Determination of Pyrrolizidine Alkaloids in Teas and Herbal Teas by LC-MS/MS

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1. Introduction

Here is presented an analysis of pyrrolizidine alkaloids in teas and herbal extracts by LC-MS/MS. Pyrrolizidine alkaloids (PAs) occur in many plants and protect the plant against herbivores. They are particularly common in asteraceae, boraginaceae, and fabaceae. Depending on the plant family, different PAs are formed (figure 2), covering a multitude of substances. In plants they occur as N-oxides (mainly for transport and storage) and in their free (active) form. PAs are of interest because their metabolites are liver toxic and carcinogenic. An agreement on target analytes is still under discussion.

2. Experimental Work

The analytical challenges result from a multitude of stereoisomers having the same mass and often a similar fragmentation pattern. Chromatographically not all isomers can be separated in a single run. Sum parameters may be problematic, as the fragmentation patterns are similar but not always identical.

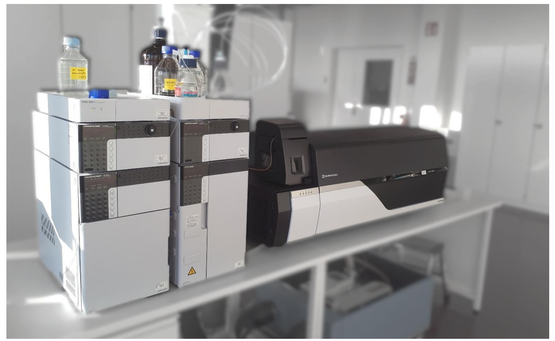


Figure 1: UHPLC-MS/MS system

2.1 Analytical Conditions

Determination of pyrrolizidine alkaloids in teas and herbal extracts is performed by LC-MS/MS on a Shimadzu Nexera UHPLC coupled to an 8060 mass spectrometer. The system parameters are listed in Table 1.

Table 1: System parameters UHPLC-MS/MS system

LC system	
LC system:	Nexera X2 (Shimadzu)
Analytical Column:	Raptor Biphenyl 100 mm x 2.1 mm, 2.7µm (RESTEK) Equisil Gold C18 200 x 2 mm, 3 µm, (Dr Maisch GmbH) or Acquity CHS Phenyl-hexyl 150 x 2.1 mm, 1,7 mm (Waters)
Mobile Phase A:	water containing 0.1% formic acid and 5 mM ammonium formate
Mobile Phase B:	methanol containing 0.1% formic acid and 5 mM ammonium formate
Injection volume:	10 µL
Column oven Temperature:	40 °C
MS	
MS system:	LCMS-8060 (Shimadzu)
Interface Voltage:	-4 kV
Nebulizing Gas Flow:	3 L/min (N_2)
Drying Gas Flow:	5 L/min (N_2)
Heating Gas Flow:	10 L/min (Air)
DL Temperature :	250 °C
Block Temperature :	300 °C
Interface Temperature :	300 °C

Table 2: MS parameters

Analyte	Precursor ion	product ions (CE)	
Jacobine (Jb)	352.1	155 (-28)	280 (-23)
Europine (Eu)	330.1	138 (-21)	254 (-19)
Intermedine (Im)	300.1	94 (-27)	138 (-19)
Echinatine (Ec)	300.1	138 (-22)	89 (-21)
Jacobine-N-oxide (JbN)	368.1	296 (-24)	120 (-35)
Indicine (Id) / Lycopsamine (Ly)	300.1	94 (-26)	156 (-27)
Europine-N-oxide (EuN)	346.1	172 (-32)	111 (-45)
Echinatine-N-oxide (EcN)	316.1	172 (-28)	102 (-21)
Indicine-N-oxide (IdN) / Intermedine-N-oxide (ImN)	316.1	172 (-26)	138 (-28)
Lycopsamine-N-oxide (LyN)	316.1	172 (-27)	138 (-28)
Retrorsine (Re)	352.2	120 (-29)	138 (-29)
Retrorsine-N-oxide (ReN)	368.2	94 (-48)	120 (-35)
Seneciphylline (Sp)	334.2	120 (-28)	94 (-36)
Heliotrine (He)	314.2	138 (-20)	156 (-27)
Seneciphylline-N-oxide (SpN)	350.2	94 (-46)	91 (-44)
Heliotrine-N-oxide (HeN)	330.2	172 (-28)	111 (-41)
Senecivernine (Sv) / Senecionine (Sc)	336.2	120 (-29)	308 (-26)
Senecivernine-N-oxide (SvN)	352.1	94 (-43)	120 (-37)
Senecionine-N-oxide (ScN)	352.2	94 (-46)	118 (-31)
Echimidine-N-oxide (EmN)	414.2	254 (-31)	396 (-24)
Echimidine (Em)	398.2	120 (-24)	220 (-17)
Senkirkine (Sk)	366.2	168 (-29)	122 (-31)
Lasiocarpine (Lc)	412.2	120 (-29)	220 (-19)
Lasiocarpine-N-oxide (LcN)	428.1	254 (-30)	94 (-44)

2.2 Sample Preparation

For extraction and clean up, we follow the BfR protocol [1] that includes an extraction with sulphuric acid. Alternatively, for tea, an extraction with hot water comparable to a preparation of a cup of tea in the kitchen provides sufficient results. In routine analysis, one protocol suitable for as many matrices as possible is required. For other foodstuff, a water' extraction may not be suitable, so we keep to the original protocol. After extraction, a clean-up on a C18-SPE material is performed prior to measurement according to the protocol.

2.3 Materials and Methods

LC separation follows the BfR protocol [1] as well. Separation is carried out on C18 material with gradient elution. If necessary, to separate critical pairs of analytes, we perform a second run with identical chromatographic conditions using a phenyl-hexyl column as suggested by other labs [3]. Still unseparated pairs are reported as a sum parameter, if the response of the transitions is comparable for the corresponding analytes (figure 3).

The BfR proposes several analytes. Some of them we discarded due to their rare occurrence. However, we added other analytes that, although rarely occurring, might cause analytical problems by being isomers of target analytes. MS/MS Transitions are optimised for the instrument (table 2).

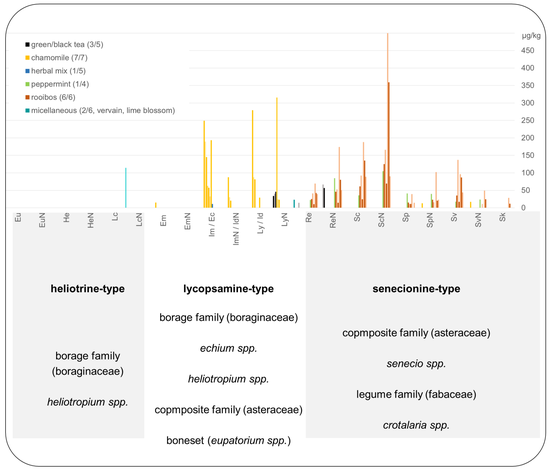


Figure 2: Occurrence pattern of pyrrolizidine alkaloids in tea - measurements 2016/2017. Positive samples out of total samples given in brackets. Occurrence in plant families are given according to [2] (exemplarily)

3. Results and Conclusion

An overview is given of some measurements in 2016/2017 (figure 2). Different patterns can be observed in different kind of teas. The patterns result from typical contaminations e. g. from contamination with Fen Ragwort (*senecio angustifolia*) in rooibos. In camomile contaminations are region-specific, often caused by common ragwort (*senecio jacobaea*). Fortunately, overall the amounts measured in 2018/2019 are lower compared to 2016/2017 (figure 4).

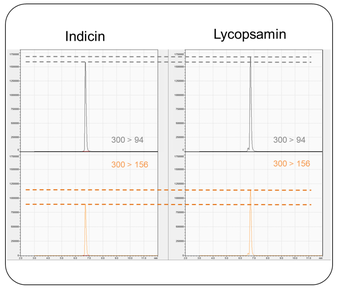
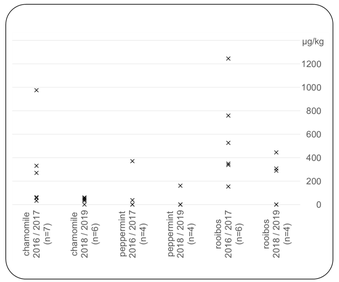


Figure 3: Comparison of responses of two transitions (300 > 94 and 300 > 156) for indicine and lycopsamine.

Figure 4: Summed up amounts of 27 pyrrolizidine alkaloids measured in 2016/17 compared to 2018/19



4. References

[1] BfR-protocol, Bestimmung von Pyrrolizidinalkaloiden (PA) in Pflanzenmaterial mittels SPE-LC-MS/MS Methodenbeschreibung, BfR-PA-Tee-2.0/2014

[2] Mulder, P. P. J.; et al., Occurrence of Pyrrolizidine Alkaloids in Food, EFSA Supporting Publication 2015: EN-859, External Scientific Report, 03.08.2015

[3] C. Czerwenka, A. Turkowitsch: Pyrrolizidine alkaloid isomers: Analytical challenges and solutions, poster presentation at RAFA symposium, 2017