Accidental Intoxication with *Veratrum album*

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**Abstract**

A 49-year-old man consumed two glasses (approximately 2 × 20 mL) of a beverage containing yellow gentian (*Gentiana lutea*). Shortly after ingestion, he developed nausea, vomiting, and oral paraesthesia. On admission to the hospital he suffered from severe bradycardia (35 beats/min) and hypotension (50/30 mm Hg), and he was treated with activated charcoal, antiemetics (metoclopramide, ondansetron), atropine, and intravenous electrolytic solution. The initial suspicion of *Veratrum* poisoning could be confirmed by identifying protoveratrines A (ProA) and protoveratrine B (ProB) in a sample from the beverage as well as in the patients serum by liquid chromatography–electrospray ionization-tandem mass spectrometry (LC–ESI–MS–MS). The yellow-colored beverage contained 25% ethanol (by headspace gas chromatography), 20.4 mg/L ProA, and 13.7 mg/L ProB. The serum concentration of ProA was 1162 ng/L and ProB was 402 ng/L. Veratridine, cevadine, and jervine were not detected, neither in the beverage nor in the serum sample. The lower limits of quantitation for all compounds is 10 µg/L (S/N > 10, beverage) and 100 ng/L (S/N > 10, serum). After treatment, the patient completely recovered from the symptoms within 24 h and was discharged from the hospital. The analytical method described was developed for the simultaneous identification and quantitation of five *Veratrum* alkaloids. The method is based on a liquid–liquid extraction followed by LC–MS–MS analysis. The time needed for analysis was 6 min.

**Introduction**

**Botany**

*Gentiana lutea* (Great Yellow Gentian) or “Yellow Gentian” is an herbaceous perennial plant, growing to 1–2 m tall, with broad lanceolate to elliptic leaves approximately 10–30 cm long and 4–12 cm broad and yellow flowers. The plant is best known for its intensely bitter properties residing in the root and every part of the herbage, hence it is used as tonic medicine. Gentian root has a long history in the treatment of digestive disorders and is an ingredient in a variety of medicines (anthelmintic, anti-inflammatory, antiseptic, bitter tonic, cholagogue, emmenagogue, febrifuge, refrigerant and stomachic). It contains some of the most bitter compounds known (e.g., amarogentine) (1).

*Veratrum album* commonly known as the White Hellebore, which is similar to *Gentiana lutea* native to central or southern Europe (800–2700 m) is a perennial plant with greenish yellow flowers and stems of 50–175 cm tall. Both species are alpine plants and when not in flower, the leaves seem to show a similar morphology at first sight. Various reports describe the ingestion of fermented tea or wine decoctions containing *Veratrum album*, which is mistaken for *Gentiana lutea* (2–5). In comparison to *Gentiana lutea*, the plant is highly toxic. Alkaloids were found in leaves (up to 1.5%), in the roots, and also in the rhizome (both up to 1.6%). For example, the rhizome of *Veratrum album* (veratri rhizoma) contain a range of alkaloids (1,6–9), which can be classified into different groups, for example, with a typical steroidal skeleton (jervine, pseud jervine, veratramine) or with a cevanine skeleton [protoveratrine A (ProA) and protoveratrline B (ProB)] (10,11). In contrast, the esteralkaloids ProA and ProB represent the largest group in *Veratrum album* (1).

Some confusion can occur, because related alkaloids are found in *Veratrum californicum*, *Veratrum viride* and *Veratrum nigrum* (12,13). Also, Amianthium muscitoxicum, Zigadenus, and Schoenocaulon officinale are other sources of *Veratrum* related alkaloids. For example, the seeds of Schoenocaulen officinale, commonly called “Sabadilla seed”, which were used as insecticides, yield a mixture of alkaloids, called “veratrine”, and contain principal constituents of veratrine, cevadine and veratridine, the two largest sabadilla components and several others like cevacine (10,14–16).

**Pharmacotoxicology**

*Veratrum* alkaloids act by increasing the permeability of sodium channels of excitable cells, causing them to fire prematurely and then leaving them refracted. The onset of symptoms occurs between 30 min and 4 h after the ingestion of the *Veratrum* plant (beverages containing *Veratrum* plant in traces). The symptoms are vomiting, nausea, and abdominal pain. They are followed by cardiovascular effects such as severe bradycardia, hypotension, and in severe cases, cardiac conduction abnormalities and death (4,12). Clinical studies monitoring the effects of *Veratrum* alkaloids on blood pressure exhibited diverse findings (17–20). With prompt supportive care, patients usually make a full recovery within the first day. In cases of
such intoxication, atropine is the treatment of choice (12).

In general, severe poisoning with Veratrum plant material is quite seldom. For example, the Swiss Toxicological Centre studied approximately 25,000 cases (period: 1966–1996) of exposure to toxic plant material. Veratrum album was involved in only eight non-fatal cases. Six intoxicated adults suffered from bradycardia (< 40 beats/min), and five presented circulatory shock (21).

Severe intoxications were reported when Veratrum album rhizomes were dried and marketed as sneezing powders (22,23). After a series of incidents (the alkaloids can be easily absorbed through undamaged skin) (22), these powders were removed from the market. In one reported case, a valerian (Valeriana officianalis) tincture contained Veratrum alkaloids (24), and in another case, a dried root of Veratrum album was mistaken for a dried valerian root to make sedative tea (25).

One or two grams of dried root powder could be fatal to humans (26). Gaillard and Pépin (27) reported two fatal poisonings due to the ingestion of plant material. The examination of the stomach contents revealed the presence of a large number of small blackish granules, which were later identified as seeds of a Veratrum species. Veratridine and cevadine were identified and quantitated by high-performance liquid chromatography–mass spectrometry (HPLC–MS). Measured blood concentrations were 170 and 400 ng/L for veratridine and 320 and 480 ng/L for cevadine.

Analytical methods

Only few analytical methods for the determination of Veratrum alkaloids in blood or urine are described in the literature (28). Veratridine and cevadine were identified and quantitated by HPLC–MS (21). Identification from plant material before 1990 was carried out by paper chromatography (16), thin-layer chromatography (TLC), and chemical ionization MS (5). Some procedures are dealing with the determination and/or quantitative analysis using HPLC–UV (15,29), HPLC–APCI-MS (14), or HPLC–electrospray ionization (ESI)-multistage MS (13). HPLC–(ESI)-MSn was performed to study the fragmentation pathways of 17 steroidal alkaloids (e.g., protoveratrine A). For the identification of new alkaloids a combination of spectral methods (IR, MS, 1H- and 13C-NMR, COSY, and NOESY) were used (30).

Herein, a rapid and sensitive method for the determination of five Veratrum alkaloids in serum with a simple extraction procedure was developed. The chemical structures of alkaloids, (identified by our method) are shown in Figure 1. Although these alkaloids have fairly close chemical structures and despite variations in their effects (17), their general mode of action is approximately the same (4).

Case History

A 49-year-old man reported s.o. an ingestion of two glasses (approx. 2 × 20 mL) of a self-made alcoholic root beverage containing what he thought was yellow gentian (Gentiana lutea). Shortly after ingestion, he developed nausea, vomiting and oral paraesthesia. On admission to the hospital he suffered from severe bradycardia (35 beats/min) and hypotension (50/30 mm Hg). Other laboratory findings were negative (except Glucose/P: 158, range: 55–110) mg/dL). For primary detoxification, he received activated charcoal. Additional medications were atropine, metoclopramide, and ondansetron, along with intravenous electrolytic solution for symptomatic treatment. With intoxication by Veratrum alkaloids suspected, the beverage and a serum sample were sent to our institute for investigation.

Materials and Methods

Because intoxication by Veratrum alkaloids was suspected, the serum and the beverage were analyzed using the described LC–MS–MS method. This method enables the identification and quantitation of five Veratrum alkaloids: ProA, ProB, veratridine, cevadine, and jervine.

For the identification of selected Veratrum alkaloids in the beverage, a precursor ion mass spectrum and fragmentation experiments were performed. The quantitation of ethanol was made by headspace gas chromatography (HS-GC) (31).

Chemicals

All chemicals, reagents, and solvents were of analytical grade. Dichloromethane, methanol, and formic acid were purchased from Riedel de Haen (Seelze, Germany). Protoveratrine A, protoveratrine B, veratrine (a mixture of alkaloids consisting of approximately 38% veratridine, 59% cevadine, and 3% other alkaloids) (14) were from Sigma-Aldrich (Seelze, Germany). Jervine and the
internal standard (IS) fentanyl-d₅ were obtained from Promochem (Wesel, Germany). Water was purified using an osmosis system (Membra Pure, Bodenheim, Germany).

Sample preparation

Beverage (precursor ion mass spectrum/fragmentation experiments). The sample/analytical standard was diluted 1:10 with a methanol/water mixture (1:2) and directly infused at a flow rate of 10 µL/min into the LC–MS–MS system.

For the quantification of ethanol by HS-GC, the sample was previously diluted with water (1:100 and 1:200).

Serum. A total of 1.0 mL serum, 0.4 mL extraction reagent [50 µL fentanyl-d₅ (c = 0.01 mg/mL in methanol) dissolved in 50 mL dichloromethane] were mixed in a 1.5-mL eppendorf cup for 2 min. The sample was centrifuged for 2 min at 15,000 × g and 0.3 mL of the organic layer was evaporated to dryness under a stream of nitrogen at 30°C. The residue was redissolved in 0.1 mL of methanol.

Quantitation

Beverage. A methanol/water mixture (1:2) was spiked at five concentrations of each analyte (10, 50, 100, 250, and 500 µg/L). Quantitation followed the external calibration method. The beverage was measured undiluted and diluted (1:50, 1:100, and 1:200).

Serum. The drug concentration in the serum samples was calculated using the peak-area ratios of the base peak ions of the target ions versus IS. For quantitation, drug-free serum was spiked at six concentrations of each analyte (100, 250, 500, 1000, 1500, and 2000 ng/mL). Quantitation followed the internal standard method. In-house quality control (QC) samples were prepared in the matrix being examined (each analyte c = 300 and 800 ng/mL). QC were accepted if they were within ± 20 % of target.

LC parameters

A Shimadzu (Duisburg, Germany) LC system equipped with three pumps (LC-20AD), two solvent degasser (DGU-20A), an autosampler (SIL-HT), an oven (CTO-10AS), two 7-port, 6-position high-pressure valves (FCV-14AH), two 6-port, 2-position valves (FCV-12AH) was used. Analytical separation was carried out using a Varian Pursuit 5 pentafluorophenyl (PFP) column (150 × 3.0 mm, 5 µm) The oven temperature was 60°C. The gradient consisted of a mixture of solvent A [methanol/0.1% HAc with 10 mM NH₄Ac (97:3)] and solvent B [0.1% HAc with 5 mM NH₄Ac/methanol (90:10)] pumped at a flow rate of 0.55 mL/min. The following gradient was used: 0.0–4.0 min: 6% B linear; 4.0–4.6 min: 6% B linear; 4.6–5.0 min: 95% B linear; and 5.0–6.0 min: 95% B linear. The injection volume was 25 µL.

MS parameters

The MS ABI 3000-system was obtained from Applied Biosystems/MDS Sciex (Darmstadt, Germany). The instrument operated in the positive mode with ESI. MS parameters were optimized in tune mode using flow injection analysis (FIA). For all compounds, turbo ionspray voltage and capillary temperature were set at 5.5 kV and 450°C, respectively. Nebulizer (air), curtain (N₂), and collision gas (N₂) pressures were maintained at 13, 6, and 12 units, respectively. Drying gas flow rate was set at 8 L/min. Compounds were quantitated in the multiple reaction mode (MRM). MRM was performed with 40 ms dwell time per channel. The MS was operated at unit mass resolution (peak width at half-height set at 0.7 Da) for both Q1 and Q3. The following transitions were monitored (m/z): ProA: 794.3 → 776.3/676.3/658.3; ProB: 810.4 → 792.2/676.3/658.3, veratridine: 674.3 → 492.4/456.3/165.1; cevadine: 591.7 → 574.3/456.3/162.3; jervine: 426.0 → 405.2/114.1/84.0; and fentanyl-d₅ (IS): 342.1 → 188.1. The HPLC and the MS were controlled by Analyst™ software (Applied Biosystems/MDS Sciex, Analyst software version 1.4.1).

Results and Discussion

Corresponding to the suspected Veratrum album intoxication, the beverage and the serum sample were analysed with the described analytical method.

Beverage. Commercial gentian beverage is a clear or pale-yellow-colored liquid (does not contain less than 38% ethanol). In contrast, the beverage was deep yellow, and the ethanol concentration was 25%. We analyzed the beverage for veratrine alkaloids using our LC–MS–MS system, and we recorded a precursor ion mass spectrum from m/z 50 to 1000 (Figure 2).

As a result, prominent peaks with ion masses of supposedly protonated [M+H]+ ProA (m/z 794), ProB (m/z 810) and jervine (m/z 426) were identified but no peaks of protonated veratriine (m/z 674) or cevadine (m/z 592) could be found in the spectrum. For the confirmation of the findings, analytical standards and the beverage were directly but separately infused into the ESI source for fragmentation experiments. The analytical standards were found to produce predominately [M+H]+ ions molecular adducts in positive-ion mode by ESI-MS. Consequently, the [M+H]+ base peak was used in further investigations. ProA was clearly identified in the beverage. The fragmentation patterns (or fingerprints) of ProA and the beverage were exactly the same (m/z 794 → 776/676/658/556/436/164/162) (Figure 3).
Although there was a high signal of the ion mass at m/z 426 in the precursor ion mass spectrum of the beverage, the fragmentation pattern of the analytical standard jervine and the beverage were not identical. In comparison to ProA, the fingerprint of jervine is less complex. However, three fragments of the analytical standard (m/z 426 → 408/145/128/114/112) were missing in the cracking spectrum of the beverage (m/z 426 → –/–/–/114/–) (Figure 4). Hence, jervine could not be identified in the beverage.

Consequently, ProB, veratridine and cevadine were fragmented in the same manner. As expected, the presence of ProB could be confirmed (fingerprint standard/beverage, m/z 810 → 792/676/658/556/162), whereas veratridine (fingerprint standard, m/z 674 → 656/492/474/456/165; beverage: 674 → 656/–/474/–/–) and cevadine (fingerprint standard, m/z 592 → 575/474/188/162; beverage: 592 → 575/474/–/–/162) were not in the beverage.

Most published cases related to Veratrum poisonings contain no information of blood level concentrations. To our knowledge, only one LC–MS method has been reported for the determination of two Veratrum alkaloids (veratridine and cevadine) from gastric content and heart blood (27).

This paper presents a selective and sensitive method for the simultaneous identification and quantification of five Veratrum alkaloids by LC–(ESI)-MS–MS. The chosen conditions allowed a fast elution with good peak shapes in 6 min. The following retention times were recorded (min): jervine (4.82), ProB (5.04), cevadine (5.14), veratridine (5.21), and ProA (5.35). The general working conditions (mobile phase, analytical column, ESI-source) corresponded to the LC–MS–MS standard configuration in our laboratory, so no extra time for set up was needed. To avoid a possible misidentification, three transitions of the former fragmentation experiments were used.

For the quantification of the alkaloids, a five-point calibration (10, 50, 100, 250, and 500 µg/L) was performed in a water/methanol mixture (1:2). The calibration curves of all compounds were linear (R ≥ 0.995). The beverage was measured undiluted and diluted (1:50, 1:100, and 1:200). The concentration of ProA was 20.4 mg/L and of ProB was 13.7 mg/L, respectively. No veratridine, cevadine or jervine were detected, neither in the diluted nor in undiluted sample [c < 10 µg/L (S/N ≥10)].

Because the alkaloids under examination show a common steroidal backbone, the beverage was tested for cross-reaction in two assays for heart glycosides. For this purpose, the beverage was diluted with water (1:10) and analyzed for digoxin (FLX/TDx) and digitoxin (FPIA) (both assays from Abbott Laboratories (Wiesbaden, Germany)). Digoxin and digitoxin resulted negative.

For serum quantification, a five-point calibration (100, 200, 500, 1000, and 1500 ng/L) was performed. The actual calibrations showed linearity for all analytes within the range of...
100–1500 µg/L (R ≥ 0.995) and the QC samples met the target level of ± 20% of the rated values (300 ng/L < 10% and 800 ng/L < 14%). The limit of quantification was 100 ng/L (S/N ≥ 10). Six drug-free human serum samples of six different sample tubes were successfully tested for the absence of Veratrum alkaloids.

The serum sample was collected approximately 6 h after the ingestion of the beverage. The serum ethanol concentration was < 0.1 g/L. Because of insufficient material, the serum sample had to be diluted with drug-free serum (1+1). It is evident from the chromatograms that ProA was clearly identified through its three transitions and their three equal retention times (Figure 5).

The concentration of ProA was 1162 ng/L and of ProB was 402 ng/L, respectively. To our knowledge, ProA and ProB could be quantitated for the first time in human serum using LC-MS-MS. Veratridine, cevadine and jervine were not detected in the serum (c < 100 ng/L).

The patient was continuously monitored in hospital (e.g., heart rate, blood pressure). Heart rate returned to normal within 8 h after admission. After a consequent treatment the patient completely recovered from symptoms within 24 h.

Severe Veratrum album poisoning is a rare event. In most cases the plant was mistaken for yellow gentian. Quatrehomme et al. (4) summarized 32 cases from the medical literature (period: 1912–1987) when Veratrum album was involved. Eight of 25,000 cases of exposure to toxic plant material were reported to the Swiss Toxicological Centre (period: 1966–96). Between 1976 and 1981, seven cases of plant material ingestion, including leaves, parts of the root, and supposedly gentian wine have been reported to the Austrian Poison Information Centre (32). In Italy, two men consumed a supposed yellow gentian beverage that was freshly prepared by a friend. Both developed similar symptoms: nausea, abdominal discomfort, and bradycardia (< 44 beat/min). The patients received standard therapy (antiemetics and volume, and one patient received activated charcoal as well) and recovered within 8 and 48 h, respectively (2).

In France, four of five people showed sinus bradycardia after the ingestion of homemade, supposedly gentian wine. In one patient, complete atrioventricular block with an ectopic atrial bradycardia and an intermittent idioventricular rhythm were recorded. Symptomatic treatment with an emphasis on hemodynamic stability with fluid replacement and atropine led to recovery within a few hours.

Figure 5. Ion chromatogram of the serum sample containing protoveratrine A (cPro A = 1162 ng/L) (A, 3 transitions) and the internal standard fentanyl-d₅ (B).
Conclusions

A case of Veratrum album intoxication is reported. A man ingested self-made alcoholic beverage (25% ethanol) containing Veratrum album that was mistaken for Gentiana lutea (yellow gentian) and developed severe bradycardia (35 beats/min) and hypotension (50/30 mm Hg). Veratrum species contain a mixture of steroidal alkaloids that process such symptoms. Using three transitions, we could identify the inotropically acting Veratrum album protoveratrine A and protoveratrine B in the beverage and in the patient’s serum. The beverage contained 20.4 mg/L ProA and 13.7 mg/L ProB, the serum 1162 ng/L ProA and 402 ng/L ProB. Veratridine, cevadine, and jervine were not detected, neither in the beverage nor in the serum sample.

After a consequent symptomatic treatment, the patient completely recovered and was discharged from the hospital within 24 h.

The LC–MS–MS method described is capable of simultaneous determination and quantitation of five Veratrum alkaloids in human serum. The method is based on a simple, fast liquid–liquid extraction. The time for analysis is 6 min.

References


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