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SHORT COMMUNICATION

Toxic plants—Detection of colchicine in a fast systematic clinical toxicology screening using liquid chromatography–mass spectrometry

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Abstract

Colchicum autumnale, which can be mistaken for *Allium ursinum*, contains the alkaloid colchicine potentially leading to life-threatening up to fatal intoxications. We report two cases of acute intoxications with unexplained circumstances. Using the authors' systematic screening approaches, colchicine could be detected in blood plasma and urine samples using liquid chromatography coupled to linear ion trap mass spectrometry (LC-ITMS^n) and high-resolution tandem mass spectrometry (LC-HRMS/MS). Metabolites of colchicine could be identified in urine for confirmation of screening results. Gas chromatography–mass spectrometry (GC-MS) analysis was also conducted, but colchicine could not be detected. Furthermore, colchicine concentration was estimated via LC-HRMS/MS in plasma samples. Results of the systematic screening indicated the ingestion of colchicine from both subjects. In both cases, the parent compound was detected in blood plasma and urine using the LC-HRMS/MS and LC-ITMS^n system. An *O*-demethylation metabolite was identified in urine samples of both subjects using LC-HRMS/MS; the *N*-deacetylation product was also found in urine samples of both cases via LC-HRMS/MS and LC-ITMS^n. The use of LC-ITMS^n resulted only in the detection of the *O*-demethylation product in case 2. Plasma concentrations were estimated at 2.5 ng/ml and 4.7 ng/ml for cases 1 and 2, respectively. We demonstrated the detection of this highly toxic alkaloid in blood plasma and urine using a time-saving and reliable clinical systematic screening. Furthermore, we identified metabolites of colchicine being rarely discussed in literature, which can be used as additional screening targets.

KEYWORDS
colchicine, liquid chromatography, mass spectrometry, systematic screening

1 | INTRODUCTION

Abuse, suicide, homicide, and accidents are amongst the reasons for the intake of toxic plants, which may lead to severe intoxications or death.[1] Colchicine is a naturally occurring, potent alkaloid present in the autumn crocus (*Colchicum autumnale*) and the glory lily (*Gloriosa superba*).[2] Colchicine is used as a drug for the treatment of acute gout and the familial Mediterranean fever as well as gout prophylaxis.

However, leaves of the autumn crocus may be mistaken for bear's garlic (*Allium ursinum*).[3] An ingestion exceeding a colchicine dose of 0.5 mg/kg leads to high fatality rates.[3] Intoxications with colchicine are characterized by gastrointestinal complaints within 4–12 h followed by life-threatening complications like renal failure, cardiovascular collapse, or respiratory insufficiencies.[4] Leukocytosis, alopecia, or multiorgan dysfunction occur after 5 to 7 days often followed by death.[5] Suspected poisonings with toxic plants can be confirmed using compound-specific analytical methods being often time-consuming.[6,7] In literature, some case reports about the detection and/or quantification of colchicine in forensic toxicology were published,[2,8] but to best of our knowledge, no publication deals with the identification of colchicine routinely involving its metabolites. Therefore, the aims of this study were the determination of colchicine and its metabolites in plasma and urine samples of two subjects after suspected intake of autumn crocus leaves. Analysis should be done by means of the authors' systematic toxicological screening approaches including LC-ITMS^n, LC-HRMS/MS, and gas chromatography–mass spectrometry (GC-MS).

2 | EXPERIMENTAL

2.1 | Chemicals and reagents

Trimipramine-d_3 and diazepam-d_5 as internal standards (IS) were purchased from LGC Standards (Wesel, Germany) and colchicine from Sigma (Taufkirchen, Germany). Methanol, diethyl ether, ethyl acetate, dichloromethane, ammonium formate, formic acid, and all other chemicals were obtained from VWR International GmbH (Darmstadt, Germany). Drug-free pooled human plasma was obtained from a local blood bank, and blank urine was provided by the authors' laboratory. All colchicine containing solutions used were kept in dark-colored flasks and stored at 4°C in the refrigerator.

2.2 | Sample preparation for systematic blood plasma screening

EDTA-stabilized blood was centrifuged and the blood plasma processed according to a published liquid–liquid extraction (LLE).[9] Briefly, 1 ml of blood plasma, 100-µl internal standard (0.01 mg/ml trimipramine-d_3 in methanol), and 2-ml saturated natrium sulfate solution were mixed. Extraction was performed using 5 ml of diethyl ether-ethyl acetate (1:1, *v/v*) followed by manually shaking for 20 s and centrifugation for 2 min at 3000 × *g*. After separation, the organic layer was transferred into a glass flask, the residue was basified with 500 µl of sodium hydroxide solution (1 mol/L), and extraction was again performed with 5 ml of diethyl ether-ethyl acetate (1:1, *v/v*). After shaking manually for 20 s and centrifugation for 2 min at 3000 × *g*, the organic layer was transferred into the same glass flask and evaporated to dryness at 60°C. The residue was reconstituted in 100-µl methanol and transferred into autosampler vials. Injection volumes differ regarding the different instruments. On the LC-HRMS/MS as well as the LC-ITMS^n system, 10 µL of the processed samples were injected whereas on the GC-MS apparatus just 1 µL was injected.

2.3 | Sample preparation for systematic GC-MS urine screening

Samples were extracted using published procedures with a minor modification.[10] Urine samples were divided into two aliquots of 2.5-ml. One aliquot was mixed with 100-µl internal standard (0.1 mg/ml diazepam-d_5 in methanol) and 1-ml hydrochloric acid (37%) followed by an acidic hydrolysis for 15 min under reflux. Afterwards, 2 ml of ammonium sulfate solution (30%) and 1.5 ml of sodium hydroxide solution (10 mol/L) were added to obtain a pH value of 8–9. Then, the second aliquot of native urine (2.5 ml) was added. Extraction was performed by using 5 ml of a mixture of ethyl acetate-dichloromethane-isopropanol (3:1:1; *v/v/v*) followed by manually shaking for 20 s and centrifugation (2 min at 3000 × *g*). The organic layer was transferred into a glass flask and evaporated to dryness at 60°C. For derivatization, 100-µl of a mixture of acetic anhydride-pyridine (3:2, *v/v*) was used coupled to microwave irradiation for 5 min at 440 W. The acetylated extract was evaporated to dryness at 60°C, and the residue was reconstituted in 100-µl methanol. One µl was injected onto the GC-MS system.

2.4 | Sample preparation for LC-ITMS^n and LC-HRMS/MS urine screening

Sample preparation was based on published procedures with minor modifications.[11,12] Using a 1.5-ml reaction tube, 100-µl urine were mixed with 10-µl internal standard (0.1 mg/ml diazepam-d_5 in methanol) and 500-µl acetonitrile. After vortexing for 2 min and centrifugation for 2 min at 10,000 × *g*, the supernatant was transferred into a glass vial and evaporated to dryness at 70°C under a gentle stream of nitrogen. The residue was reconstituted in 50-µl of a mixture of eluent A and B of the LC-ITMS^n system (1:1, *v/v*) and 5-µl of the extract were injected onto the LC-ITMS^n and LC-HRMS/MS system.

2.5 | Sample preparation for colchicine concentration estimation in blood plasma

Extraction and concentration estimation of colchicine from blood plasma was based on published procedures, [6,9] which was similar to the LLE mentioned above with the difference of using 100-µl of eluent A and B from the LC-ITMS^n system (1:1, *v/v*) for reconstitution instead of methanol. Additionally, a quality control (QC) and a 1-point calibrator in blood plasma were extracted. For the QC as well as the 1-point calibrator, 1 ml of blank plasma, 100-µl internal standard (0.01 mg/ml trimipramine-d_3 in methanol), and 100-µl colchicine spike

solution were mixed. The QC and the 1-point calibrator contained 1 ng/ml (QC) and 8 ng/ml (1-point) of colchicine.

2.6 | Quantification of urinary creatinine

Urinary creatinine was quantified using the P.I.A.^{2} device (Protzek, Lörrach, Germany). Briefly, 100-µl of urine were diluted with 900-µl buffer provided by Protzek followed by shaking. Afterwards, 100-µl of the diluted urine were pipetted into the reaction chamber. After incubation for 3 min, the test cassette was inserted into the P.I.A.^{2} device. Results were indicated automatically.

2.7 | GC-MS apparatus for systematic blood plasma and urine screening

Apparatus conditions were in line with previously published data.[9] Details can be found in the Supporting Information.

2.8 | LC-ITMS^n apparatus for systematic blood plasma and urine screening

Apparatus conditions were in line with a previously published study.[11] Details can be found in the Supporting Information.

2.9 | LC-HRMS/MS apparatus for systematic blood plasma and urine screening

Apparatus conditions were in line with previously published studies.[13,14] Details can be found in the Supporting Information.

2.10 | LC-HRMS/MS apparatus for colchicine blood plasma concentration estimation

Apparatus conditions were in line with a previously published study.[15] Details can be found in the Supporting Information.

3 | RESULTS AND DISCUSSION

3.1 | Systematic screening approaches for blood plasma and urine samples

Systematic screening analysis in clinical toxicology is performed to investigate, which pharmaceuticals, drugs, or other substances might be responsible for, for example, an acute intoxication. Results have to be available within a limited period of time. Blood plasma and urine samples are mostly analyzed in systematic screening approaches unlike to forensic toxicology where postmortem samples are used (e.g., femoral blood, bile, and gastric content). Human blood and urine samples of two subjects were submitted to the authors' laboratory for toxicological analysis. Table 1 summarizes the detection results for colchicine and its metabolites. Regarding cases 1 and 2, the parent compound could already be detected in blood plasma, whereas its metabolites were only found in urine samples. GC-MS analysis revealed negative results. This is in line with Peters et al. reporting that colchicine could not be detected via GC-MS.[16]

3.2 | Metabolites of colchicine

Colchicine used as drug is known for a narrow therapeutic window and adverse effects like hepatological and hematological toxicity, or renal issues.[17] Guo et al. investigated the hepatotoxicity of the demethyl metabolites of colchicine (2-*O*-demethyl colchicine, 3-*O*-demethyl colchicine, and 10-*O*-demethyl colchicine).[17] Compared with the parent compound, these metabolites do not enhance liver toxicity. In another project of Guo et al., colchicine was incubated in rat liver microsomes with the result that four *O*-demethylation products could also be detected (1-*O*-demethyl colchicine, 2-*O*-demethyl colchicine, 3-*O*-demethyl colchicine, and 10-*O*-demethyl colchicine).[18]

Regarding case 2 in this study, the parent compound could be detected in blood plasma and urine using LC-HRMS/MS as well as LC-ITMS^n. Metabolites after *O*-demethylation were identified in urine samples of both subjects with both LC-MS systems likewise the *N*-deacetylation product at *m/z* 358.1639. The *O*-demethylation can occur at four possible positions whereby it cannot be determined which *O*-demethylation product was formed. ITMS^2 and HRMS^2

TABLE 1 Detection of colchicine and its metabolites in blood plasma and urine samples for case 1 and 2 using LC-HRMS/MS, LC-ITMS^n, and GC-MS

		Plasma			Urine		
		HRMS/MS	ITMS^n	GC-MS	HRMS/MS	ITMS^n	GC-MS
Case 1	COL	x	x	-	x	x	-
	O-demethyl-COL	-	-	-	x	-	-
	N-deacetyl-COL	-	-	-	x	x	-
Case 2	COL	x	x	-	x	x	-
	O-demethyl-COL	-	-	-	x	x	-
	N-deacetyl-COL	-	-	-	x	x	-

Abbreviations: COL, colchicine; x, detected; –, not detected.

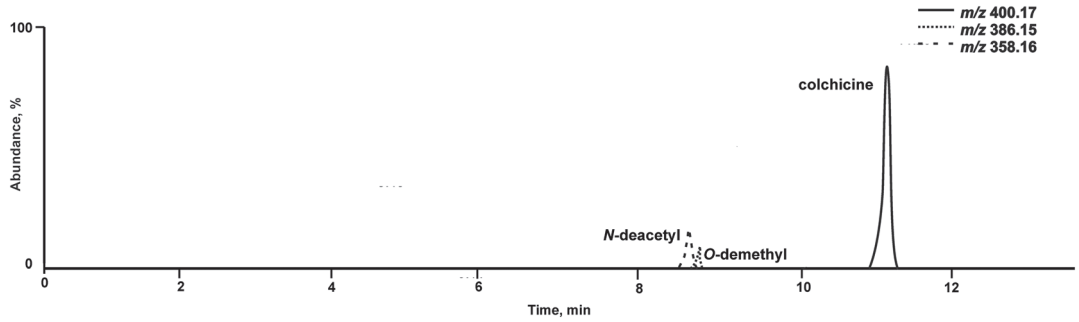

FIGURE 1 Reconstructed ion chromatograms of colchicine and its metabolites in urine using the LC-ITMS^n system, exemplified for case 2

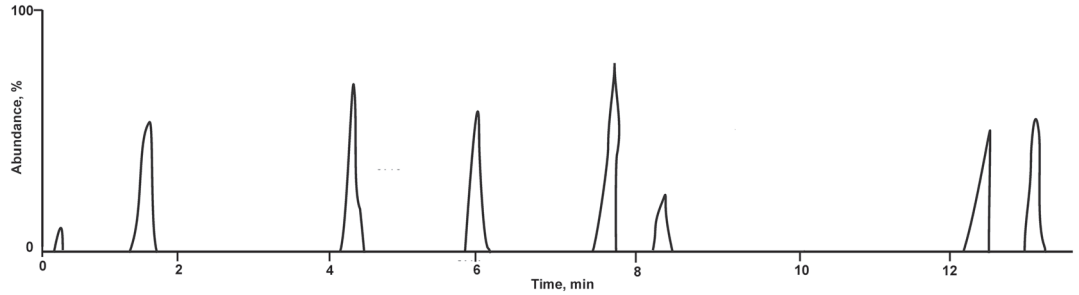

FIGURE 2 Reconstructed ion chromatograms after analysis of a blank urine sample using the LC-ITMS^n system

spectra of the metabolites are depicted in Figures S1 and S2. In case reports, colchicine was identified/quantified by its parent compound, but in one case, Abe et al.[19] used the *N*-deacetylation product for quantification. As indicated in Table 1, the *O*-demethylation product could not be detected in case 1 using the LC-ITMS^n system probably due to lower sensitivity compared with LC-HRMS/MS. However, LC-ITMS^n was able to identify metabolites of colchicine even at higher concentrations. Figure 1 shows the reconstructed ion chromatograms of colchicine and its metabolites in urine analyzed by LC-ITMS^n. Figure 2 the reconstructed ion chromatograms of a blank urine analyzed by LC-ITMS^n.

3.3 | Estimation of colchicine concentrations in blood plasma

Beyer et al. developed and validated a multi-analyte procedure for toxic alkaloids in human plasma using solid-phase extraction. Results have shown that colchicine indicated better extraction efficiency using LLE.[6] Based on this procedure, a 1-point calibration was performed. After full scan analysis, the peak area of the protonated precursor ion (*m/z* of $M + H^+$ 400.1785) and the peak area of the IS (*m/z* of $M + H^+$ 298.2357) were integrated. The peak area ratio of colchicine and IS was compared to the 1-point calibrator followed by calculation of blood plasma concentrations. Plasma concentrations obtained were 2.5 ng/ml (case 1) and 4.7 ng/ml (case 2). The deviation of the QC was 1.9% (1.02 ng/ml). The amount of colchicine estimated in case 1 was half the amount of case 2 which might have had an impact on detecting metabolites. Those low plasma concentrations were in line with the supposed time of colchicine ingestion, which was about 3 days before the analysis. Schulz et al.[20] reported plasma concentrations of colchicine in the context of gout therapy up to 2.5 ng/ml and toxic values starting at 5 ng/ml. Urinary creatinine values were <20 mg/dl for case 1, most likely due to urine dilution via medical interventions and 174 mg/dl for case 2.

4 | CONCLUSIONS

Colchicine intake may not only lead to life-threatening complications but can also result in fatalities. The syndrome includes nonspecific gastrointestinal symptoms, which also may appear after ingestion of many other compounds.[4] Hence, it should be of importance to be able to detect colchicine in biological specimens routinely even without any suspicion. In our study, detection of colchicine and/or its metabolites in blood plasma and urine samples could be realized by using the authors' systematic toxicological screening approaches based on LC-HRMS/MS and LC-ITMS^n. Including metabolites in the screening is important in case of ion suppression or interference of co-eluting compounds impacting the reliable detection of the parent compound. However, as demonstrated earlier, a detection of colchicine and/or its metabolites was not possible using GC-MS. In conclusion, this report will help toxicologists to update and evaluate their screening strategies by providing a LC-MS based screening solution as well as additional screening targets for detection of colchicine.

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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 may be found in the online version of the article at the publisher's website.

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