Received: 5 July 2022 | Revised: 18 August 2022 | Accepted: 19 August 2022

DOI: 10.1002/dta.3360

SHORT COMMUNICATION

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Analytical toxicology of yew constituents in human blood and urine by liquid chromatography-high-resolution tandem mass spectrometry

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Abstract

The active, poisonous constituents in *Taxus baccata*, the yew plants, are taxine alkaloids whose main action is suggested to be a block of calcium and sodium channels. The main alkaloids are taxine B (30%) and taxine A (1.3%). Symptoms can include bradycardia, bradypnea, diastolic, and cardiac standstill. The current investigation reports the analytical toxicology of human blood and urine to confirm a suspected ingestion of yew needles. This includes the qualitative detection of several yew ingredients, including the main alkaloids, the validated quantification of 3,5-dimethoxyphenol, and the discussion of suitable analytical targets. After analyzing human specimens and yew needle extracts using the developed procedures, the five alkaloids 1-deotaxine B, taxicatin, taxine A, taxine B, and taxine I could be detected and tentatively identified. Finally, taxine A and B can be recommended as analytical targets besides 3,5-dimethoxyphenol.

KEYWORDS
analytical toxicology, clinical toxicology, liquid chromatography, mass spectrometry, yew intoxication

1 | INTRODUCTION

The coniferous *Taxus baccata* (family of Taxaceae) is an evergreen poisonous tree or shrub, which is commonly used for landscaping.[1] The poisonousness of the yew plant is known since the second century B.C.E. Yew already played an important role in the celtic mythology and as poison for hunting. The active, poisonous constituents in yew plants are taxine alkaloids whose main action is suggested to be a block of calcium and sodium channels. The main alkaloids are taxine B (30%) and taxine A (1.3%).[2] The content of cephalomannine, paclitaxel, and 10-deacetylbaccatin-III is much lower.[3,4] Taxines are present in all parts of the plant except the scarlet, berry-like aril. They are abundant in English yew (*T. baccata*) and Japanese yew (*Taxus cuspidata*); however, only minimal amounts of taxines are found in Pacific yew (*Taxus brevifolia*). Besides heart failure, ingestion of yew needles can cause dizziness, pupil dilation, nausea, vomiting, diffuse abdominal pain, initially tachycardia, muscle weakness, and convulsions. These symptoms can proceed to bradycardia, bradypnea, cardiac arrest, and finally death.[2] The lethal dose of yew needles for an adult was reported to be 50 g, which equals to 250-mg taxine alkaloids or approximately 3-mg taxine per kilogram body weight.[1] Because no antidote for yew needle intoxication is known, the clinical management is essentially symptomatic and supportive. Intensive treatment, for example, with admission of antiarrhythmic drugs, atropine, and anti-digitalis Fab-Fragment, as well as a temporary pacemaker and excessive diuresis were discussed.[5] *T. baccata* contains

further constituents such as nonalkaloidal diterpenoids (e.g., 10-deacetetylbaccation-III) or phenolic continents (e.g., 3,5-dimethoxyphenol), but also flavonoids.[1] The determination of different yew constituents by liquid chromatography (LC) or gas chromatography coupled to mass spectrometry (MS) has been reported.[1,6–9]

The current study presents a fast analytical method using LC-high-resolution tandem MS (LC-HRMS/MS) for the detection of yew constituents in human biosamples. Furthermore, suitable analytical targets were identified and recommended after also analyzing yew needle extracts and available analytical reference standards.

2 | EXPERIMENTAL

Information on chemicals and reagents can be found in the Supporting Information (1. Chemicals and reagents).

2.1 | Samples and sample preparation

A person supposedly ate 100 g of yew needles with suicidal intent and developed nausea, vomited several times, became somnolent, and was brought to hospital. The electrocardiogram showed a sinus-rhythm, a broad QRS-complex, a prolonged QT interval, a complete right bundle brunch block, and stable rhythm. An esophago-gastro-duodenoscopy was carried out, and a large quantity of plant material assumed to be yew needles could be removed. A poison information center was consulted, and anti-digitalis Fab-Fragment, lidocaine, and atropine were recommended. During the course, the QRS-complexes became narrower; however, QT interval was prolonged with 468 ms but the subject finally completely recovered.

Blood and urine samples taken approximately 50 h after supposed yew needle intake were submitted to the laboratory for regular clinical toxicological analysis. To 100 µl of either plasma or urine, a volume of 10 µl of internal standard (trimipramine-d_3, 0.01 mg/l, for plasma, diazepam-d_5, 0.01 mg/l, for urine) was added, and a dilution with 500 µl of acetonitrile was carried out. Samples were shaken for 2 min at 1500 rpm at room temperature (24°C) using a Thermoshaker Pro (CellMedia, Elsteraue, Germany) before 2 min of centrifugation at 10,000×*g*. The supernatant was evaporated to dryness under a nitrogen flow at a temperature of 30°C and samples were afterwards reconstituted in 50 µl of methanol.

2.2 | Yew needle extraction

Four fresh yew needles (±0.08 g, $n = 2$, from two different yew trees) were cut into small pieces with a scissor and extracted with 5 ml of methanol in a glass vial, which was gently shaken manually for 5 min. A volume of 1.5 ml of the supernatant was dried under a gentle stream of nitrogen at 30°C, and the samples were reconstituted in 100 µl of methanol.

2.3 | Analytical procedure for qualitative detection of yew constituents in human blood and urine

The analysis was performed using a Thermo Fisher Scientific (TF, Dreieich, Germany) Dionex UltiMate 3000 Rapid Separation LC system consisting of a degasser, a quaternary pump, and an HTC PAL autosampler (CTC Analytics AG, Zwingen, Switzerland) coupled to a TF Q-Exactive Plus mass spectrometer with a heated electrospray ionization (HESI)-II source. An external mass calibration was performed prior to analysis according to the manufacturer's recommendations. The injection volume was 5 µl for all samples. Gradient elution was performed on a TF Accucore Phenyl-Hexyl column (100 mm × 2.1 mm, 2.6 µm) at 40°C. The mobile phases were composed of 2-mM aqueous ammonium formate containing formic acid (0.1%, v/v, pH 3, eluent A) and 2-mM ammonium formate solution with acetonitrile:methanol (1:1, v/v), water (1%, v/v), and formic acid (0.1%, v/v, eluent B). The gradient was programmed as follows: 0–0.5 min hold 99% A, 0.5–2 min to 70% A, 2–6 min hold at 70% A, 6–7 min to 1% A, 7–7.5 min hold 1% A, 7.5–9 min hold 99% A. The flow rate was set to 500 µl/min (0–7.5 min) and 800 µl/min (7.5–9 min). The following HESI-II source conditions were used: heater temperature, 320°C; ion transfer capillary temperature, 320°C; spray voltage, 4.0 kV; ionization mode, positive; sheath gas, 60 arbitrary units (AU); auxiliary gas, 10 AU; sweep gas, 0 AU and S-lens RF level, 60.0.

Mass spectrometry was performed in full scan with a subsequent data-dependent MS^2 ($ddMS^2$) with priority to *m/z* of yew constituents (see Table 1). The settings for full scan data acquisition were the following: resolution, 35,000; microscans, 1; automatic gain control (AGC) target, 1e6; maximum injection time (IT), 120 ms; scan range,

TABLE 1 Selected constituents of yew, their sum formula, and exact protonated masses

Compound	Sum formula	$[M + H]^+$, *m/z*
10-Deacetyl-7-xylosylpaclitaxel (10-DAXP)	$C_{50}H_{57}NO_{17}$	944.3699
10-Deacetylbaccatin III	$C_{29}H_{36}O_{10}$	545.2381
10-Deacetyltaxol (10-DAP)	$C_{45}H_{49}NO_{13}$	812.3277
1-Deoxytaxine B	$C_{33}H_{45}NO_7$	568.3269
2-Deacetyltaxine A	$C_{33}H_{45}NO_9$	600.3167
3,5-Dimethoxylphenol	$C_8H_{10}O_3$	155.0703
Baccatin III	$C_{31}H_{38}O_{11}$	587.2487
Itaxine B	$C_{33}H_{45}NO_8$	584.3218
Monoacetlytaxin	$C_{29}H_{27}NO_9$	534.1759
Taxicatin (3,5-dimethoxylphenol-glucosid)	$C_{14}H_{20}O_8$	317.1231
Taxine I	$C_{37}H_{49}NO_{10}$	668.3429
Taxine A	$C_{35}H_{47}NO_{10}$	642.3273
Taxine B	$C_{33}H_{45}NO_8$	584.3218
Taxol A (paclitaxel)	$C_{47}H_{51}NO_{14}$	854.3382
Taxol B (cephalomannine)	$C_{45}H_{53}NO_{14}$	832.3539

m/z 130 to 860 due to the *m/z* of expected yew constituents. The following settings were selected: Option "pick others," enabled; dynamic exclusion, feature not used; resolution, 17,500; microscans, 1; isolation window, 1.0 *m/z*; loop count, 5; AGC target, 2e5; maximum IT, 250 ms; high collision dissociation cell with stepped normalized collision energy, 17.5, 35.0 and 52.5; exclude isotopes, on and spectrum data type, profile. ChemSketch 2010 12.01 (ACD/Labs, Toronto, Canada) was used for drawing of chemical structures of yew constituents, as well as for calculation of their exact masses. TF Xcalibur Qual Browser software version 4.0.27.19 was used for MS data analysis. Selectivity of the method for the suspected targets (see Table 1) was tested against blank urine and plasma samples.

2.4 | Method validation for the quantitative detection of 3,5-DMP

Samples were prepared and analyzed as described under 2.1 and 2.3. Because 3,5-DMP was a proposed marker for yew needle intoxication and quantitative data are available in literature,[10,11] a basic validation for a quantitative method was done (details in the Supporting Information).

3 | RESULTS AND DISCUSSION

3.1 | Qualitative detection of different yew constituents in plasma and urine

An easy sample preparation and a fast chromatographic separation (see Figure 1) could be developed for analysis of blood and urine samples after suspected intake of yew needles. It allowed the detection of several signals corresponding to the exact protonated masses (see Table 1) of 1-deotaxine B ($n = 3$ peaks), 3,5-DMP, taxicatin, taxine A, taxine B ($n = 4$), and taxine I ($n = 2$) after full scan analysis of plasma extracts. After full scan analysis of urine extracts, several signals corresponding to the exact masses of 3,5-DMP and taxine B ($n = 2$) could be detected (see Table 2). Interpretation of MS^2 spectra, which can be found in Figures S1–S7, was used for tentative identification of analytes. Additional spectra were recorded at a normalized collision energy of 60 eV (Figures S8 and S9). For further analysis and identification, the reference compound of taxicatin (3,5-dimethoxylphenol-glucosid) could be purchased and the identity in the samples could be confirmed. An in-source fragmentation of taxicatin was additionally observed, which was already assumed after analysis of the case samples. Hereby, the glycoside is removed resulting in formation of 3,5-DMP (*m/z* 155.0703).

The MS^2 data and retention times (RTs) acquired after additional analysis of the yew needle extracts were compared with those after analysis of the subject's samples. The RT and the MS^2 data of 1-deotaxine B, taxicatin, taxine A, taxine B, and taxine I could be confirmed. Interestingly, signals corresponding to the *m/z* of 1-deotaxine B, taxine B, and taxine I could be observed at different RTs in the chromatogram of the subject's samples and the yew needle extracts. Underlying MS^2-spectra at least of taxine B matched to the respective library entries.[12] Thus, they were considered as isomers.

3.2 | Qualitative and quantitative determination of 3,5-dimethoxyphenol in human plasma

3,5-DMP, the aglycone of taxicatin, is formed in the human intestine by cleavage of a glycosidic bond and afterwards absorbed into the

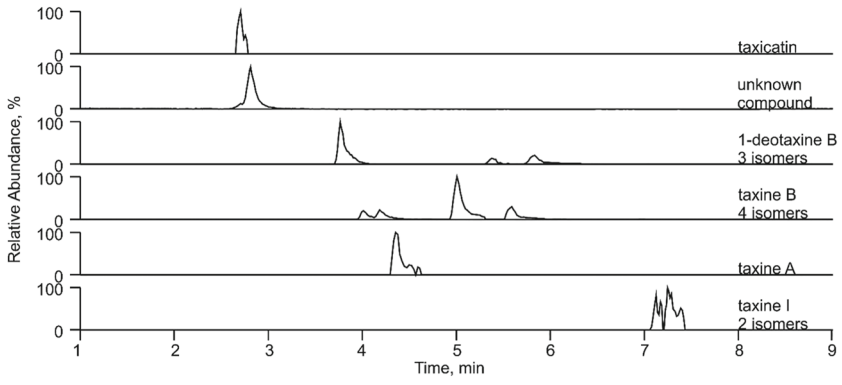

FIGURE 1 $[M + H]^+$ chromatograms of yew constituents after analyzing human plasma extracts

TABLE 2 Yew constituents detected in human samples (x: not detected; ✓: detected)

	1-Deotaxine B	Taxicatin	Taxine A	Taxine B	Taxine I	Unknown compound, *m/z* $[M + H]^+$ 155.0703
Plasma	✓ (3 isomers)	✓	✓	✓ (4 isomers)	✓ (2 isomers)	✓
Urine	x	x	x	✓ (2 isomers)	x	✓

blood.[13] Because 3,5-DMP was proposed as marker for yew needle intoxications and reference concentrations after intake were available, a quantitative method should be developed.[10,11] Detailed results of the validation can be found in the Supporting Information. Briefly, selectivity of the method was given and no carry-over could be detected after injection of a processed plasma sample containing 100 ng/ml of 3,5-DMP. Within-day accuracy and precision, as well as, dilution integrity testing, and benchtop stability in plasma (36 h, 24°C) were in line with recommendations.[14]

Analysis of the clinical toxicological samples revealed a shift in RT (+0.6 min) between the signal of the reference compound and the suspected 3,5-DMP signal in subject's plasma and urine samples (see Table S3). Because the MS^2 data were identical, the presence of an isomer was suspected. All isomers of 3,5-DMP, namely 2,3-DMP, 2,4-DMP, 2,5-DMP, 2,6-DMP, and 3,4-DMP were thus purchased and analyzed. However, all isomers showed an RT shift compared with the signal in the subject samples (see Table S3), which lead to the conclusion that the observed signal may come from a still unidentified compound and the identity of this compound remains unclear. It cannot be excluded that the peak at m/z 155.0407 at RT 2.8 min resulted from a 3,5-DMP conjugate after its ion source fragmentation. Furthermore, the signal of the unknown compound but also of 3,5-DMP could not be observed in the yew needle extracts. In summary, we developed a quantitative method suitable for detection and quantification of 3,5-DMP, the proposed marker for yew needle intoxication. However, we were not able to detect 3,5-DMP in the samples nor in the yew extracts. Absence of a signal due to instability of 3,5-DMP in patients' plasma could be excluded because benchtop stability testing revealed no degradation of the compound. A possible explanation for the absence of 3,5-DMP in the samples could be the origin and species of yew but also the delayed sampling of approximately 50 h. Furthermore, concentration of yew constituents can vary during seasons. Grobosch et al. also described cases were 3,5-DMP could not be detected even though taxines were identified after the ingestion of a few yew needles.[15] It should also be noted that 3.5-DMP is a component of many plants and even fruits like grapes and is therefore not specific for yew intoxications.[13] We would therefore recommend to also include other yew ingredients such as taxine A and B into screening strategies, for example, as long-time marker of a yew (needle) ingestion.

4 | CONCLUSION

In case of intoxications, fast and reliable analytical procedures facilitate a rational treatment of the patient. The presented ad hoc LC-HRMS/MS method could detect the yew constituents 1-deotaxine, taxicatin, taxine A, taxine B, and taxine I in plasma and/or urine. The previously proposed marker for yew needle intoxication, 3,5-DMP, could not be detected in specimens for different reasons, but alternatives were recommended.

ACKNOWLEDGEMENTS

The authors like to thank Sascha K. Manier, Aline C. Vollmer, Fabian Frankenfeld, Selina Hemmer, and Gabriele Ulrich for their support and/or helpful discussion. Open Access funding enabled and organized by Projekt DEAL.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Jacobs CM, Wagmann L, Meyer MR. Analytical toxicology of yew constituents in human blood and urine by liquid chromatography-high-resolution tandem mass spectrometry. *Drug Test Anal*. 2023;15(1):123-127. doi:10.1002/dta.3360