

# Noninvasive Detection of Chemical Defenses in Poison Frogs Using the MasSpec Pen

Anna C. Krieger, Sydney C. Povilaitis, Prajwal Gowda, Lauren A. O'Connell,\* and Livia S. Eberlin\*

Cite This: *ACS Meas. Sci. Au* 2022, 2, 475–484

Read Online

ACCESS |



Metrics &amp; More



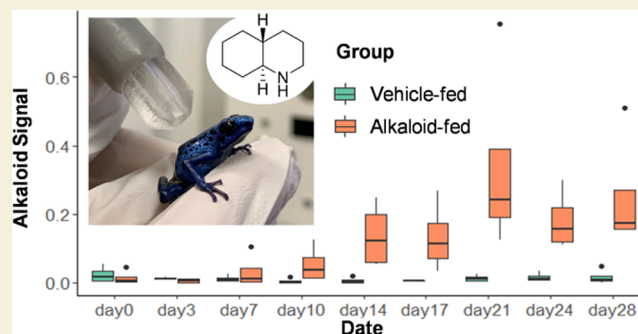
Article Recommendations



Supporting Information

**ABSTRACT:** Poison frogs are well-known for their fascinating ability to store alkaloids in their skin as chemical defense against predators. Chemical methods used to study these alkaloids are limited by requirements for euthanasia or stress during sampling. Here, we demonstrate sensitive and biocompatible alkaloid detection and monitoring *in vivo* using the MasSpec Pen, a handheld, noninvasive chemical detection device coupled to a mass spectrometer. The MasSpec Pen allowed rapid (<15 s), gentle, and consecutive molecular analysis without harm or undue stress to the animals. Through a month-long alkaloid-feeding study with the dyeing poison frog, we observed temporal dynamics of chemical sequestration *in vivo* by comparing frogs fed the alkaloid decahydroquinoline (DHQ) to vehicle-fed frogs. We also demonstrate the feasibility of the MasSpec Pen for the untargeted detection of rich alkaloid profiles from skin extracts of the Diablotto poison frog, collected from two distinct geographical populations in Ecuador. The results obtained in this study demonstrate the utility of the MasSpec Pen for direct, rapid, and biocompatible analysis of poison frog alkaloids.

**KEYWORDS:** alkaloid, biocompatible sampling, dendrobatidae, direct analysis, mass spectrometry



## INTRODUCTION

The name “poison frog” refers to several genera of frogs from anuran families including dendrobatidae, bufonidae, mantellidae, and myobatrachidae, all of which sequester alkaloids from their diet of arthropod prey.<sup>1–4</sup> Chemists and biologists have great interest in studying poison frogs for the medicinal implications of alkaloids<sup>5,6</sup> and better understanding how the physiological mechanisms allowing alkaloid sequestration evolved.<sup>7–10</sup> Alkaloids can be detected on the mucous membrane of the mouth, stomach, intestine, and liver of the frog,<sup>11</sup> prior to storage in granular glands in the skin and excretion upon threat of predation. The mechanism of this chemical sequestration is under investigation but has been found to occur rapidly within 4 days of alkaloid ingestion and may involve plasma carrier proteins and proteins involved in small molecule transport and metabolism.<sup>10,12</sup> Over 800 different alkaloid compounds have been described in poisonous amphibians,<sup>13</sup> and alkaloid profiles vary between species, sexes, populations, habitats, and seasons, processes that are largely related to arthropod species availability and diversity.<sup>14–18</sup>

Several methods for sample collection have been employed to study alkaloid chemical defenses in poison frogs, with varying degrees of invasiveness for the animal. Approaches for sample collection include injection of neurotransmitters to stimulate evacuation of granular gland contents,<sup>19</sup> whole skin

extraction requiring euthanasia prior to excision,<sup>20</sup> dermal electrical stimulation,<sup>21,22</sup> and manual expression of glands in certain frog species.<sup>22</sup> In terms of animal impact, sampling modalities for alkaloid sequestration studies require numerous time points, often necessitating multiple biological replicates for each point, leading to a substantial animal burden as euthanasia is required for sampling. Alternatively, sampling modalities that stimulate total or near-total evacuation of granular glands may preclude monitoring of alkaloid sequestration over time. Once collected, chemical characterization of alkaloid extracts has been predominantly accomplished *via* gas chromatography-mass spectrometry (GC-MS) and Fourier transform infrared spectroscopy followed by spectral comparisons with molecular libraries described by Daly and co-workers.<sup>13</sup> More sensitive techniques such as liquid chromatography-MS (LC-MS) have also been employed for alkaloid analyses, although the lack of commercially available internal standards has presented a challenge for structural confirmation and quantitative analysis.<sup>18</sup>

Received: June 10, 2022

Revised: July 25, 2022

Accepted: July 27, 2022

Published: August 10, 2022



We have previously described the development of the MasSpec Pen technology for rapid and direct sample analysis utilizing a gentle liquid extraction process.<sup>23</sup> The handheld, pen-like device is a user-friendly system that employs a solvent droplet to gently extract molecules from a sample surface upon 3 s of contact time. The solvent containing the analytes is then directly transferred to a mass spectrometer for rapid chemical analysis. The MasSpec Pen has previously been employed for *in vivo* molecular characterization of murine, porcine, and human tissues, with no observed macroscopic damage to the tissues analyzed.<sup>23–26</sup> While many applications of the MasSpec Pen have involved qualitative analysis of small molecules and lipids, the platform has also been adapted and applied for the semiquantitative analysis of the alkaloid drug cocaine and the alkaloid-derived oxycodone.<sup>27</sup>

Here, we describe the development of the MasSpec Pen integrated with an orbitrap mass spectrometer for the detection of an alkaloid standard, as well as the *in vivo* detection of a model alkaloid from the skin of alkaloid-fed poison frogs. Further, we conducted a 4-week alkaloid-feeding study using the optimized MasSpec Pen platform to detect temporal dynamics of alkaloid sequestration to the skin *in vivo* by feeding decahydroquinoline (DHQ) to the dyeing poison frog, *Dendrobates tinctorius*. Additionally, we demonstrate the ability of this platform for untargeted chemical detection of rich alkaloid profiles from field-collected frog skin extracts.

## EXPERIMENTAL SECTION

### Materials

*Trans*-decahydroquinoline and caffeine were purchased from Sigma-Aldrich (St. Louis, MO). Ethanol (EtOH), ACS grade, was purchased from Pharmco by Greenfield Global (Toronto, CA), and LC-MS grade water used for the MasSpec Pen solvent system was purchased from Fisher Scientific (Waltham, MA).

### MasSpec Pen Platform Optimization

We have previously reported the design, component materials, and experimental setup of the MasSpec Pen platform.<sup>23</sup> Adaptations to the original parameters of the pen platform for this work included poly(dimethylsiloxane) pen tips with a 4 mm reservoir diameter, a solvent droplet volume of 30  $\mu$ L, and poly(tetrafluoroethylene) (PTFE) tubing length of 0.5 m, which were used for all experiments. The MasSpec Pen was coupled to a Q Exactive Hybrid Quadrupole-Orbitrap and to a Q Exactive Orbitrap or a Q Exactive HF Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Scientific, San Jose, CA) for all analyses. Except for MS/MS experiments, all data was collected in full scan mode.

Standard solutions of DHQ and caffeine were used for solvent, S-lens, and data extraction procedure optimization. Various % concentrations of EtOH in LC-MS grade H<sub>2</sub>O solvent blends (0, 1, 5, 10, 25, and 50% EtOH in H<sub>2</sub>O) were used to analyze three concentrations of decahydroquinoline (DHQ). DHQ standards were dried onto glass slides in triplicate to determine the optimal solvent blends for signal intensity and reproducibility. We also evaluated each EtOH solvent blend for DHQ signal intensity.

### Animals

Animal protocols were approved by the Institutional Animal Care and Use Committee of the University of Texas at Austin (Animal Use Protocol 2018–00156). *D. tinctorius* “*azureus*” juveniles ( $N = 8$ ) were purchased from Josh's Frogs (Owosso, MI) and allowed to acclimate for 1 week prior to the feeding experiment. Frogs were housed individually on a 12 h:12 h light/dark cycle, misted once daily, and fed *ad libitum* fruit flies dusted with vitamin supplement (DendroCare Vitamin/Mineral Powder) thrice weekly.

### DHQ Feeding Experiment Design

After acclimation, frogs were randomly assigned to one of two treatment groups, one alkaloid-fed group and one vehicle-fed group ( $N = 4$  per group). The alkaloid-fed group was administered 10  $\mu$ L of 0.05 mg/mL DHQ in 9:1 H<sub>2</sub>O/EtOH orally twice weekly for 4 weeks, for a total of 4  $\mu$ g of DHQ. The control group received oral administration of the vehicle solution, 9:1 H<sub>2</sub>O/EtOH, twice weekly for the same duration. During oral administration, we observed that the 10  $\mu$ L volume was sufficiently small to avoid spitting or regurgitation of the vehicle or alkaloid dose. After the completion of the feeding experiment (day 28), frogs were euthanized by anesthetization with 20% benzocaine gel applied to the ventral skin, followed by decapitation.

### In Vivo MasSpec Pen Analysis

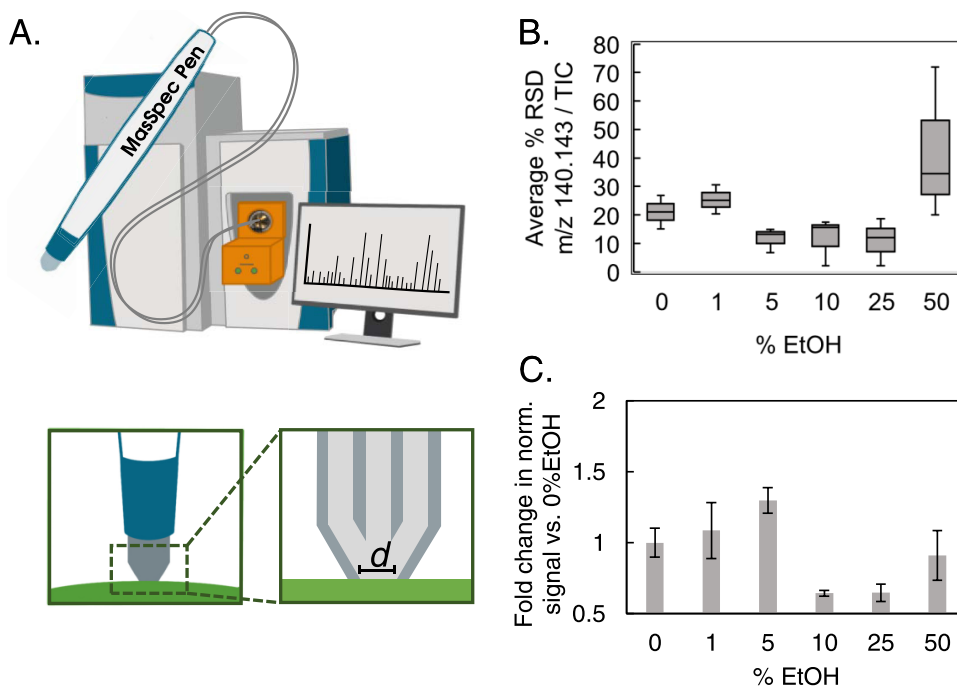
*In vivo* analyses were performed using the MasSpec Pen platform described previously. Experiments were scheduled such that analysis was performed prior to initial toxin or vehicle administration (day 0) and on a twice-weekly basis thereafter (days 2, 7, 10, 14, 17, 21, 24, and 28). MasSpec Pen experiments were performed using a solvent system of 5% EtOH in DI H<sub>2</sub>O with 0.1  $\mu$ g/mL caffeine from  $m/z$  100–300 in positive ion mode, a resolving power of 70,000 (at  $m/z$  200), and an S-lens radio frequency (RF) level of 55. On day 28, two additional analyses were performed after obtaining alkaloid monitoring data to obtain untargeted molecular profiles of frog skin in positive and negative ion modes. These analyses were performed in both ion modes from  $m/z$  150–1800. All other MasSpec Pen and instrument parameters were conserved. A clean pen tip and tubing system were used for each analysis of each frog to minimize carry-over between analyses. The MasSpec Pen analysis workflow for the feeding study was performed as follows. First, a washing step was performed where the pen system was flushed with the solvent system. Following the washing step, the frog was removed from its terrarium, and any leaf litter or other debris was gently wiped away from the dorsal skin using a gloved finger. The pen tip was gently held in contact with the dorsal skin of the frog such that the entire edge of the reservoir was in contact with the skin, and an analysis was triggered. After the analysis (<15 s), the frog was returned to its terrarium. The MasSpec Pen tip and tubing were then replaced prior to the analysis of another individual. To monitor data quality, including mass accuracy, background signal intensity, and proper instrument and platform functioning, a background analysis was performed prior to all *in vivo* analyses. On day 28, two additional MasSpec Pen analyses were performed with each frog to collect mass spectra from  $m/z$  120–1800 in both positive and negative ion modes.

### DHQ Extraction from Skin Samples

Dorsal skin was excised from each frog and immediately flash frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until use. DHQ and other small endogenous molecules were extracted from the skin samples by thawing samples to room temperature and homogenizing with 400  $\mu$ L of MeOH using an Omni International bead mill homogenizer (Kennesaw, GA) and ceramic bead tubes for 4 min at speed setting 5. Homogenates were centrifuged to remove particulate matter, and the supernatant was removed for LC-MS analysis.

### LC-MS Quantification of DHQ

Methods for liquid chromatography-mass spectrometry (LC-MS) quantification of DHQ were adapted from McGugan et al.<sup>18</sup> Analysis was performed on an Agilent G6530A Q-TOF system (Santa Clara, CA) with an Agilent 1260 Infinity LC system (Santa Clara, CA). A reversed-phase LC gradient method was used with an Agilent Eclipse Plus C18, 5  $\mu$ m particle size, 50  $\times$  2.1 mm<sup>2</sup> column, and an Agilent Zorbax guard column. The sample injection volume was 1.00  $\mu$ L. Mobile phase A was composed of LC-MS grade water, and mobile phase B was composed of LC-MS grade methanol. The flow rate was 0.7 mL/min. The gradient proceeded with 95% A/5% B at 0 min, 40% A/60% B at 3.5 min, and 0% A/100% B at 3.6 min. This composition was held until 8.1 min, when the solvent was reverted to 95% A/5% B, which was held until 12 min to re-equilibrate the column for the next injection (Table S1). Electrospray ionization was



**Figure 1.** Optimization of the MasSpec Pen platform for the detection of decahydroquinoline (DHQ). (A) Scheme showing MasSpec Pen platform (top) and pen tip reservoir diameter ( $d$ ) cutaway (bottom). (B) Average % relative standard deviation of DHQ signal normalized by total ion current for EtOH solvent blends. Triplicate analyses were performed for three concentrations of DHQ standards dried onto glass slides for each solvent blend analyzed. Error bars show standard deviation. (C) Fold change in DHQ signal normalized by total ion current for each EtOH solvent blend compared with 0% EtOH (pure DI water). Triplicate analyses were performed for three concentrations of DHQ standards dried onto glass slides for each solvent blend analyzed. Error bars show standard deviation.

used in the positive ionization mode with a source gas of 10 L/min at 350 °C, a nebulizer at 60 psi, and a sheath gas of 11 L/min at 350 °C. The capillary voltage was set to 3500 V, the nozzle voltage to 2000 V, and fragmentor at 180 V. Mass spectrometry data were continually acquired from  $m/z$  50–1000 with a scan rate of 1 spectrum/s. The limit of detection (LOD) for DHQ for the LC-MS method was calculated using 3.3 times the standard error in  $y$  ( $s_y$ ) divided by the slope of the calibration curve.<sup>28</sup>

### MasSpec Pen Analysis of Skin Extracts from Field-Collected Frogs

To determine if the MasSpec Pen could reliably detect the more complex alkaloid cocktail present on poison frogs in an ecologically relevant context, we utilized skin extracts from two populations of the Diablito frog, *Oophaga sylvatica* ( $N = 12$ ), previously collected in Ecuador. The collection and description of alkaloids from these samples, representing the Felfa and Cristóbal Colón populations, have been published elsewhere.<sup>18</sup> The MasSpec Pen platform described above and a solvent system of 5% EtOH in H<sub>2</sub>O and 0.01% formic acid were used to analyze extracts dried onto PTFE-coated glass slides.

### Tandem MS

Tandem MS (MS/MS) analyses were performed using the MasSpec Pen coupled with a Q Exactive HF Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Scientific, San Jose, CA). Collisional-induced dissociation was performed in the higher-energy collision dissociation cell of the mass spectrometer with normalized collision energies from 10 to 50 (units arbitrary).

### Data Extraction and Statistical Analysis

Feeding study data were extracted by integrating the extracted ion chromatogram (XIC) for  $m/z$  140.143 (DHQ) and 195.088 (Caffeine) for each MasSpec Pen analysis. Analyses of these data were performed in RStudio (1.1.383) using the R programming language (3.6.1). A generalized linear mixed model was built to describe differences in DHQ XIC area among treatment groups over

time using the glmmTMB R package (1.1.2.3), with the effects being treatment group, analysis date, and the interaction between treatment group and analysis date. Log-transformed caffeine XIC area was used as an offset to account for signal variability not related to treatment effects. Frog identifier was included as a random effect to account for repeated measurements of individuals for the duration of the study. The emmeans package (version 1.7.2) was used for post hoc pairwise analyses to determine which days showed significant differences between treatment groups adjusted for multiple testing using Tukey.

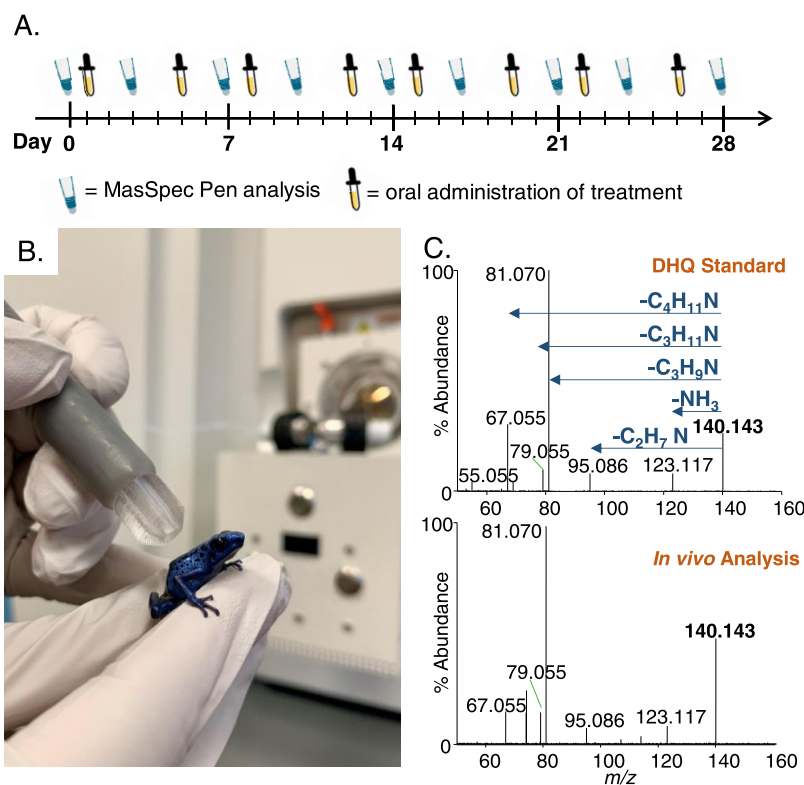
Field-collected frog molecular profiles were extracted and uploaded to MetaboAnalyst 5.0 (<https://www.metaboanalyst.ca/MetaboAnalyst/home.xhtml>) for partial least squares-dimensional analysis (PLS-DA). Data were binned to  $m/z$  0.025, and ions appearing in fewer than 20% of spectra were removed; spectra were normalized by the summed total ion intensity, log-transformed, and mean-centered. The two-dimensional (2D) score plot and variable importance in projection plots and tables were exported from MetaboAnalyst. Putative molecular assignments were based on high mass accuracy (<5 ppm) measurements and MS/MS analysis for specified assignments and were obtained by searching metabolite and lipid libraries on the METASPACE metabolite annotation platform (<https://metaspace2020.eu/>), as well as comparing with literature reports.<sup>13,14,18</sup>

## RESULTS AND DISCUSSION

### Optimization of the MasSpec Pen Platform for Detection of Alkaloids

The MasSpec Pen platform has been implemented in numerous applications including *in vivo* tissue analysis in operating rooms,<sup>23–25</sup> consumer product fraud,<sup>29</sup> and forensics.<sup>27</sup> Due to the versatility and ease of use of the device and its appealing capabilities for *in vivo* sampling and chemical analysis, we adapted and applied this technology for the detection and monitoring of alkaloids from poison frog skin,





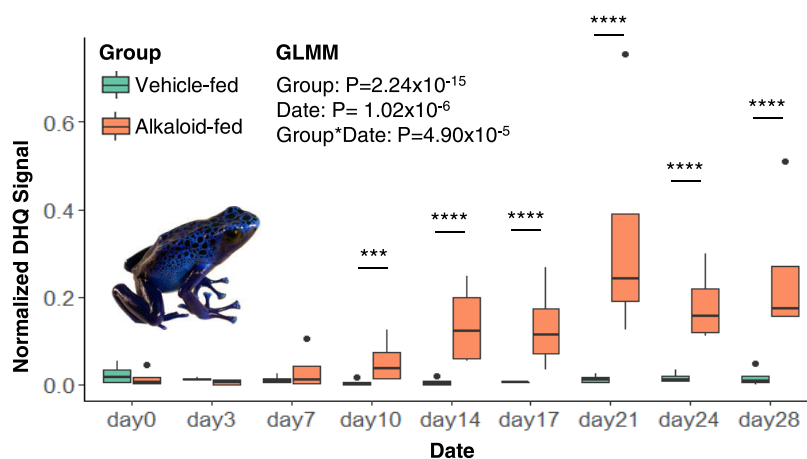
**Figure 2.** Alkaloid-feeding study design and *in vivo* detection of decahydroquinoline (DHQ) sequestration using the MasSpec Pen. (A) Scheme showing the timeline of the 4-week feeding study. MasSpec Pen analysis and treatment administration were performed twice weekly. (B) Photograph depicting *in vivo* MasSpec Pen analysis of *D. tinctorius*. (C) Collision-induced dissociation (CID) MS/MS analysis of *m/z* 140.143 from MasSpec Pen analysis of a DHQ standard (top) and from *in vivo* analysis of *D. tinctorius* dorsal skin (bottom).

both *ex vivo* and *in vivo*, with the goal of developing a chemical analysis platform to study chemical defenses in amphibians. To adapt the MasSpec Pen technology (Figure 1A) for the detection of alkaloids, we made several alterations to platform parameters and optimized the MasSpec Pen solvent system and instrument ion optics. We opted for a 4.0 mm pen tip reservoir diameter to facilitate maximal sampling of granular glands in the skin while ensuring the sampling device remained relevant to the size of the frog. A PTFE transfer tubing length of 0.5 m was used for efficient analyte transfer to the mass spectrometer. As alkaloids are lipophilic, and MasSpec Pen sampling is based on a liquid–solid chemical extraction process, we evaluated if incorporating EtOH into the solvent system would facilitate alkaloid extraction while maintaining biocompatibility. Optimal DHQ signal reproducibility,  $12 \pm 4\%$  relative standard deviation (% RSD), was observed for the 5% EtOH in H<sub>2</sub>O solvent blend (Figure 1B). We also evaluated each EtOH solvent blend for DHQ signal intensity (Figure 1C), which was observed with the 5% EtOH solvent, with an increase of  $1.29 \pm 0.09$ -fold change in signal intensity versus the 0% EtOH solvent. Thus, the solvent system 5% EtOH in DI H<sub>2</sub>O (v/v%) was chosen for all consecutive experiments as it afforded both optimal DHQ signal intensity and % RSD while minimizing % EtOH. All other components of the MasSpec Pen that contact the analysis surface or solvent system are comprised of biocompatible materials.<sup>23</sup> This platform was then used to obtain a limit of detection for a DHQ standard deposited and dried on glass slides, which was determined to be 0.86 pg DHQ (Figure S1).

#### Use of the MasSpec Pen Platform for Detection and Monitoring of DHQ *In Vivo*

We next employed the optimized MasSpec Pen platform to study *in vivo* temporal dynamics of alkaloid sequestration in poison frogs (Video S1). Previous alkaloid-feeding studies have been performed to support the “dietary hypothesis” that alkaloids are obtained by poison frogs from their arthropod diet,<sup>2</sup> as well as to study temporal dynamics and physiological implications of alkaloid sequestration.<sup>10,30</sup> These studies have ranged in duration from several weeks<sup>10</sup> to several months,<sup>30</sup> and alkaloid administration is commonly performed using a 1% alkaloid in vitamin powder to dust fruit flies, making the exact dosage of alkaloid difficult to determine. Individual alkaloids have been quantified from frogs collected in the field and range from <5 to >50  $\mu\text{g}$  per 100 mg skin.<sup>4</sup> Here, we opted for oral administration of a DHQ solution to facilitate a more accurate estimate of alkaloid dosage. DHQ was selected as a model alkaloid as it is one of few commercially available poison frog alkaloids, and we administered 0.5  $\mu\text{g}$  of DHQ twice weekly for a total of 4  $\mu\text{g}$  of DHQ over the course of the 4-week feeding study. Due to a lack of readily available deuterated reference molecules for DHQ, we incorporated caffeine (0.1  $\mu\text{g}/\text{mL}$ ) into the analysis solvent to account for day-to-day signal variability throughout the feeding study, and instrument optics were operated at optimal RF levels for transmission of both DHQ and caffeine (Table S2). Finally, *D. tinctorius* was selected as the model species due to its commercial availability.

On “Day 0” of the feeding study, the MasSpec Pen was used to sample the dorsal skin of each frog prior to the beginning of the feeding study (Figure 2A). After this initial MasSpec Pen analysis, frogs were orally administered either 0.5  $\mu\text{g}$  of DHQ



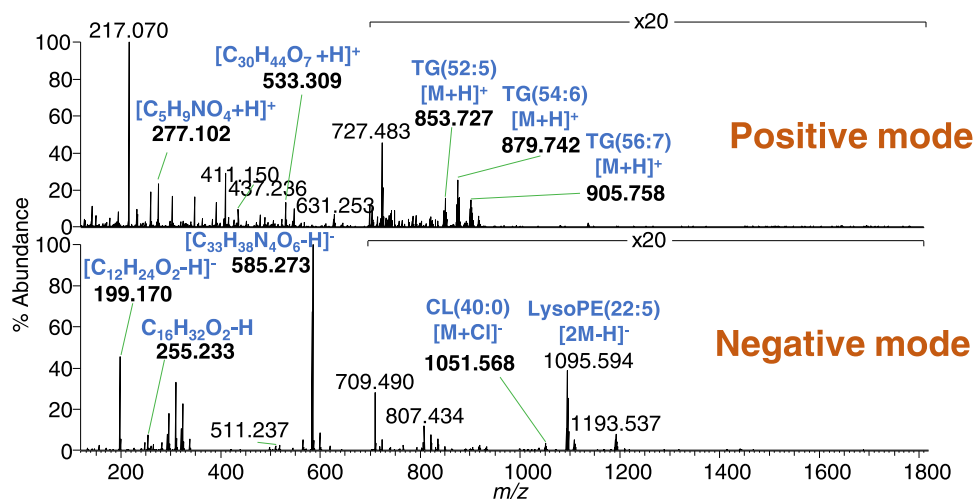
**Figure 3.** Monitoring of decahydroquinoline (DHQ) sequestration during 4-week feeding study using the MasSpec Pen. Caffeine-normalized DHQ signal monitored among alkaloid- (orange) and vehicle-fed (teal) frogs ( $N = 4$  frogs per treatment group) over the course of 4 weeks of the alkaloid-feeding study. Boxplots show upper and lower quartiles, and whiskers indicate nonoutlier maximum and minimum values. Dots indicate outliers above  $1.5 \times$  interquartile range. A generalized linear mixed model (GLMM) showed a significant effect for the treatment group ( $P = 2.24 \times 10^{-15}$ ), date ( $P = 1.02 \times 10^{-6}$ ), and the interaction of the treatment group and date ( $P = 4.90 \times 10^{-5}$ ). Post hoc pairwise analyses with Tukey adjustment show significant differences between treatment groups (\*\* $P < 0.01$ , \*\*\*\* $P < 0.001$ ).

or vehicle dose according to their randomly assigned treatment groups twice weekly for the duration of the feeding experiment. MasSpec Pen analysis was performed twice weekly for the duration of the 4-week study to quantify alkaloid uptake. A photo showing the *in vivo* analysis of *D. tinctorius* is shown in Figure 2B. Frogs were not purposely stressed, stroked, or manipulated with the purpose of inducing toxin secretion during any experiment, nor did we observe any milky or clear secretions on the skin during MasSpec Pen analyses that would indicate granular gland secretion. The average approximate duration of MasSpec Pen analysis for each frog was  $<15$  s, and after analysis, we observed no damage, disruption, or discoloration of the skin within the area analyzed. Dorsal skin of all frogs was also examined on the final day of MasSpec Pen analysis, and no macroscopic changes to the dorsal skin were observed, demonstrating the feasibility for a gentle, biocompatible sampling of poison frog skin. Based on these observations, we expect that the MasSpec Pen platform as described would also be suitable for use in studies with similar durations where the frogs are allowed to rest for 3–4 days between analyses without undue stress to the animal. Additional tests should be done to determine the effects of increasing the number or frequency of MasSpec Pen analyses. Additionally, similarities in granular gland anatomy among poison frogs<sup>31</sup> suggests this platform could easily be applied to study alkaloid profiles or temporal dynamics of alkaloid sequestration in other poison frogs and other amphibian species.

During the feeding experiment, an ion detected in the positive ionization mode at  $m/z$  140.143 *in vivo* from toxin-fed frog skin was putatively assigned to the protonated DHQ molecule with a small mass error of 4.02 ppm. To confirm the identity of the ion, MS/MS experiments were performed on a standard solution of DHQ for comparison with the ion detected from the skin of toxin-fed *D. tinctorius* individuals (Figure 2C). MS/MS analysis of the DHQ standard, detected as the protonated species at  $m/z$  140.143 (Figure 2C, top), yielded a major fragment ion at  $m/z$  81.070 and additional fragment ions at  $m/z$  123.117,  $m/z$  95.086,  $m/z$  79.055, and  $m/z$  67.055. A similar fragmentation pattern was observed

from MS/MS analysis of  $m/z$  140.143 from frog skin (Figure 2C, bottom), verifying that the MasSpec Pen can detect DHQ *in vivo* from the granular glands in the skin of *D. tinctorius*. Note that additional ions present in the mass spectrum collected *in vivo* are likely due to coisolated ions during the MS/MS experiment, a common observation when performing MS/MS during a direct analysis experiment of a complex sample such as skin.

During the feeding study, we observed an increase in normalized DHQ signal intensity between alkaloid-fed frogs compared with vehicle-fed frogs as well before and after treatment (Figure 3). There was a significant interaction between the treatment group and time (GLMM,  $X^2(8) = 33.553$ ,  $P = 4.90 \times 10^{-5}$ ), where DHQ increased in the alkaloid-fed group over time compared to the control group. While alkaloid and control treatment groups did not differ in DHQ abundance within the first week, there were significant group differences at all other time points in the experiment (Table S3). Although all of the analyses required extraction of analyte molecules for ionization and subsequent detection, we observed an increasing normalized DHQ signal over the course of the 4-week feeding study, suggesting that the amount of DHQ extracted during a single MasSpec Pen analysis is less than the  $0.5 \mu\text{g}$  of DHQ administered. More experiments are needed to quantify alkaloids extracted from granular glands during MasSpec Pen analysis; however, due to the low alkaloid dosage we used in our feeding study relative to alkaloid quantities observed in frogs collected in the field, our results suggest that this platform is suitable for experiments where minimal removal of granular gland contents is desirable, such as accumulation studies. It should be noted that signal intensity among control frogs was greater than 0; the average normalized integrated XIC area among the control group for the feeding experiment was  $1.17 \times 10^{-3} \pm 1.41 \times 10^{-3}$ . This signal can likely be ascribed to chemical noise present in the solvent system that was detected at a similar  $m/z$  value compared to DHQ and was not due to the presence of the alkaloid in the granular glands of these individuals. Additionally, we did not observe a significant increase in this signal over



**Figure 4.** Representative MasSpec Pen molecular profiles of *D. tinctorius* dorsal skin *in vivo* in positive (top) and negative (bottom) ion modes. Spectra were obtained at the conclusion of the alkaloid-feeding experiment from  $m/z$  150–1800 and are an average of 10 scans. Ion abundance from  $m/z$  700–1800 is shown at 20 $\times$  scale to aid visualization of lower abundance species. Molecular candidates shown above  $m/z$  flags are putative attributions based on high mass accuracy measurements (<5 ppm) and were obtained by searching the METASPACE metabolite annotation platform.

**Table 1.** LC-MS Validation Sample Preparation and Raw and Calculated Results<sup>a</sup>

treatment group	frog ID	body mass (mg) <sup>b</sup>	mass of excised skin (mg) <sup>b</sup>	DHQ XIC area <sup>c</sup>	calculated [DHQ] (ng/mL) <sup>d</sup>	calculated DHQ in skin sample (ng/mg)
vehicle-fed	1	0.647	0.011	$4.8 \times 10^3$	–44	below LOD
	3	0.740	0.012	$4.9 \times 10^3$	–44	below LOD
	4	0.717	0.012	$2.9 \times 10^3$	–44	below LOD
	7	0.732	0.016	$3.6 \times 10^3$	–44	below LOD
alkaloid-fed	2	0.686	0.015	$1.1 \times 10^6$	210	0.003
	5	0.539	0.011	$2.2 \times 10^6$	463	0.017
	6	0.552	0.013	$2.0 \times 10^6$	426	0.013
	8	0.578	0.017	$3.5 \times 10^6$	759	0.018

<sup>a</sup>DHQ = decahydroquinoline, XIC = extracted ion chromatogram, LOD = limit of detection. <sup>b</sup>Measurements performed on the final day of the alkaloid-feeding study. <sup>c</sup>Integrated mass trace of  $m/z$  140.14. <sup>d</sup>Concentrations were calculated using the linear regression fit of the standard response curve.

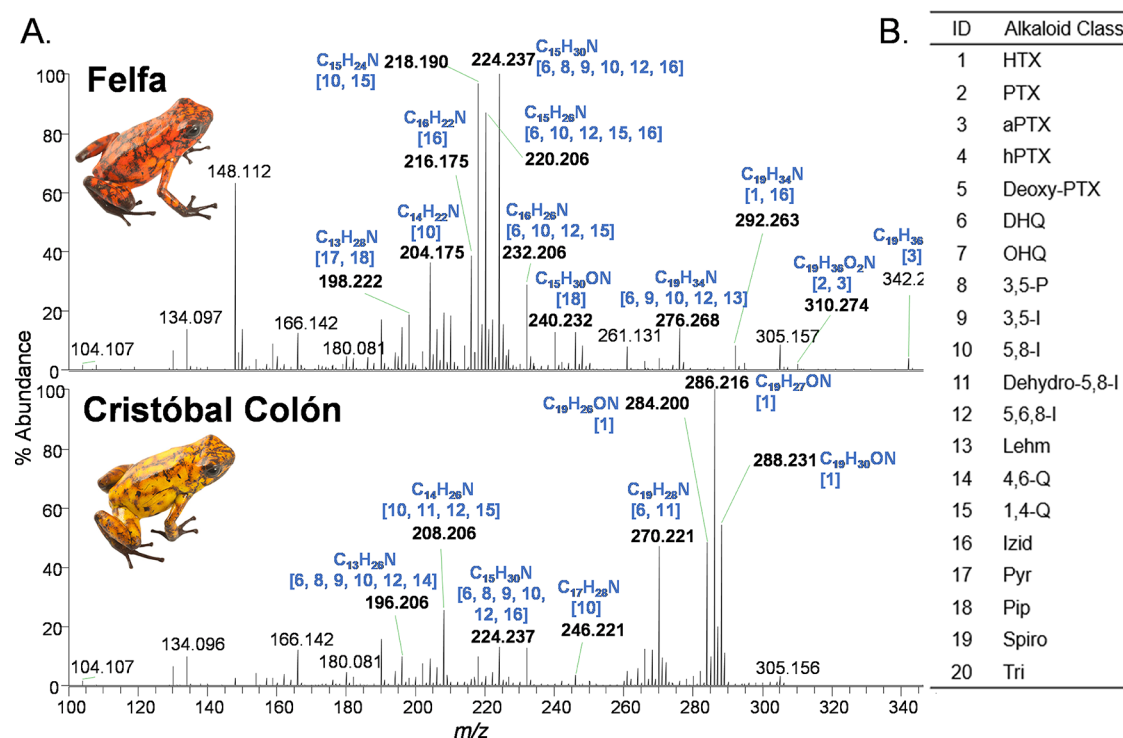
time in the control group as was observed for the alkaloid-fed group.

At the conclusion of the feeding study, two additional analyses were performed for each individual to evaluate the use of the MasSpec Pen for untargeted chemical profiling of frog skin *in vivo*. Rich molecular profiles were obtained from all of the positive and negative ion mode analyses performed from  $m/z$  150–1800 (Figure 4). In the positive ion mode, highly abundant, putatively identified species included choline ( $m/z$  104.107), ganoderic acid ( $m/z$  533.310), and several triacylglyceride (TG) species including TG(52:5), TG(54:6), and TG(56:7) at  $m/z$  853.727, 879.742, and 905.758, respectively. In the negative ion mode, abundant, putatively identified species included dodecanoic acid ( $m/z$  199.170), several phosphatidic acid (PA) species including PA(42:8) at  $m/z$  807.472 and PA(44:8) at  $m/z$  835.504, and a chlorinated cardiolipin (CL), CL(40:0) + Cl at  $m/z$  1051.568. Triacylglycerol, cardiolipin, and phosphatidylethanolamine are all important lipid components in cellular and organelle membranes. Although we did not monitor these species throughout the alkaloid-feeding study, these results support the technology's ability to monitor metabolites present in the skin during the alkaloid sequestration process. Note that some of the compounds detected may originate from plant material

present in terraria that were in contact with the frog's skin, and thus additional experiments from whole skin extracts could be performed to verify these results obtained.

#### LC-MS Validation of Feeding Study Results

To validate the MasSpec Pen feeding study results in which significantly greater DHQ signals in alkaloid-fed frogs *versus* vehicle-fed frogs were detected at the conclusion of the 28-day study, we quantified DHQ in excised skin samples using LC-MS. The mass of each individual and the mass of excised dorsal skin were obtained on day 28 (Table 1). A response curve was constructed with DHQ standard solutions at 0, 200, 400, 600, 800, 1000, and 1500 ng DHQ/mL. Standards were diluted in excess skin matrix from vehicle-fed frogs to account for matrix effects when analyzing the unknown alkaloid-fed frog samples. (Figure S2). The linear fit for this response curve had an  $R^2$  of 0.9962, a gradient of  $4347.3 \pm 119.9$ , and an intercept of  $1.951 \times 10^5 \pm 0.956 \times 10^5$ . The method LOD was determined to be 113.6 ng DHQ/mL. We then employed the same LC-MS method to analyze the skin extracts of all individuals included in the feeding study. As expected, we detected DHQ in all skin extracts from alkaloid-fed individuals, whereas the alkaloid was not detected in skin extracts of vehicle-fed individuals. Notably, alkaloid-fed individual 8 was found to have the highest signal intensity of DHQ by direct MasSpec Pen analysis on day 28



**Figure 5.** MasSpec Pen alkaloid profiling of field-caught *O. sylvatica* skin extracts. (A) MasSpec Pen spectra obtained from analysis of *O. sylvatica* skin extract from Felfa (top) and Cristóbal Colón populations (bottom). Putative molecular assignments are bolded in blue next to  $m/z$  flags and are based on high mass accuracy ( $<5$  ppm) measurements and alkaloid species previously isolated from the closely related *O. pumilio*. (B) Numeric code identifiers (ID) for alkaloid classes. Exact mass measurements cannot distinguish molecular isomers; as such, putative molecular assignments have associated numeric code in brackets indicating all classes of alkaloids that contain species with the same molecular formula.

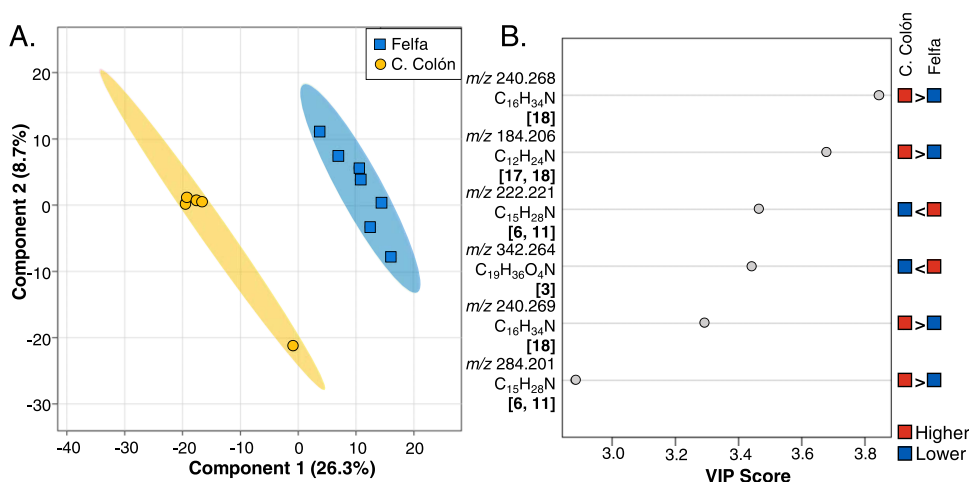
and also presented the highest concentration of DHQ in their skin by LC-MS. The concentration of DHQ for all alkaloid-fed samples was determined using the linear fit from the response curve, accounting for the mass of the dorsal skin used in the homogenate, and was determined to be  $13 \pm 7$  ng DHQ/ $\mu$ g skin. Note that while alkaloids have been detected in frog skin as soon as 4–5 days after initial administration by LC-MS,<sup>9,10</sup> we did not observe an increase in normalized DHQ signal among alkaloid-fed individuals *versus* vehicle-fed individuals until day 10 during the feeding study. As alkaloid detection depends on the sensitivity of the mode of analysis and total quantities of accumulated DHQ in skin have been shown to increase with higher doses of the alkaloid,<sup>32</sup> we expect that administration of a higher dose of DHQ would result in earlier detection of the alkaloid *in vivo* using the MasSpec Pen. It should also be noted that the efficiency of alkaloid accumulation differs among alkaloids;<sup>30,32</sup> thus, additional experiments are needed to determine the *in vivo* detection limit of the MasSpec Pen for various alkaloid compounds.

#### Application of the MasSpec Pen for Detection of Alkaloids from Field-Collected Frog Skin Extracts

We also evaluated the ability to employ the MasSpec Pen technology to detect profiles of a variety of alkaloid compounds from the skin extracts of field-caught Diablito frog (*O. sylvatica*) specimens from two geographical locations in Ecuador: “Felfa” ( $N = 5$ ) and “Cristóbal Colón” ( $N = 7$ ). Collection and characterization of these samples were previously described by McGugan and co-workers.<sup>18</sup> Formic acid (0.01%) was incorporated into the solvent system of 5% EtOH in  $H_2O$  (v/v%) to facilitate the protonation of alkaloid species (Table S4). Remarkably, rich molecular profiles were

obtained from MasSpec Pen analysis of all samples. Figure 5A shows representative MasSpec Pen molecular profiles from skin extracts collected in Felfa and Cristóbal Colón regions. Alkaloid species in these profiles were putatively identified using an exact monoisotopic mass of alkaloids isolated from the closely related Strawberry poison frog (*Oophaga pumilio*)<sup>14</sup> and previously reported molecular formulas.<sup>13</sup> As exact mass measurements cannot distinguish molecular isomers, for each putative molecular assignment, we indicate a numeric code in brackets indicating each class of alkaloid that contains a described species with the same molecular formula (Figure 5B). MasSpec Pen spectra of skin extracts from the Felfa region were characterized by high relative abundance ( $>30\%$ ) of  $m/z$  204.175 ( $C_{14}H_{22}N$  [10]),  $m/z$  218.190 ( $C_{15}H_{24}N$  [10, 15]),  $m/z$  220.206 ( $C_{15}H_{26}N$  [6, 10, 12, 15, 16]),  $m/z$  224.237 ( $C_{15}H_{30}N$  [6, 8, 9, 10, 12, 16]), and  $m/z$  232.206 ( $C_{16}H_{26}N$  [6, 10, 12, 15]). Skin extracts from the Cristóbal Colón region were characterized by high relative abundance ( $>30\%$ ) of  $m/z$  270.221 ( $C_{19}H_{28}N$  [6, 11]),  $m/z$  282.200 ( $C_{19}H_{26}ON$  [1]),  $m/z$  286.216 ( $C_{19}H_{27}ON$  [1]), and  $m/z$  288.231 ( $C_{19}H_{30}ON$  [1]). Among all skin extracts analyzed using the MasSpec Pen, we detected 67 unique ions with putative alkaloid attributions, lending feasibility to the utility of the MasSpec Pen as a sampling and analysis method for additional feeding studies using one or more alkaloids other than DHQ. Further development of this platform will be necessary to perform alkaloid profiling of field-caught specimens for correlation with other biobehavioral or environmental factors, as this platform has not been validated for the identification or quantification of alkaloids. A complete table of molecular attributions and detected  $m/z$  values from MasSpec Pen analysis of field-collected frog skin extracts can be found in Table S5.





**Figure 6.** PLS-DA of alkaloid profiles from field-caught *Oophaga sylvatica* skin extracts obtained using the MasSpec Pen. (A) PLS-DA scores plot for MasSpec Pen alkaloid profiles from the Felfa (light green) and Cristóbal Colón (pink). Shaded ovals represent 95% confidence intervals. (B) Variable importance in projection plot showing alkaloid features contributing to partial least squares dimensional analysis separation. Numeric code identifiers indicating all classes of alkaloids (defined in Figure S5B) that contain species with the same molecular formula are shown in brackets for all features shown. Legend (right) indicates the population in which the alkaloid feature was detected at higher (red) or lower (blue) relative abundance.

We also performed partial least squares dimensional analysis on the alkaloid profiles obtained using the MasSpec Pen to determine important alkaloids for differentiating the two frog populations. Figure 6A shows the 2D score plot from PLS-DA of MasSpec Pen spectra from analysis of the field-caught frog skin extract samples. Complete separation was observed among the two populations based on spectral features, and the six features with the greatest variable importance in projection scores are shown in Figure 6B. These features are the greatest contributors to separation among the two groups, where  $m/z$  222.221 (C<sub>15</sub>H<sub>28</sub>N [6, 11]) and  $m/z$  342.264 (C<sub>19</sub>H<sub>36</sub>O<sub>4</sub>N [3]) were detected at the greater relative abundance in Felfa extracts, while  $m/z$  240.268 (C<sub>16</sub>H<sub>34</sub>N [18]),  $m/z$  184.206 (C<sub>12</sub>H<sub>24</sub>N [17, 18]), and  $m/z$  284.201 (C<sub>15</sub>H<sub>28</sub>N [6, 11]) were detected at the greater relative abundance in Cristóbal Colón extracts.

To further confirm the identity of several alkaloid species detected, we performed MasSpec Pen MS/MS experiments to compare the fragmentation mass spectra of  $m/z$  224.238,  $m/z$  278.284, and  $m/z$  292.264 (Figure S2) to those reported by McGugan and colleagues from LC-MS/MS analysis of the same field-collected frog extracts.<sup>18</sup> Major fragment ions observed in the MasSpec Pen fragmentation mass spectrum of  $m/z$  224.238 included  $m/z$  55.055,  $m/z$  67.055,  $m/z$  81.071, and  $m/z$  126.128. Major fragment ions observed for  $m/z$  278.285 included  $m/z$  67.055,  $m/z$  95.086, and  $m/z$  112.113. Fragment ions observed for  $m/z$  292.263 included  $m/z$  55.055,  $m/z$  67.055, and  $m/z$  91.055. These fragment ions were also observed in the LC-MS/MS experiments performed on the same precursor ions as described by McGugan and colleagues, thus corroborating the chemical identity of these molecules. Additional ions appearing in fragmentation spectra obtained with the MasSpec Pen are likely due to coisolated species.

## CONCLUSIONS

In this work, we have demonstrated the development and application of the MasSpec Pen technology for the detection and *in vivo* monitoring of alkaloid sequestration directly from the skin of poison frogs. The optimized platform enabled

gentle and biocompatible chemical analysis of the skins of the living animals, including untargeted analysis of various biomolecules. We also demonstrated the feasibility of using the MasSpec Pen for the detection of rich profiles of alkaloids from skin extracts of poison frogs collected from two distinct geographical populations in Ecuador, thus showing evidence for its use in the untargeted detection of various alkaloid molecules. Ongoing developments include coupling of the MasSpec Pen system with portable mass spectrometers to enable the translation of this platform for alkaloid profiling of poison frogs in the field. Collectively, this study demonstrates the value of *in vivo* chemical analysis using the MasSpec Pen technology to detect, monitor, and study alkaloid chemical defenses in poison frogs and opens new opportunities for further applications in chemical and natural product analyses within living organisms.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsmesureciau.2c00035>.

Tables S1–S4 and Figures S1 and S2; data to support MasSpec Pen detection of DHQ; statistical significance values associated with the alkaloid-feeding study; and data to support the detection of alkaloids from field-caught *O. sylvatica* skin extracts (PDF)

*In vivo* analysis of alkaloid sequestration in poison frogs with the MasSpec Pen (Video S1) (MP4)

## AUTHOR INFORMATION

### Corresponding Authors

Lauren A. O'Connell – Department of Biology, Stanford University, Stanford, California 94305, United States; Email: [loconnel@stanford.edu](mailto:loconnel@stanford.edu)

Livia S. Eberlin – Department of Chemistry, University of Texas at Austin, Austin, Texas 78712, United States; Department of Surgery, Baylor College of Medicine, Houston,



Texas 77030, United States; [orcid.org/0000-0002-3885-3215](https://orcid.org/0000-0002-3885-3215); Email: [livia.eberlin@bcm.edu](mailto:livia.eberlin@bcm.edu)

## Authors

**Anna C. Krieger** – Department of Chemistry, University of Texas at Austin, Austin, Texas 78712, United States  
**Sydney C. Povilaitis** – Department of Chemistry, University of Texas at Austin, Austin, Texas 78712, United States  
**Prajwal Gowda** – Department of Chemistry, University of Texas at Austin, Austin, Texas 78712, United States

Complete contact information is available at:  
<https://pubs.acs.org/10.1021/acsmeasuresci.2c00035>

## Author Contributions

L.S.E. and L.A.O. conceived the application of the MasSpec Pen for *in vivo* alkaloid analysis. A.C.K. performed MasSpec Pen platform optimization experiments. L.A.O. and A.C.K. designed the alkaloid-feeding study. A.C.K., S.C.P., and P.G. performed animal care duties, performed MasSpec Pen experiments, and extracted data. A.C.K. analyzed the data, and A.C.K. and L.A.O. performed statistical analyses. A.C.K., L.S.E., and L.A.O. wrote and edited the manuscript.

## Notes

The authors declare the following competing financial interest(s): L.S.E. is an inventor in US Patent No. 10,643,832 and/or in other patent applications related to the MasSpec Pen Technology licensed by the University of Texas to MS Pen Technologies, Inc. L.S.E. is a shareholder in MS Pen Technologies, Inc. and serves as chief scientific officer for MS Pen Technologies, Inc. All other authors declare no competing interests.

The data that support the findings of this study will be available in a public repository hosted by the Harvard Dataverse upon acceptance of the manuscript.

## ACKNOWLEDGMENTS

Funding for this work was generously provided by the Welch Foundation, Eli Lilly and Company, the Moore Foundation to L.S.E., and the National Science Foundation (IOS-1557684) to L.A.O. The authors thank Aurora Alvarez-Bullya for helpful advice on frog handling and care; Samuel Chen, Iñaki Sanz Gurruchaga, and Ashish Chakraborty for their experimental assistance; and Dr. David Cannatella and Dr. Mike Ryan for allowing them space to house animals in their frog room. L.A.O. is a New York Stem Cell Foundation—Robertson Investigator and is grateful to Dr. Luis Coloma at Centro Jambatu for a decade-long partnership studying poison frog chemical defense in Ecuador. L.S.E. and L.A.O. would like to acknowledge the L'Oréal USA For Women in Science Program for previous support and for enabling the conception of this collaboration.

## REFERENCES

- (1) Daly, J. W.; Kaneko, T.; Wilham, J.; Garraffo, H. M.; Spande, T. F.; Espinosa, A.; Donnelly, M. A. Bioactive alkaloids of frog skin: combinatorial bioprospecting reveals that pumiliotoxins have an arthropod source. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 13996–14001.
- (2) Saporito, R. A.; Spande, T. F.; Garraffo, H. M. Arthropod alkaloids in poison frogs: a review of the 'dietary hypothesis'. *Heterocycles* **2009**, *79*, 277–297.
- (3) Saporito, R. A.; Donnelly, M. A.; Spande, T. F.; Garraffo, H. M. A review of chemical ecology in poison frogs. *Chemoecology* **2012**, *22*, 159–168.
- (4) Santos, J. C.; Tarvin, R. D.; O'Connell, L. A. A review of chemical defense in poison frogs (Dendrobatidae): ecology, pharmacokinetics, and autoresistance. *Chem. Signals Vertebr.* **2016**, *13*, 305–337.
- (5) Salehi, B.; Sestito, S.; Rapposelli, S.; Peron, G.; Calina, D.; Sharifi-Rad, M.; Sharopov, F.; Martins, N.; Sharifi-Rad, J. Epibatidine: A Promising Natural Alkaloid in Health. *Biomolecules* **2019**, *9*, No. 6.
- (6) Daly, J. W.; Martin Garraffo, H.; Spande, T. F.; Decker, M. W.; Sullivan, J. P.; Williams, M. Alkaloids from frog skin: the discovery of epibatidine and the potential for developing novel non-opioid analgesics. *Nat. Prod. Rep.* **2000**, *17*, 131–135.
- (7) Darst, C. R.; Menéndez-Guerrero, P. A.; Coloma, L. A.; Cannatella, D. C. Evolution of Dietary Specialization and Chemical Defense in Poison Frogs (Dendrobatidae): A Comparative Analysis. *Am. Nat.* **2005**, *165*, 56–69.
- (8) Clark, V. C.; Raxworthy, C. J.; Rakotomalala, V.; Sierwald, P.; Fisher, B. L. Convergent evolution of chemical defense in poison frogs and arthropod prey between Madagascar and the Neotropics. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 11617–11622.
- (9) Alvarez-Buylla, A.; Payne, C. Y.; Vidoudez, C.; Trauger, S. A.; O'Connell, L. A. Molecular physiology of pumiliotoxin sequestration in a poison frog. *PLoS One* **2022**, *17*, No. e0264540.
- (10) O'Connell, L. A.; Course, L. I. S. L.; O'Connell, J. D.; Paulo, J. A.; Trauger, S. A.; Gygi, S. P.; Murray, A. W. Rapid toxin sequestration modifies poison frog physiology. *J. Exp. Biol.* **2021**, *224*, No. jeb230342.
- (11) Jeckel, A. M.; Matsumura, K.; Nishikawa, K.; Morimoto, Y.; Saporito, R. A.; Grant, T.; Ifa, D. R. Use of whole-body cryosectioning and desorption electrospray ionization mass spectrometry imaging to visualize alkaloid distribution in poison frogs. *J. Mass Spectrom.* **2020**, *55*, No. e4520.
- (12) Sanchez, E.; Rodríguez, A.; Grau, J. H.; Lötters, S.; Künzel, S.; Saporito, R. A.; Ringler, E.; Schulz, S.; Wollenberg Valero, K. C.; Vences, M. Transcriptomic Signatures of Experimental Alkaloid Consumption in a Poison Frog. *Genes* **2019**, *10*, 733.
- (13) Daly, J. W.; Spande, T. F.; Garraffo, H. M. Alkaloids from amphibian skin: a tabulation of over eight-hundred compounds. *J. Nat. Prod.* **2005**, *68*, 1556–1575.
- (14) Saporito, R. A.; Donnelly, M. A.; Jain, P.; Martin Garraffo, H.; Spande, T. F.; Daly, J. W. Spatial and temporal patterns of alkaloid variation in the poison frog *Oophaga pumilio* in Costa Rica and Panama over 30 years. *Toxicon* **2007**, *50*, 757–778.
- (15) Daly, J. W.; Wilham, J.; Spande, T. F.; Garraffo, H. M.; Gil, R.; Silva, G.; Vaira, M. Alkaloids in bufonid toads (*Melanophryniscus*): temporal and geographic determinants for two Argentinian species. *J. Chem. Ecol.* **2007**, *33*, 871–887.
- (16) Daly, J. W.; Garraffo, H. M.; Spande, T. F.; Giddings, L. A.; Saporito, R. A.; Vieites, D. R.; Vences, M. Individual and geographic variation of skin alkaloids in three species of Madagascan poison frogs (Mantella). *J. Chem. Ecol.* **2008**, *34*, 252–279.
- (17) Saporito, R. A.; Donnelly, M. A.; Madden, A. A.; Garraffo, H. M.; Spande, T. F. Sex-related differences in alkaloid chemical defenses of the dendrobatid frog *Oophaga pumilio* from Cayo Nancy, Bocas del Toro, Panama. *J. Nat. Prod.* **2010**, *73*, 317–321.
- (18) McGugan, J. R.; Byrd, G. D.; Roland, A. B.; Caty, S. N.; Kabir, N.; Tapia, E. E.; Trauger, S. A.; Coloma, L. A.; O'Connell, L. A. Ant and mite diversity drives toxin variation in the Little Devil Poison frog. *J. Chem. Ecol.* **2016**, *42*, 537–551.
- (19) Bols, N. C.; Roberson, M.; Haywood-Reid, P.; Cerra, R.; Barondes, S. Secretion of a cytoplasmic lectin from *Xenopus laevis* skin. *J. Cell Biol.* **1986**, *102*, 492–499.
- (20) Daly, J. W. Thirty years of discovering arthropod alkaloids in amphibian skin. *J. Nat. Prod.* **1998**, *61*, 162–172.
- (21) Grant, J. B.; Land, B. Transcutaneous amphibian stimulator (TAS): A device of the collection of amphibian skin secretions. *Herpetol. Rev.* **2002**, *33*, No. 38.

(22) Clark, V. C. Collecting arthropod and amphibian secretions for chemical analyses. In *Behavioral and Chemical Ecology*, 1st ed.; Nova Science Pub. Inc: New York, 2010; pp 1–46.

(23) Zhang, J.; Rector, J.; Lin, J. Q.; Young, J. H.; Sans, M.; Katta, N.; Giese, N.; Yu, W.; Nagi, C.; Suliburk, J.; Liu, J.; Bensussan, A.; DeHoog, R. J.; Garza, K. Y.; Ludolph, B.; Sorace, A. G.; Syed, A.; Zahedivash, A.; Milner, T. E.; Eberlin, L. S. Nondestructive tissue analysis for ex vivo and in vivo cancer diagnosis using a handheld mass spectrometry system. *Sci. Transl. Med.* **2017**, *9*, No. ean3968.

(24) Keating, M. F.; Zhang, J.; Feider, C. L.; Retaillieu, S.; Reid, R.; Antaris, A.; Hart, B.; Tan, G.; Milner, T. E.; Miller, K.; Eberlin, L. S. Integrating the MasSpec Pen to the da Vinci Surgical System for In Vivo Tissue Analysis during a Robotic Assisted Porcine Surgery. *Anal. Chem.* **2020**, *92*, 11535–11542.

(25) King, M. E.; Zhang, J.; Lin, J. Q.; Garza, K. Y.; DeHoog, R. J.; Feider, C. L.; Bensussan, A.; Sans, M.; Krieger, A.; Badal, S.; et al. Rapid diagnosis and tumor margin assessment during pancreatic cancer surgery with the MasSpec Pen technology. *Proc. Natl. Acad. Sci. U.S.A.* **2021**, *118*, No. e2104411118.

(26) Zhang, J.; Sans, M.; Garza, K. Y.; Eberlin, L. S. Mass spectrometry technologies to advance care for cancer patients in clinical and intraoperative use. *Mass Spectrom. Rev.* **2021**, *40*, 692–720.

(27) Feider, C. L.; Gatmaitan, A. N.; Hooper, T.; Chakraborty, A.; Gowda, P.; Buchanan, E.; Eberlin, L. S. Integrating the MasSpec Pen with Sub-Atmospheric Pressure Chemical Ionization for Rapid Chemical Analysis and Forensic Applications. *Anal. Chem.* **2021**, *93*, 7549–7556.

(28) Harris, D. C. *Quantitative Chemical Analysis*; Macmillan, 2010.

(29) Gatmaitan, A. N.; Lin, J. Q.; Zhang, J.; Eberlin, L. S. Rapid Analysis and Authentication of Meat Using the MasSpec Pen Technology. *J. Agric. Food Chem.* **2021**, *69*, 3527–3536.

(30) Hantak, M. M.; Grant, T.; Reinsch, S.; McGinnity, D.; Loring, M.; Toyooka, N.; Saporito, R. A. Dietary Alkaloid Sequestration in a Poison Frog: An Experimental Test of Alkaloid Uptake in *Melanophryniscus stelnzeri* (Bufonidae). *J. Chem. Ecol.* **2013**, *39*, 1400–1406.

(31) Neuwirth, M.; Daly, J. W.; Myers, C. W.; Tice, L. W. Morphology of the granular secretory glands in skin of poison-dart frogs (Dendrobatidae). *Tissue Cell* **1979**, *11*, 755–771.

(32) Jeckel, A. M.; Bolton, S. K.; Waters, K. R.; Antoniazzi, M. M.; Jared, C.; Matsumura, K.; Nishikawa, K.; Morimoto, Y.; Grant, T.; Saporito, R. A. Dose-dependent alkaloid sequestration and N-methylation of decahydroquinoline in poison frogs. *J. Exp. Zool., Part A* **2022**, *337*, 537–546.

## Recommended by ACS

### Discriminator for Cutaneous Leishmaniasis Using MALDI-MSI in a Murine Model

Crisostomo Barajas-Solano, Enrique Mejía-Ospino, *et al.*

MAY 12, 2022

JOURNAL OF THE AMERICAN SOCIETY FOR MASS SPECTROMETRY

READ 

### Rapid In Situ Detection of THC and CBD in *Cannabis sativa* L. by 1064 nm Raman Spectroscopy

Stefania Porcu, Pier Carlo Ricci, *et al.*

JULY 18, 2022

ANALYTICAL CHEMISTRY

READ 

### Non-Invasive, Topical Sampling of Potential, Low-Molecular Weight, Skin Cancer Biomarkers: A Study on Healthy Volunteers

Skaidre Jankovskaja, Tautgirdas Ruzgas, *et al.*

APRIL 08, 2022

ANALYTICAL CHEMISTRY

READ 

### Semiquantitative Screening of THC Analogues by Silica Gel TLC with an Ag(I) Retention Zone and Chromogenic Smartphone Detection

Si Huang, Gert IJ. Salentijn, *et al.*

SEPTEMBER 30, 2022

ANALYTICAL CHEMISTRY

READ 

Get More Suggestions >