0.12)	Deviation ±5%

Efficiency	Muscimol-2DNS (9773); Ibotenic acid-2DNS (6597)	>2000
Symmetry	Muscimol-2DNS (2.217); Ibotenic acid-2DNS (2.219)	0.8-1.8
Matrix interference	No endogenous-substance peaks are observed in the analytically significant region of the chromatogram, and the detection capabilities allow these effects to be disregarded.	
Robustness	The method is robust under the following conditions: Shim-pack FC-ODS column; gradient elution from 5% B to 95% B over 15 min; mobile phase A, 0.3% v/v formic acid in water; mobile phase B, acetonitrile; flow rate, 0.5 mL/min.	

Table 1: *Chromatographic system suitability results for the MRM method according to regulatory requirements, including assessment of matrix interference and robustness.*

Based on the methods developed for detecting toxicants from fly agaric and panther cap mushrooms in biological specimens, an algorithm was proposed for laboratory confirmation of mushroom ingestion (Figure 12). This algorithm uses dansyl chloride derivatization followed by LC-MS/MS detection or SPE sample preparation on Oasis HLB cartridges followed by GC-MS.

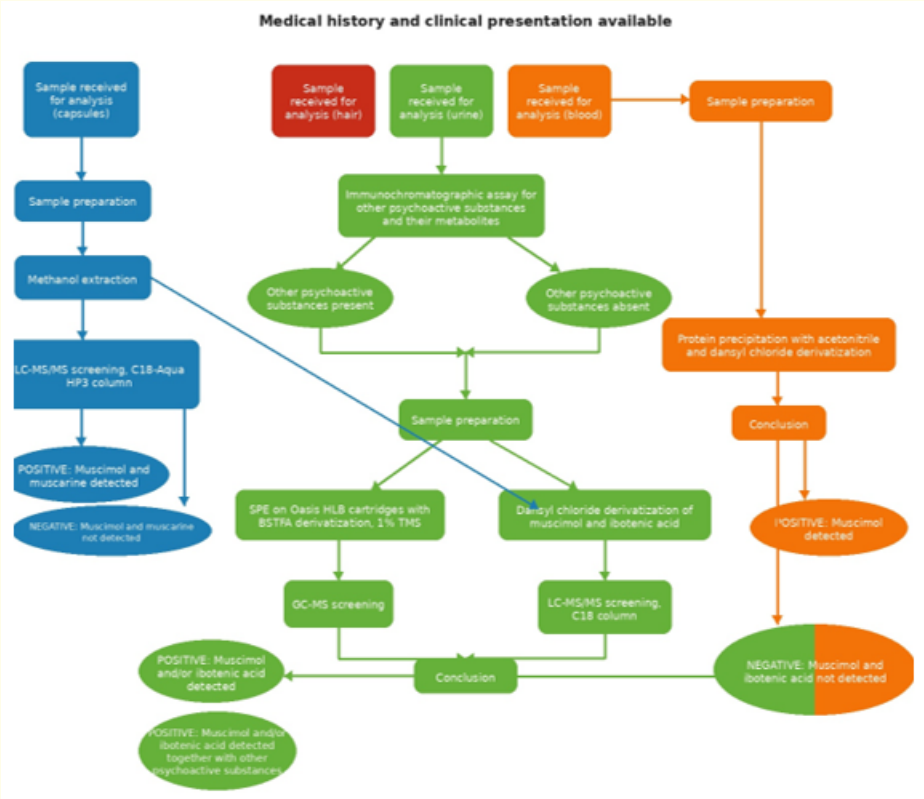


Figure 12: *Algorithm for the laboratory diagnosis of fly agaric poisoning.*

Conclusion

The botanical findings made it possible to use capsule extracts as reference samples for developing specific methods for determining the target toxicants in biological specimens. An algorithm for the laboratory diagnosis of fly agaric poisoning was proposed, and urine was shown to be the most informative specimen for chemical-toxicological analysis.

Conflict of Interest

The authors declare no conflict of interest.

Bibliography

1. Leas EC., *et al*. "Need for a public health response to the unregulated sales of *Amanita muscaria* mushrooms". *American Journal of Preventive Medicine* 67.3 (2024): 458-463.
2. Patočka J and Kočandrlová B. "Pharmacologically and toxicologically relevant components of *Amanita muscaria*". *Military Medical Science Letters* 86.3 (2017): 122-134.
3. Voynova M., *et al*. "Toxicological and pharmacological profile of *Amanita muscaria* (L.) Lam. — a new rising opportunity for biomedicine". *Pharmacia* 67.4 (2020): 317-323.
4. Zelenshchikova VA., *et al*. "Toxicologically significant properties of fly agarics and chemical-toxicological analysis in poisoning: a scientific review". *Forensic Medicine* 11.1 (2025): 63-75.
5. Bykov ZR. "Biological effects of *Amanita* sp. alkaloids on the human body" (2024).
6. Evdokimova EA., *et al*. "The problem of legalized use of fly agarics and diagnosis based on the results of clinical laboratory diagnostics: a literature review". *Issues of Biological, Medical and Pharmaceutical Chemistry* 28.6 (2025): 46-52.
7. Xu X-M., *et al*. "Determination of ibotenic acid and muscimol in plasma by liquid chromatography-triple quadrupole mass spectrometry with bimolecular dansylation". *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences* 1146 (2020): 122128.
8. USP <621> Chromatography.
9. General Pharmacopoeia Monograph 1.2.1.2.0001.15 Chromatography.
10. European Pharmacopoeia 2.2.46 Chromatographic Separation Techniques.
11. ICH M10. Bioanalytical Method Validation and Study Sample Analysis (2022).

Volume 14 Issue 8 November 2026
©All rights reserved by O Yu Strelova., *et al.*