# Analyses of the Psychoactive Poison of the Colorado River Toad *(Incilius alvarius)* using LC-QTOF-MS and LC-MS/MS


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## Introduction and Aims

The Colorado River Toad *(Incilius alvarius)* is the only toad with a special enzyme that converts bufotenin (= 5-hydroxy-*N,N*-dimethyltryptamine) into 5-methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT), an even more potent hallucinogen. That is why the toads' poison is smoked as a drug.[1] To our knowledge, the skin of this toad has so far only been examined by Erspamer *et al.* in 1967 using paper chromatography and thin-layer chromatography. With these methods, 5-MeO-DMT has been described in estimated amounts of 50 to 150 mg/g, and 10 other indolalkyl derivatives have been found.[2] We propose new approaches for the comprehensive analysis of the poison including the enrichment of compounds other than 5-MeO-DMT. Methods for LC-QToF-MS and LC-MS/MS analyses to detect both known and unknown substances in the toads' poison were developed. The LC-MS/MS method was then used to compare the concentrations of different tryptamine derivatives in zoo and wild toad poison samples, which to our knowledge has not been done before.

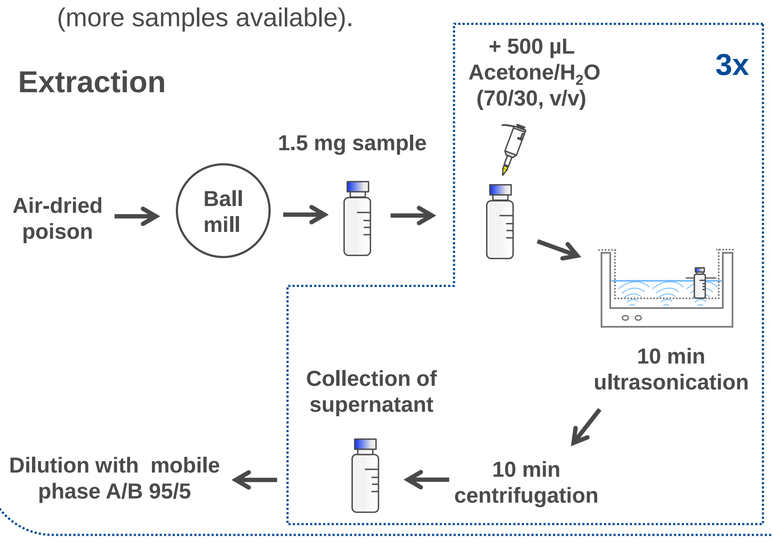

## Methods

### Sample Preparation

#### Sample collection

- Paratoid glands and the glands on the upper and lower legs of the toads were gently squeezed ('milking of the toads').
- Poison was collected in Falcon tubes.
- Samples were air-dried for several days.
- Three toad poison samples from Zoo Leipzig and three wild toad poison samples from Arizona, USA, were analyzed (more samples available).

#### Extraction

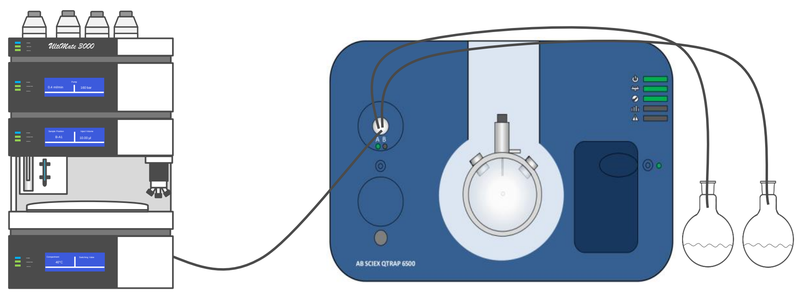



### Qualitative Analysis

Due to the large amounts of 5-MeO-DMT and subsequent chromatographic and MS system overload, further tryptamine derivatives could not be detected. For this reason, the following method was developed.

#### Enrichment of compounds other than 5-MeO-DMT

- Fractionation using an analytical HPLC and the valve of the mass spectrometer; injection 20 x 10 µL



- Evaporation of ACN, lyophilization of the remaining solution
- Residue taken up with 1 mL ethyl acetate, evaporation
- Residue dissolved with 20 µL mobile phase A/B 95/5
- Analyses using LC-QToF-MS and LC-MS/MS

### Quantitative Analysis

- Analysis using LC-MS/MS
- Use of MRM transitions of reference standards and hypothetical MRM transitions of further substances
- Comparison of zoo and wild toad poison samples

### Liquid Chromatography

- C18 column (100 mm × 2.1 mm, 3 µm)
- Mobile phase A: $H_2O$, 0.1 % HCOOH, 2 mmol/L $NH_4^+HCOO^-$, 1 % ACN
- Mobile phase B: ACN, 0.1 % HCOOH, 2 mmol/L $NH_4^+HCOO^-$
- Duration: 10 min
- Bruker Elute OLE HPLC for LC-QTOF-MS
- Dionex UltiMate® 3000 HPLC for LC-MS/MS

### Mass Spectrometry

**QToF**

- Bruker Impact II™
- Ionization: ESI(+)
- Full Scan & bbCID

**MS/MS**

- Sciex QTRAP® 6500
- Ionization: ESI(+)
- MRM & EPI scan mode

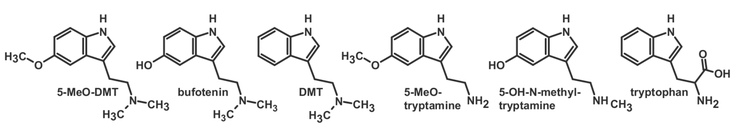
## Results and Discussion

### Qualitative Analysis

#### Analytes included in the MRM method

- **With standards:** 5-hydroxyindoleacetic acid, 5-OH-tryptophol, 5-MeO-DMT, 5-MeO-tryptamine, 5-methoxyindoleacetic acid, 5-OH-*N*-methyltryptamine, bufalin, bufotenin, DMT, marinobufagenin, serotonin, tryptophan
- **With hypothetical MRM transitions:** 5-OH-tryptophan, 5-MeO-DMT *N*-sulfate, 5-MeO-*N*-methyltryptamine, bufotenidin, bufotenin glucuronide, bufotenin *N*-sulfate, bufothionin, bufotoxin, bufoviridin, dehydrobufotenin, histamine, noradrenaline, hydroxylated MeO-DMT, di-OH-MeO-DMT, tri-OH-MeO-DMT, di-MeO-DMT, tri-MeO-DMT

#### Identified substances (standards available)




#### 5-MeO-*N*-methyltryptamine tentatively identified

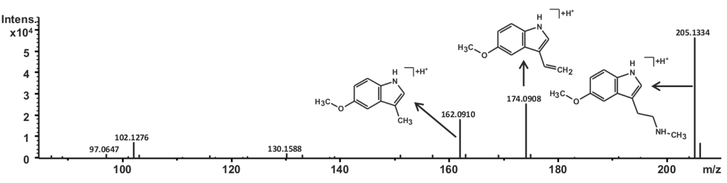



***Figure**: mass spectrum at 2.9 min; 5-MeO-N-methyltryptamine and fragments*

- 5-MeO-*N*-methyltryptamine: isomer of bufotenin
- Fragments of 174.0909 Da and 162.0910 Da confirm the substitution of the indole ring with a methoxy group
- No standard available, but also found by Erspamer *et al.*[2]

#### Hydroxylated MeO-DMT tentatively identified by QToF-Screening

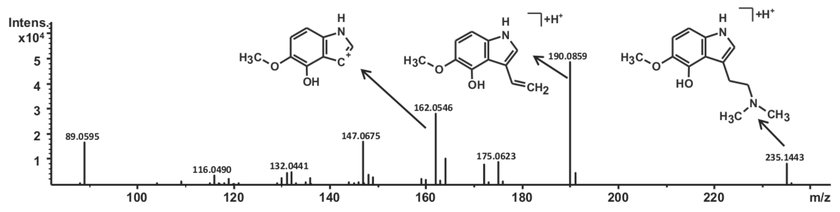
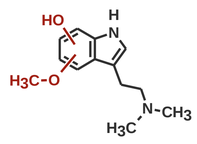



***Figure**: mass spectrum at 2.1 min; 4-OH-5-MeO-DMT (as one possible structure) and fragments*

- MS fragmentation: α- and β- cleavage of the alkyl chain
- Fragments of 162,0550 and 190,0863 Da suggest that the indole ring is substituted with the hydroxy group and with the methoxy group, not the side chain.
- Exact position of the hydroxy and the methoxy group cannot be derived from the QToF measurement; several isomers with the following basic structure are possible:



- Assumption: position 5 is substituted with a hydroxy or methoxy group
- Position of the second substitution unknown
- MRM transitions show two peaks at different retention times → measured extract contained at least two different isomeres
- No standards available

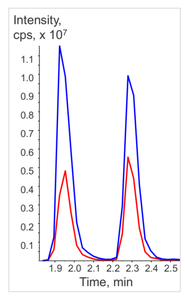



***Figure:** MRM transitions: 235/190 blue, 235/162 red*

### Quantitative Analysis

#### Comparison of zoo and wild toads' poison

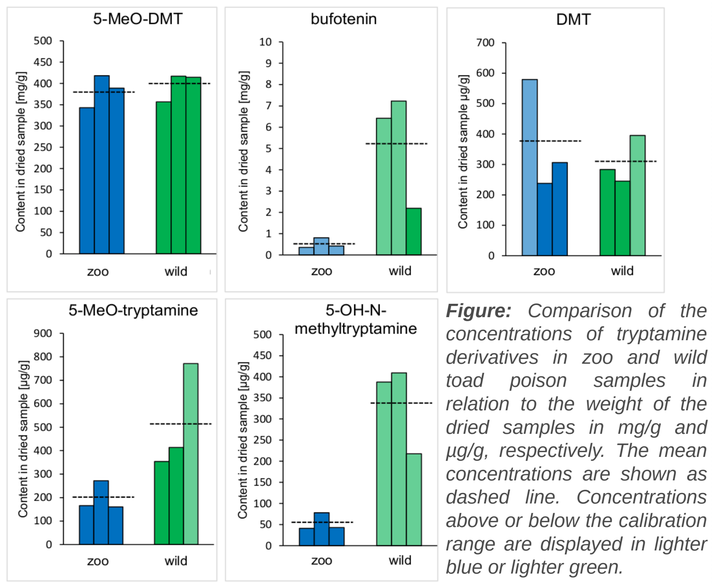



***Figure:** Comparison of the concentrations of tryptamine derivatives in zoo and wild toad poison samples in relation to the weight of the dried samples in mg/g and µg/g, respectively. The mean concentrations are shown as dashed line. Concentrations above or below the calibration range are displayed in lighter blue or lighter green.*

- Higher concentrations of bufotenin, 5-MeO-tryptamine and 5-OH-*N*-methyltryptamine in wild toad poison samples than in zoo samples
- Concentration of 5-MeO-DMT: ca. 340 – 420 mg/g in poison → high concentration in comparison to 50 – 150 mg/g estimated by Erspamer *et al.*[2] for skin

## Outlook

In order to verify the observation that wild toad poison samples contain higher concentrations of certain tryptamine derivatives, more samples should be measured using the developed MRM method. The high concentration of 5-MeO-DMT shall also be verified by further measurements. By synthesis of all possible isomers of the hydroxylated MeO-DMT, the isomers contained in the toads' poison can be identified. The confirmation of the presence of 5-MeO-*N*-methyltryptamine in the extracts is also still pending. Further studies will include the vaporization of the poison with a special device to simulate the consumption process and to assess the content of tryptamine derivatives in smoke condensates by using the developed methodology.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## References


[1] Weil A & Davis W **(1994)**: *Bufo alvarius*: a potent hallucinogen of animal origin. *Journal of Ethnopharmacology* 41 : 1–8.

[2] Erspamer V *et al.* **(1967)**: 5-Methoxy- and 5-hydroxyindoles in the skin of *Bufo alvarius*. *Biochemical Pharmacology* 16 : 1149–1164.


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