

New Findings on Type and Amount of Tryptamine Derivatives in the Poison of the Colorado River Toad (*Incilius alvarius*) using LC-HR-QTOF-MS and LC-MS/MS

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Introduction and Aims

The Colorado River Toad (*Incilius alvarius*) is the only toad having an 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 recreational 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 (captive kept) and wild toad poison samples**, which to our knowledge has not been done before.



Methods

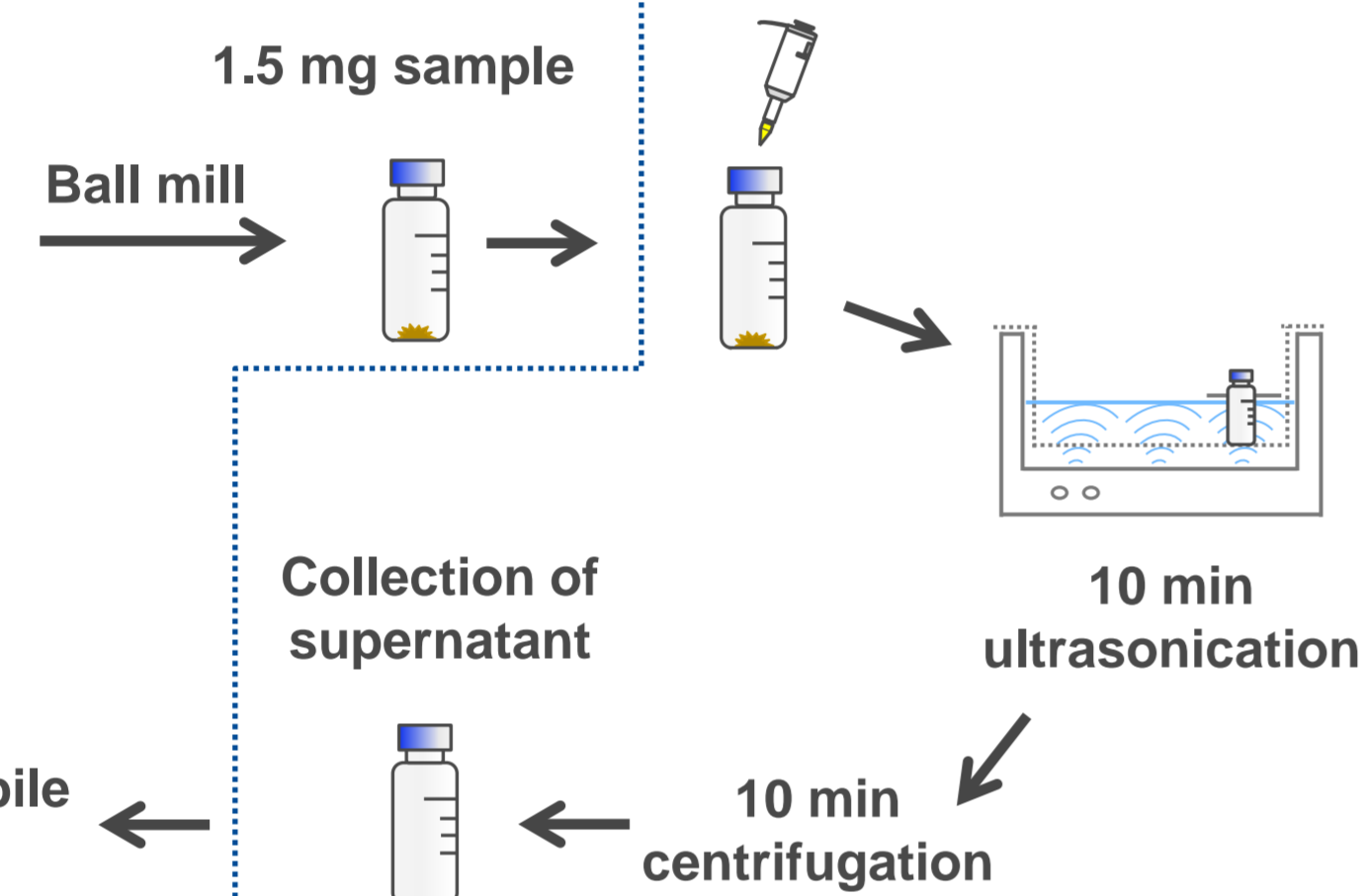
Sample Preparation

Sample collection

'Milking of the toads': Paratoid glands and the glands at the upper and lower legs of the toads were gently squeezed.



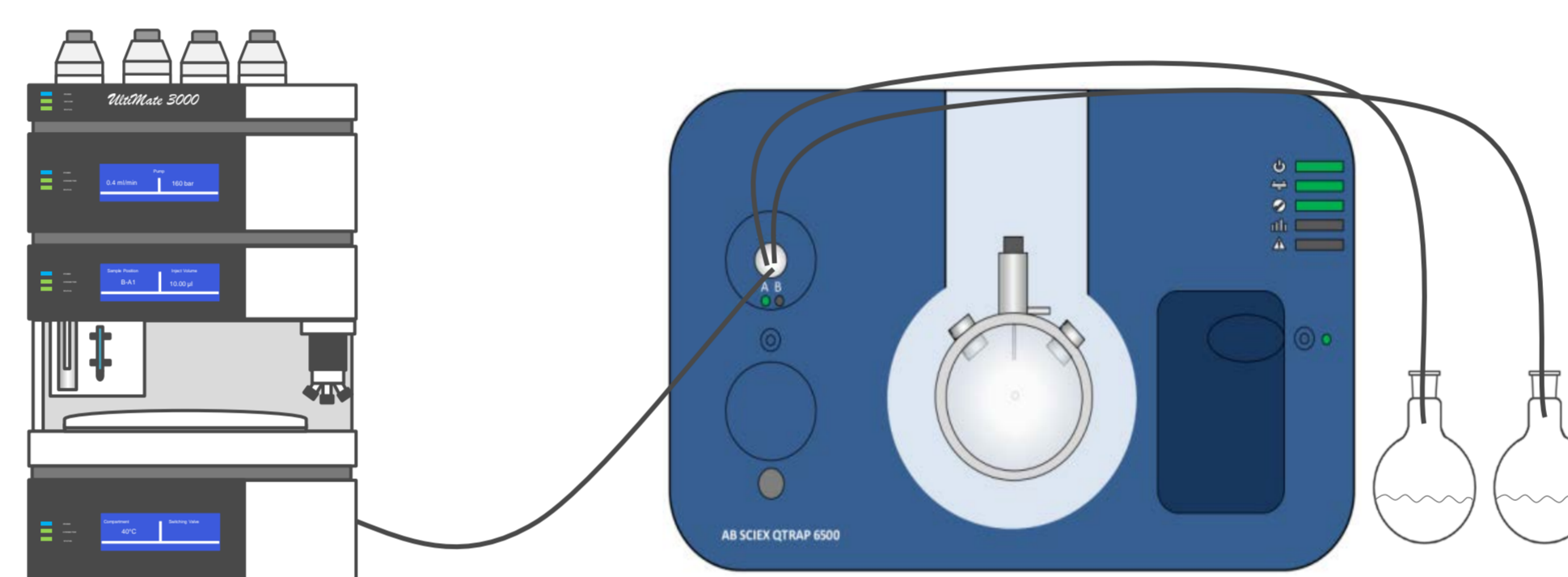
Extraction



Enrichment of compounds other than 5-MeO-DMT

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.

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



- Acetonitrile evaporated, remaining aqueous solution lyophilized
- Residue resuspended in 1 mL ethyl acetate, evaporated
- Residue dissolved in 20 µL mobile phase A/B 95/5

LC-QToF-MS and LC-MS/MS Analysis

- Quantitative 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 x 2.1 mm, 3 µm)
- Mobile phase A: H₂O, 0.1 % HCOOH, 2 mmol/L NH₄⁺HCOO⁻, 1 % ACN
- Mobile phase B: ACN, 0.1 % HCOOH, 2 mmol/L NH₄⁺HCOO⁻
- Run time: 10 min
- Bruker Elute OLE HPLC for LC-QToF-MS
- Dionex UltiMate[®] 3000 HPLC for LC-MS/MS

Mass Spectrometry

QToF

- Bruker Impact III[™]
- Ionization: ESI(+)
- Full Scan & bbCID

MS/MS

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

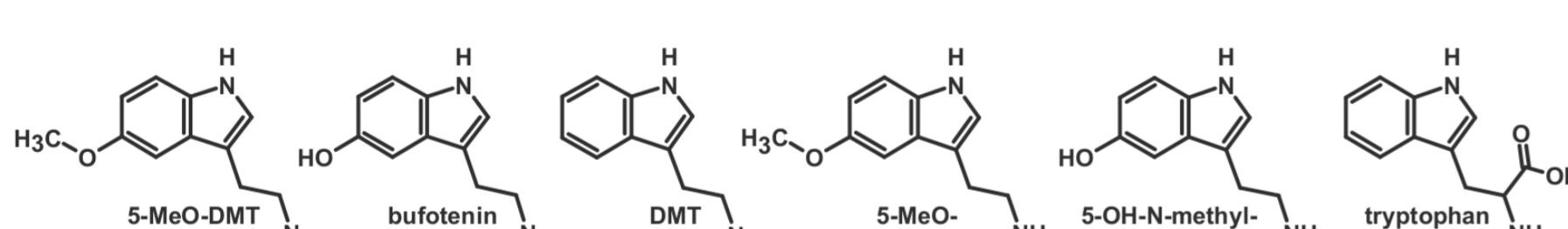
Results and Discussion

Qualitative Analysis

Analytes included in the MRM method

- Reference standards available: 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, bufotoniin, 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

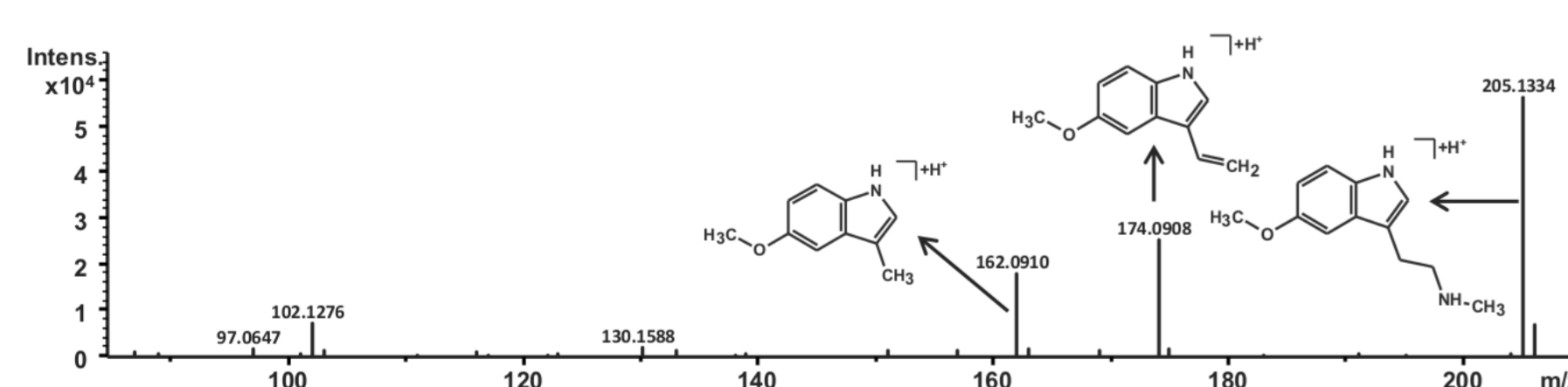


Figure: mass spectrum at 2.9 min; 5-MeO-N-methyltryptamine and fragments

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

Hydroxylated MeO-DMT tentatively identified by QToF-Screening

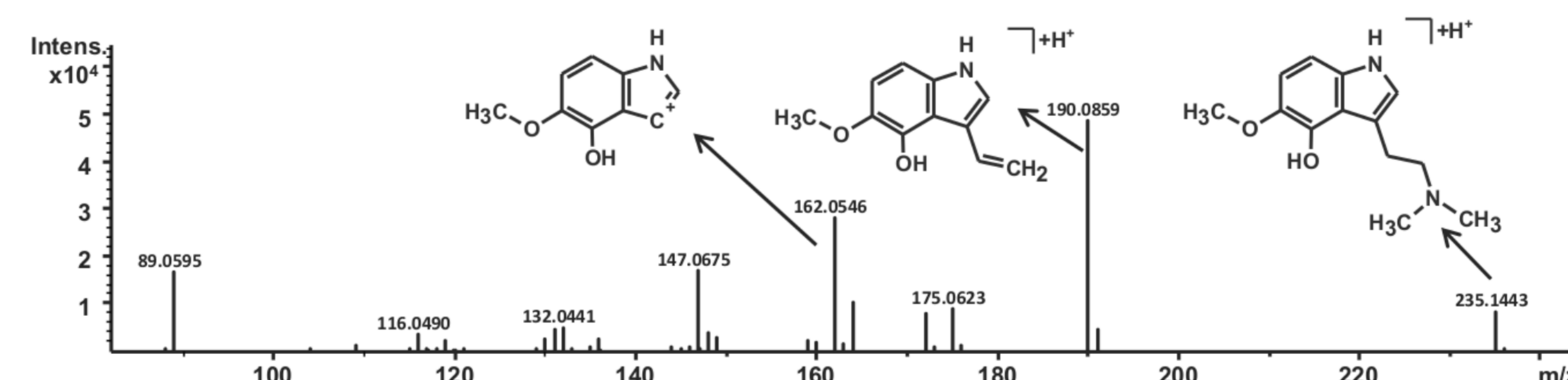
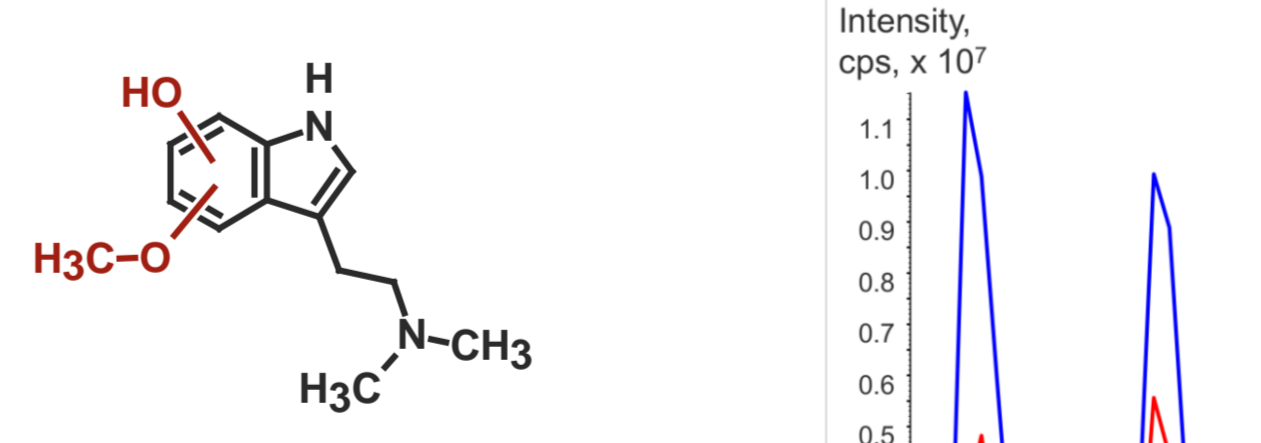


Figure: mass spectrum at 2.1 min; 4-OH-5-MeO-DMT (as one possible structure) and fragments

- MS fragmentation: α- and β- cleavage of the alkyl chain
- Fragments of 162,0550 and 190,0863 Da suggest that the indole ring and not the side chain is hydroxy and methoxy substituted.
- 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
- MRM transitions show two peaks at different retention times → at least two different isomers
- No standards available

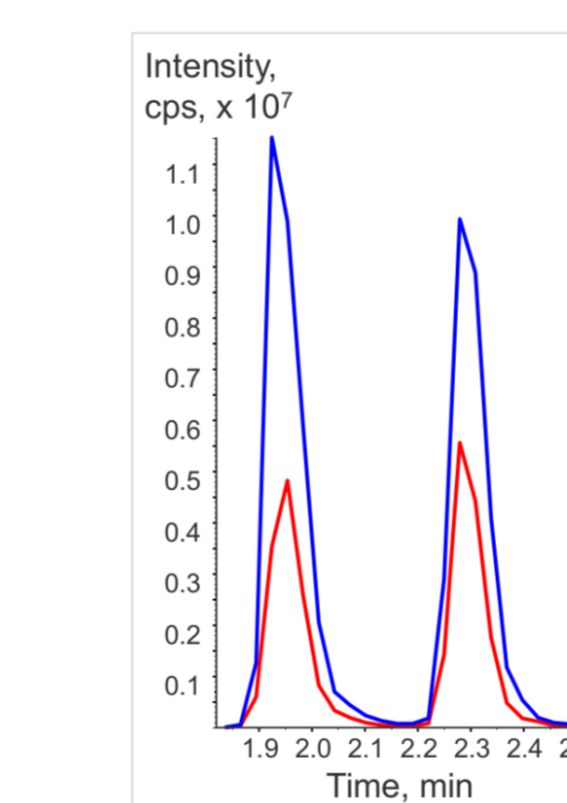


Figure: MRM transitions: 235/190 blue, 235/162 red

Quantitative Analysis

Comparison of zoo and wild toads' poison

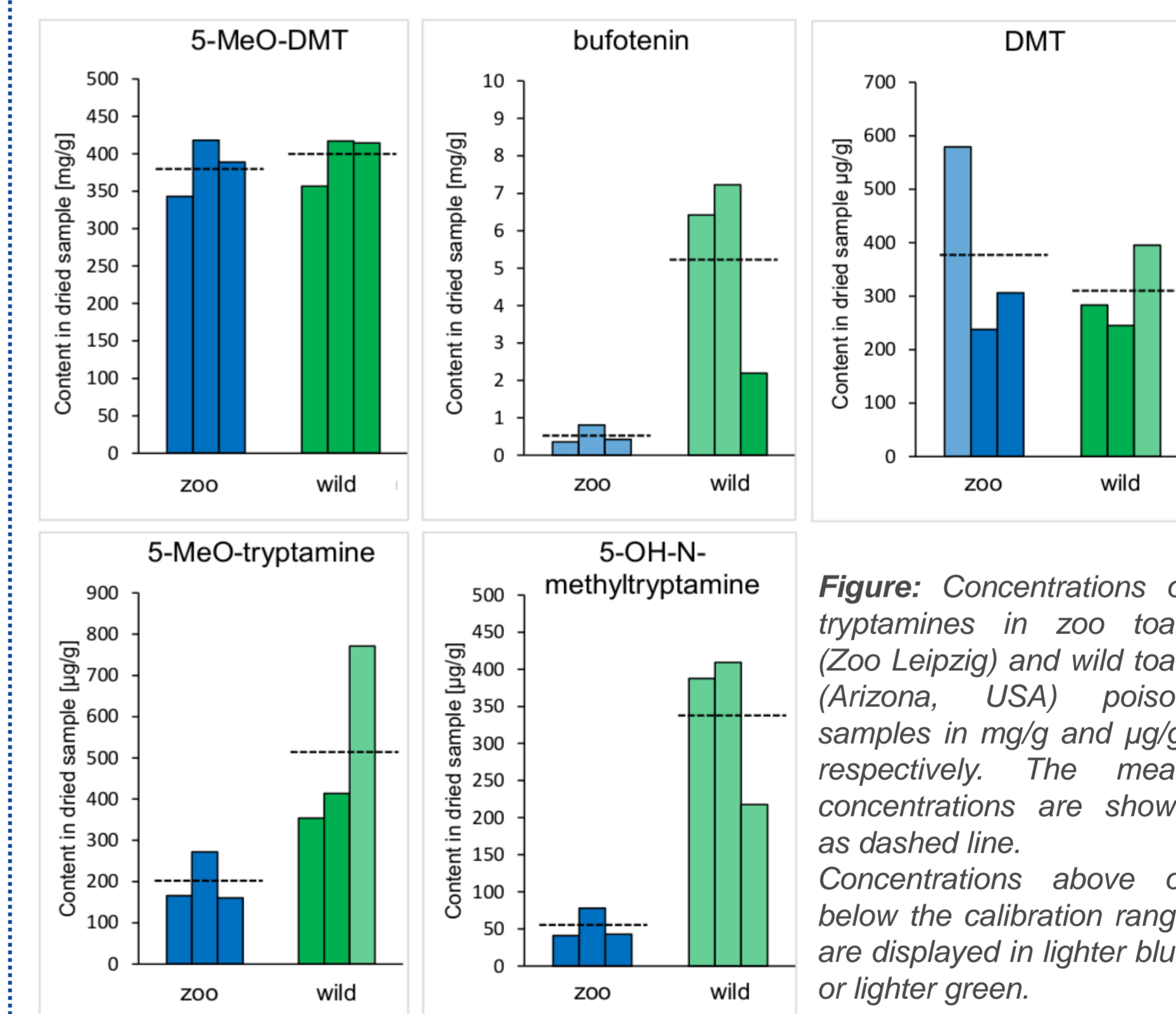


Figure: Concentrations of tryptamines in zoo toad (Zoo Leipzig) and wild toad (Arizona, USA) poison samples in mg/g and µg/g, respectively. The mean concentrations are shown as dashed line. Concentrations above or below the calibration range are displayed in lighter blue or lighter green.

- More bufotenin, 5-MeO-tryptamine and 5-OH-N-methyltryptamine in wild toads than in zoo toads
- 5-MeO-DMT: ca. 340 – 420 mg/g → high in comparison to 50 – 150 mg/g estimated by Erspamer et al.[2] for skin

Outlook

To verify that **wild toad poison contains higher concentrations of certain tryptamine derivatives** than zoo toad poison, more samples should be measured using the developed MRM method. The **high concentration of 5-MeO-DMT compared to the literature** shall also be verified by further measurements. By synthesis of all possible isomers of hydroxylated MeO-DMT, the tentative

isomers in the toads' poison can be identified. The confirmation 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 consumption and to assess the content of tryptamine derivatives in smoke condensates by using the developed methodology.

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