# Alkaloids of *Ranitomeya imitator*, a Mullerian Mimic of a Poison Frogs

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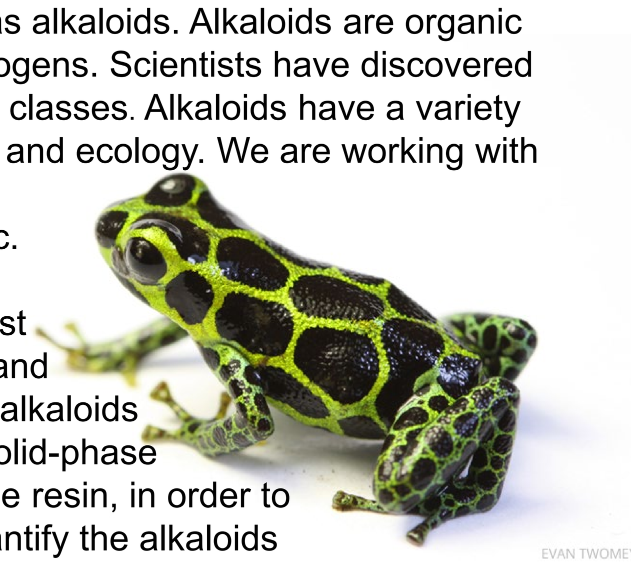
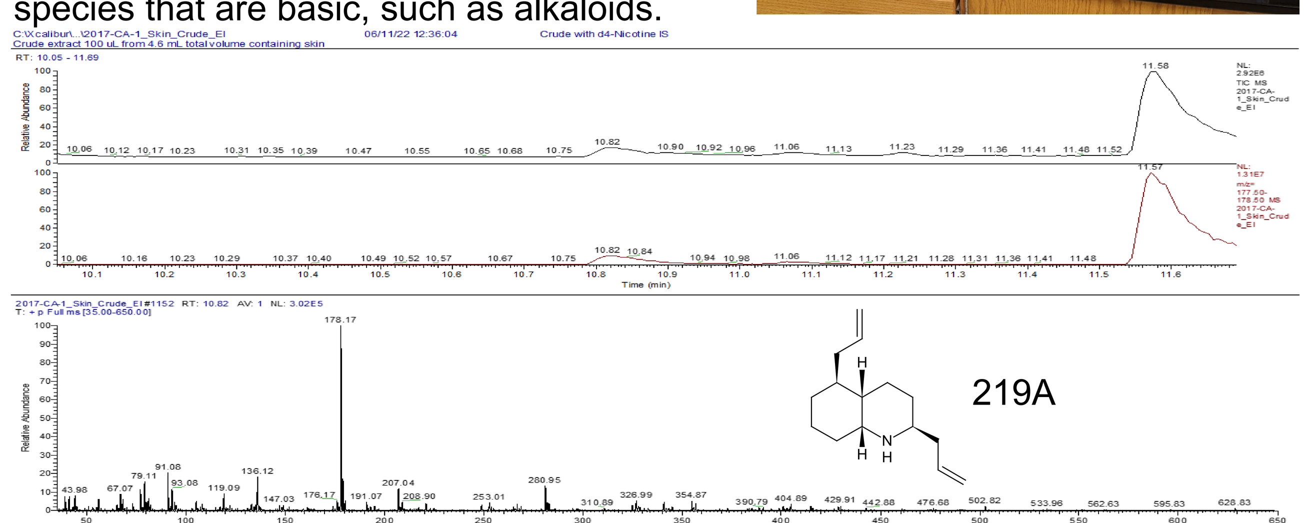
## Background

Poison frogs belong to the Dendrobatid family. They are small brightly colored amphibians and secrete toxic alkaloids from their skin. They are named for the use of their secretions by natives for poisoning darts for hunting. The frogs obtain alkaloids from dietary arthropods, also known as alkaloids. Alkaloids are organic compounds that contain one or more basic nitrogens. Scientists have discovered over 1000 different alkaloids in 25-30 structural classes. Alkaloids have a variety of biological activities relevant to human health and ecology. We are working with collaborators who study a Peruvian mimic frog, *Ranitomeya imitator*, which is a Mullerian mimic. Such mimics are similar in appearance to co-occurring poison frogs but are less toxic. We first did a crude analysis of our samples using gas and liquid chromatography to separate and identify alkaloids in the crude extracts. We will then follow with solid-phase extraction (SPE) using a strong cation exchange resin, in order to isolate alkaloids. Our goal is to identify and quantify the alkaloids present in each of our samples. Our results to date are presented herein.

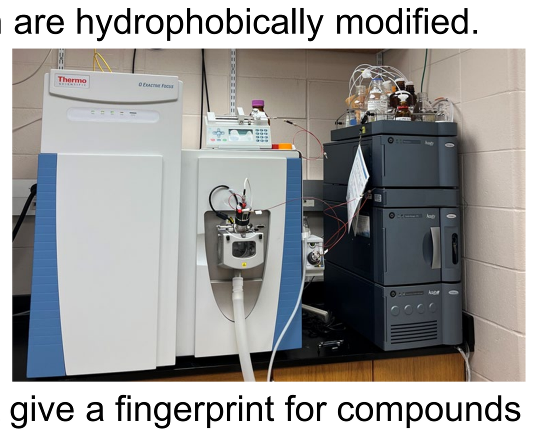


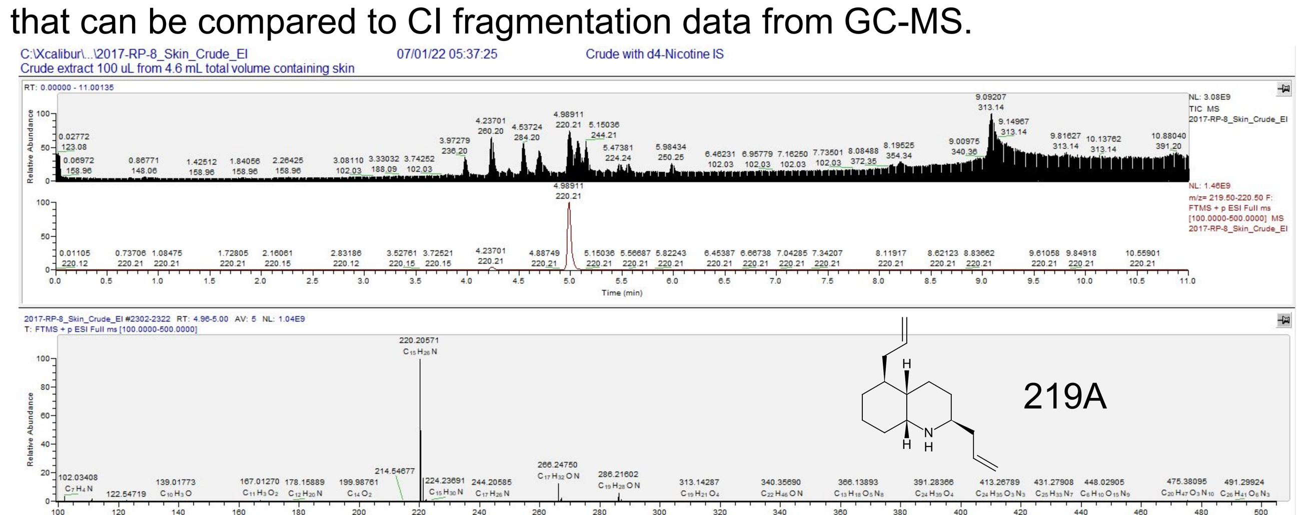

## Gas and Liquid Chromatography-Mass Spectrometry (GC-MS and LC-MS)

Gas chromatography separates volatile compound mixtures by vapor pressure by flowing through a column where they can reversibly condense and evaporate as well as dissolve in a bonded stationary phase, The MS uses electron ionization (EI), which makes unstable radical ions that fragment as shown below, giving structural information from the fragmentation pattern. It also performs chemical ionization (CI), which protonates molecules, allowing calculation of molecular weight by subtracting H. The CI reagent gas used is $NH_3$, producing the weak acid $NH_4^+$, selectively protonating species that are basic, such as alkaloids.

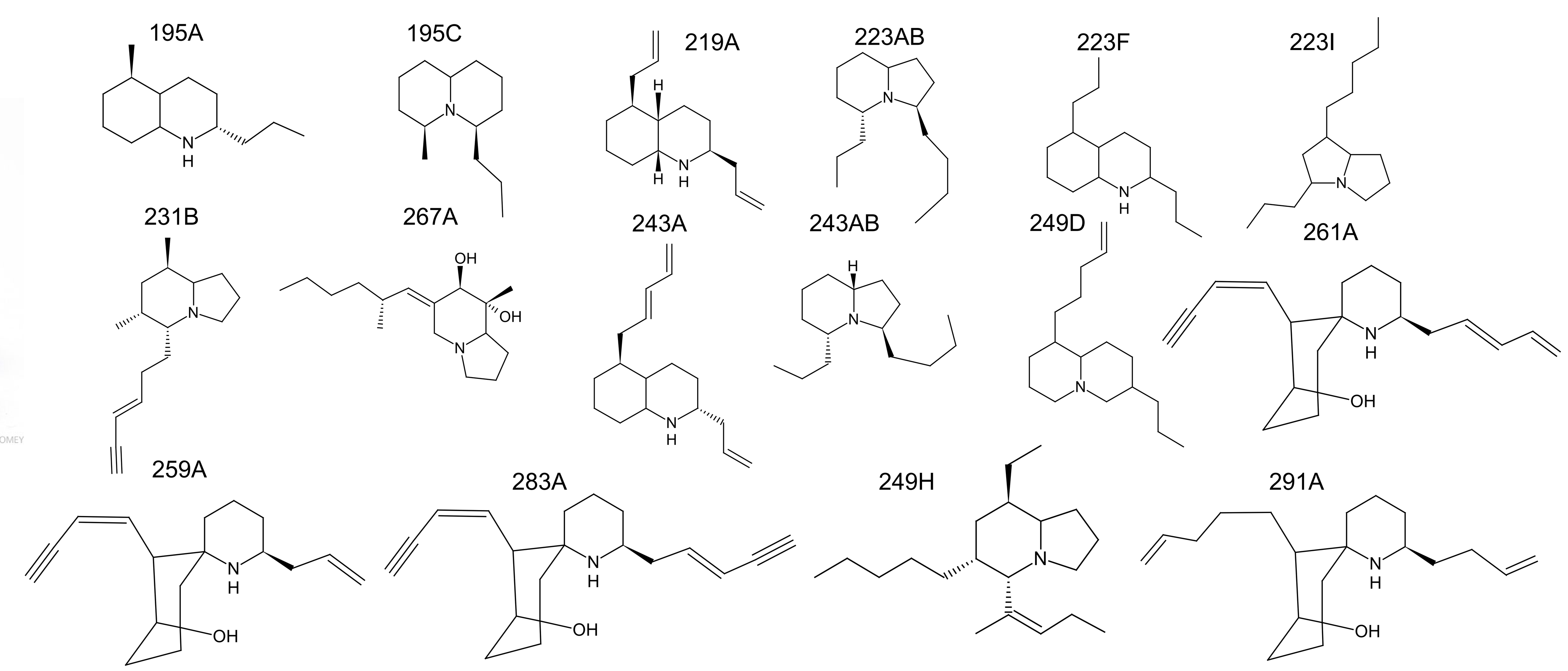



LC-MS analyzes both volatile and nonvolatile compounds. Compound mixtures in aqueous solution flow through a column and reversibly bind to it. The column is a short tube packed with small particles which are hydrophobically modified. It separates compounds by their solubility and polarity. Higher polarity compounds exit first. The MS uses electrospray ionization (ESI), a different form of chemical ionization that adds protons to the molecule. The MS we have is an orbitrap which has high resolution and mass accuracy, which allows us to determine the molecular formula. It also can do collision Induced dissociation which gives fragments that give a fingerprint for compounds that can be compared to CI fragmentation data from GC-MS.






## Structures of Alkaloids Identified




| RT | 8.1 | 8.5 | 8.9 | 9.1 | 9.8 | 9.9 | 9.9 | 10.2 | 10.8 | 11 | 11 | 11.2 | 11.5 | 12 | 12.3 | 13.1 | 13.1 | 13.2 | 13.55 | 13.45 | 13.8 | 13.9 | 14.1 | 14.2 | 14.4 | 14.88 | 14.97 | 16.88 | 16.95 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| EI | 152 | 152 | 152 | 138 | 166 | 180 | 180 | 180 | 178 | 152 | 180 | 178 | 178 | 190 | 166 | 206 | 194 | 152 | 180 | 202 | 202 | 202 | 202 | 70 | 190 | 96-218 | 96-220 | 178 | 96-218 |
| CI | 208 | 196 | 196 | 210 | 224 | 224 | 234 | 224 | 220 | 218 | 224 | 220 | 220 | 232 | 250 | 250 | 236 | 274 | 266 | 244 | 244 | 244 | 244 | 268 | 258 | 260 | 262 | 292 | 284 |
| ID | 207N | 195C | 195A | 209G | 223AB_1 | 223AB_2 | 223I | 223AB_3 | 219A_1 | 217G | 223F | 219A_2 | 219A_3 | 231M | 249H | 249D | 235BB | 273 | New 265 | 243A_1 | 243A_2 | 243A_3 | 243A_4 | 267A | 257D | 259A | 261A | 291A | 283A |
| Type | Uncl | 4,6-Q | DHQ | Uncl | 3,5-I | 3,5-I | Izidine | 3,5-I | DHQ | 5,6,8-I | DHQ | DHQ | DHQ | Uncl | 5,6,8_I | DHQ | 5,8-I | | Izindine | DHQ | DHQ | DHQ | DHQ | aPTX | 1,4-Q | HTX | HTX | HTX | HTX |
| SR_1_Skin | - | - | - | - | - | - | ++ | - | - | - | - | - | - | - | - | - | | + | - | ++ | - | - | - | - | - | - | - | - | - |
| SR_1_Ovary | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | - | - | - | - | - | - | - | - | - | - | - | - |
| SR_2_Skin | - | - | - | - | - | - | +++ | - | - | - | - | - | ++ | +++ | - | - | | ++ | - | +++ | ++ | +++ | - | - | - | - | - | - | - |
| SR_3_Skin | - | - | - | +++ | - | - | ++ | - | - | - | +++ | - | ++++ | +++ | - | - | | ++ | - | +++ | + | +++ | - | - | - | - | - | - | - |
| SR_3_Ovary | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | - | - | - | - | - | - | - | - | - | - | - | - |
| SR_4_Skin | - | - | - | +++ | + | - | +++ | - | - | - | - | - | ++++ | +++ | - | - | | ++ | - | +++ | - | +++ | - | ++ | - | - | - | - | - |
| SR-5_Skin | - | - | - | - | ++ | - | + | + | - | - | ++ | - | ++++ | +++ | - | - | | ++ | - | ++++ | ++ | +++ | - | - | - | - | - | - | - |
| SR_6_Skin | - | - | - | +++ | ++ | - | +++ | ++ | - | - | + | - | ++++ | +++ | - | - | | ++ | - | ++++ | - | +++ | - | - | - | - | - | - | - |
| SR-7_Skin | - | - | - | - | - | - | ++ | + | - | - | - | - | ++++ | +++ | - | - | | ++ | - | +++ | - | +++ | - | - | - | - | - | - | - |
| SR_8_Skin | - | - | - | - | - | - | +++ | - | - | - | - | - | +++ | ++ | - | - | | ++ | - | +++ | - | ++ | - | - | - | - | - | - | - |
| SR_8_Ovary | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | - | - | - | - | - | - | - | - | - | - | - | - |
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| SR-10_Skin | - | - | - | - | ++ | - | ++ | - | - | - | - | - | ++++ | +++ | - | - | | ++ | - | +++ | - | +++ | - | - | - | - | - | - | - |
| SA_1_Skin | - | ++ | - | +++ | +++ | +++ | - | +++ | - | - | - | - | +++ | - | - | - | | ++ | - | ++ | ++ | ++ | - | + | ++ | - | - | - | - |
| SA_2_Skin | - | - | - | +++ | ++++ | - | - | - | - | - | - | - | - | - | - | - | | ++ | - | ++ | +++ | +++ | - | - | ++ | ++ | ++ | - | ++ |
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| SA_4_Skin | - | - | - | - | ++++ | - | - | - | - | - | - | - | - | - | - | - | | ++ | - | + | ++++ | ++++ | - | - | ++ | ++ | ++ | - | ++ |
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| SA_10_Skin | - | - | - | - | ++ | ++ | - | ++ | - | - | + | - | +++ | - | - | - | | ++ | - | ++ | +++ | +++ | - | - | + | - | - | - | - |
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| PO_2_Ovary | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | - | - | - | - | - | - | - | - | - | - | - | - |
| PO_3_Skin | - | - | - | + | ++ | - | - | + | ++ | - | +++ | - | +++ | ++ | - | ++ | | - | - | - | - | - | - | ++ | - | - | - | ++ | - |
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| CA_1_Skin | - | - | - | - | + | ++ | - | + | ++ | + | - | - | +++ | ++ | ++ | - | | - | ++ | +++ | + | ++ | - | - | - | - | - | - | - |
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| CA_5_Ovary | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | - | - | - | - | - | - | - | - | - | - | - | - |
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| CA_7_Skin | - | - | - | - | - | +++ | - | - | ++ | + | - | - | +++ | ++ | ++ | - | | - | ++ | +++ | + | ++ | - | ++ | - | - | - | - | - |
| CA_8_Skin | + | - | - | + | ++ | ++ | - | - | ++ | + | - | - | ++ | - | ++ | - | | - | ++ | - | - | - | +++ | ++ | - | - | - | - | - |
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| AC_7_Ovary | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | - | - | - | - | - | - | - | - | - | - | - | - |
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| RP-2_Oldeggs | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | - | - | - | - | - | - | - | - | - | - | - | - |
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| RP_4_Skin | - | - | - | - | ++ | + | - | - | - | - | - | - | +++ | ++ | - | - | | + | + | +++ | +++ | ++ | - | ++ | +++ | ++ | ++ | +++ | ++ |
| RP_5_Skin | - | - | - | +++ | ++ | - | + | - | - | - | - | - | +++ | ++ | - | - | | ++ | ++ | +++ | +++ | - | ++++ | + | ++ | +++ | ++ | - | - |
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| RP_9_Skin | + | + | + | +++ | + | - | ++ | + | - | - | - | - | +++ | ++ | - | - | | ++ | ++ | ++ | - | + | ++ | ++ | ++ | + | - | - | - |
| RP_9_Ovary | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | | - | - | - | - | - | - | - | - | - | - | - | - |
| RP_10_Skin | - | - | - | - | - | - | + | - | - | - | ++ | - | ++ | ++ | - | - | | - | - | ++ | - | ++ | - | + | - | - | - | - | - |
| RP_11_Skin | - | - | - | - | + | - | + | - | - | - | + | - | ++++ | +++ | - | - | | - | - | +++ | - | +++ | - | - | - | - | - | - | - |
| 2017_Methanol_Blank | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

## Results and Discussion

We examined alkaloids in 82 samples of frog skins and ovaries for alkaloids. In the table are those we have identified thus far. A total of 29 alkaloids. The majority of alkaloids found were decahydroquinolines (DHQ), indolizidines (3,5-I, 5,6,8-I) and histrionicotoxins (HTX). Our most abundant alkaloids were DHQ **223AB** and **219A**. They are present in most of our samples. Multiple stereoisomers for DHQ **219A** and **243A** as well as 3,5-I **223AB**. Alkaloid abundances are reported by the integrated areas of extracted ion chromatograms of the EI base peak by using – for those with no area or below the limit of detection. and incremental numbers + signs for those detected in orders of magnitude, so ++ has 10 times the amount of +. We were not able to detect alkaloids in the ovaries, likely because ovaries are so small and were diluted in too much methanol. We hope to see more following SPE.

## .Conclusions

We were able to observe 29 alkaloids, from five different structural classes in these frogs. Alkaloid abundances varied widely between frogs, from virtually absent to highly abundant. Decahydroquinolines were the most abundant in our samples. We also a lot of isomers, sometimes we had 4 isomers for one alkaloid. Some were unclassified or may be new alkaloids. From those mimic frogs, even known they are less poisonous than the regular poison frogs, we were able to find and identify a good amount of alkaloids. We saw good correlation between our GC-MS and LC-MS data. LC-MS gives better sensitivity and we are working to correlate ESI MS/MS data with that from GC-CI data. Once the solid-phase extraction proceeded will probably find more alkaloids, that were not able to be detected in our crude analysis.

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## References

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