Analysis of diastereomeric pyrrolizidine alkaloids in tea samples by liquid chromatography and supercritical fluid chromatography vacuum differential mobility spectrometry-mass spectrometry

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Recently, the monitoring of pyrrolizidine alkaloids (PAs), classified as phytotoxic compounds, in food matrices has gained of interest. PAs are a complex mixture of diastereomers and more than 600 PAs and their N-oxides forms were identified in over 6000 plants. Many LC-MS and GC-MS assays have been developed, but requires extensive sample preparation and long analyses times, and methods with higher throughput are needed. In the present work, the potential of vacuum DMS (vDMS) for reducing LC analysis time while maintaining good selectivity is investigated for the analysis of diastereomeric PAs in tea samples. In addition, the performance of supercritical fluid chromatography-mass spectrometry (SFC-MS) is an attractive alternative to LC for reducing analysis time and is capable of resolving the diastereomeric PAs.

Diastereomeric PAs were analysed in tea samples. Epi-jacobine and atropine were used as internal standards. Samples were extracted using H_2SO_4, centrifuged and filtered. Extracts were analysed on various systems: 1) short LC-vDMS-MS, 50 x 1.0 mm C_{18} column (5 min) in trap/eluted setup using MeOH/H_2O (15/85; v/v), 0.1%FA 2) LC-MS/MS method using C_{18} column, 150 × 2.1 mm (12 min). 3) SFC-MS/MS method (7 min) using CHIRALPAK® IG-3, 3.0 x 100 mm, 3 µm SFC column. Analyte detection was performed either on LCMS-8050 QqQ or on 8060 QqQ mass spectrometer (Shimadzu) and prototype vDMS cell (33 mbar pressure).

Four sets of PA diastereomers were investigated. Intermedine, echinatine, lycopsamine, indicine (*m/z* 300) and their N-oxide forms; intermedine-N-oxide, echinatine-N-oxide, indicine-N-oxide, lycopsamine-N-oxide (*m/z* 316) have no selective fragments under CID and coeluted in LC. Senecivernine and senecionine (*m/z* 336) cannot be distinguished by MS/MS and they partially coeluted in LC. Jacobine is the only diastereomer (*m/z* 352) that has a selective fragment (*m/z* 352>155) and can be distinguished from retrorsine, while senecivernine-N-oxide and senecionine-N-oxide (*m/z* 352); have different retention times.
For SFC-MRM/MS all diastereomers are resolved using the CHIRALPAK® IG-3 SFC column under ternary gradient. vDMS has shown to be powerful approach for the separation of isomeric analytes and to improve detection selectivity. Solvent, correction and dispersion voltage, temperature and pressure were optimized in vDMS to achieve separation of diastereomers. The combination of short LC column with trap/elute setup and vDMS and detection in SIM mode enabled simplified sample preparation, reduced the analysis time and improved selectivity compared to classical LC-MS method. In addition, LC-vDMS-MS enables accurate quantitation of 10/14 diastereomeric PAs with higher throughput (<1h), compare to reported LC-MRM/MS method in which 9/14 diastereomers could be separated and SFC-MRM/MS in which all diastereomers are fully resolved but requires time consuming sample preparation (>3h). Finally, PA diastereomers were successfully analysed in 20 tea samples using the short LC-vDMS-MS method and compared to LC-MRM/MS and SFC-MRM/MS methods.