Method Development and Validation for the Determination of Pyrrolizidine Alkaloids in a Range of Plant-Based Foods and Honey Using LC-MS/MS

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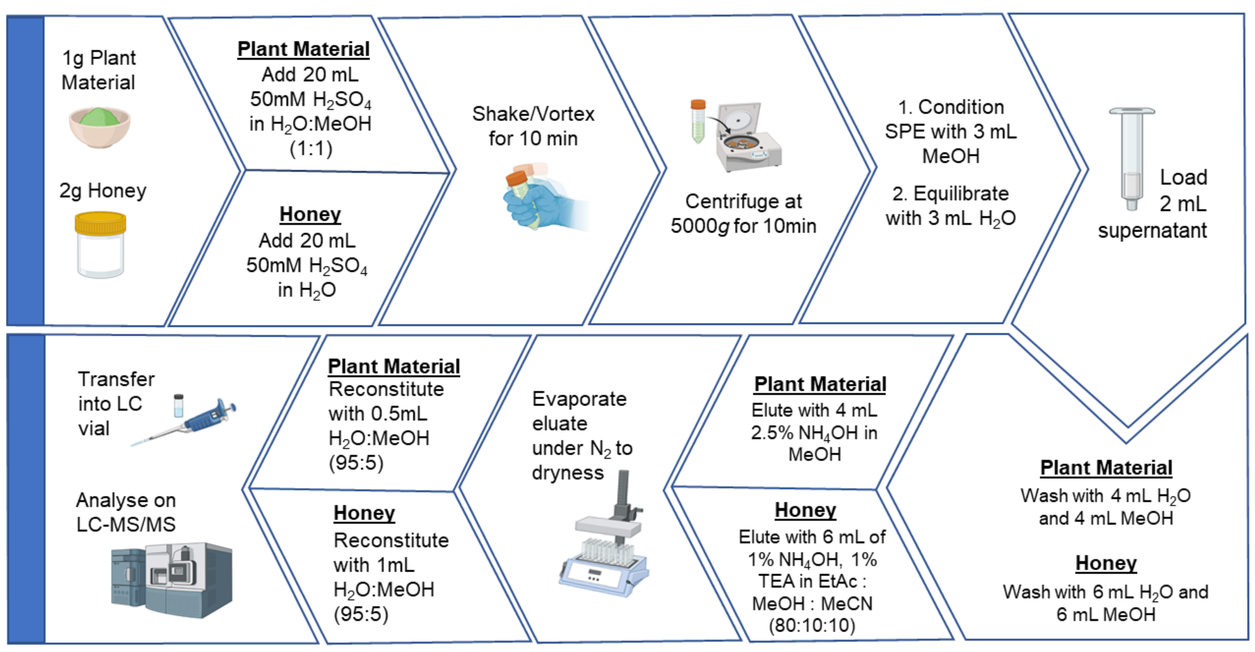
INTRODUCTION

PAs are toxins exclusively biosynthesised by plants. They are typical plant secondary metabolites against herbivores. PAs are regarded as undesirable substances in food and feed, due to their genotoxic and carcinogenic properties.

The European Commission has set maximum levels of PAs in certain foodstuff, such as herbs, spices, teas, herbal infusions, and pollen products. Maximum levels refer to the "lower bound" sum of 35 PAs and are set in EC Reg. (EU) 2020/20405 enforced from 1st July 2022 and amending Regulation (EC) 1881/2006. For example, the maximum level for PAs in most tea is 150 µg/kg, whereas the value for cumin seeds is set at 400 µg/kg.

In this work we describe a simplified approach for the quantification of 35 EU regulated PAs in plant-based food and honey using UHPLC-MS/MS and address the separation of several of the isomers in a single chromatographic run (see Figure 1).

Figure 2. Scheme of the sample preparation protocol.



RESULTS

Trueness was assessed by recovery experiments which involved spiking a blank sample at three concentration levels, as seen in Figure 4. When considering the whole panel of PAs together, mean percentage recovery across all spike levels was 85 ± 10% for green tea, 91 ± 8% for chamomile tea, 92 ± 10% for rooibos tea, 87 ± 10% for oregano, 103 ± 6% for cumin seeds, and 77 ± 10% for honey.

Recoveries were above 62% for all PAs in plant-based samples, while for Echinatine N-oxide, Europine N-oxide, Lycopsamine N-oxide, and Rinderine N-oxide in honey recoveries were between 50 and 60%. Nevertheless, since RSD% under repeatability conditions for all compounds were below 10% across all spiking levels, it would be possible to apply a recovery correction factor as precision is not compromised. Alternatively, in the case of honey, procedural calibration can be used to compensate for recovery losses.
Good repeatability was achieved, with RSDr% mostly below 10% and not exceeding 20% in all cases.

Figure 4. Bar-plot illustrating percentage recoveries in Chamomile Tea across three spiking levels (error bars = %RSD, n = 3).

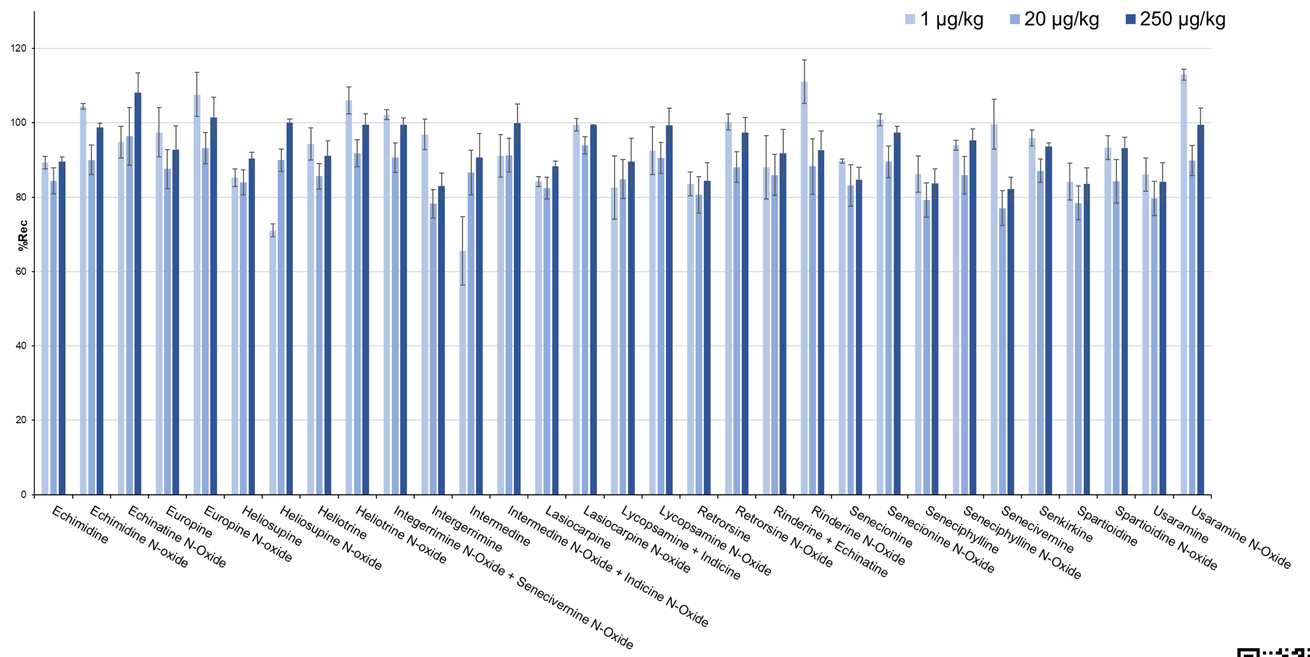
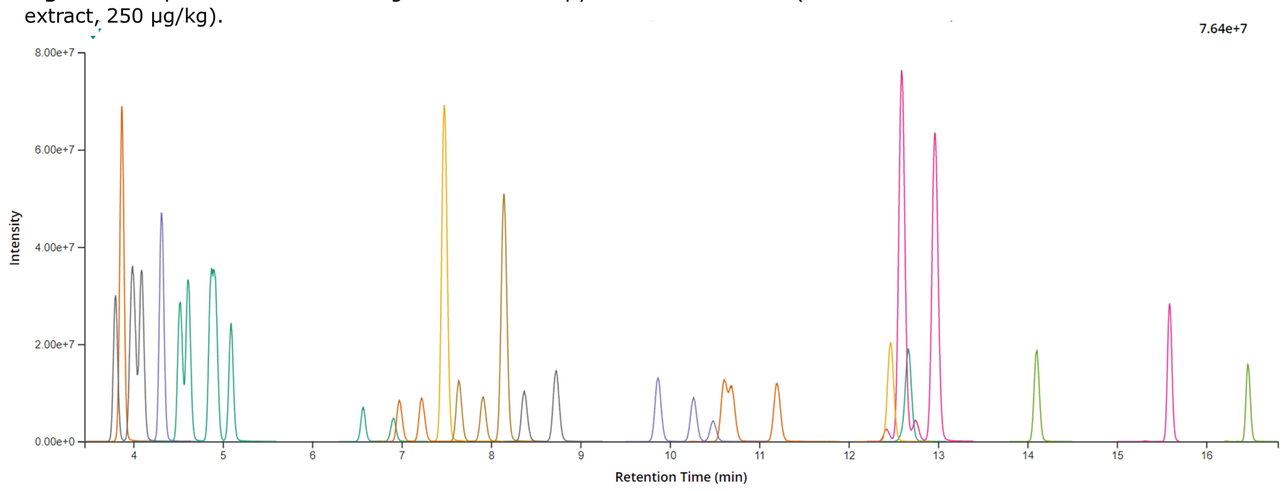


Figure 1. Representative chromatogram of the 35 pyrrolizidine alkaloids (matrix-matched standard on tea extract, 250 µg/kg).



METHOD

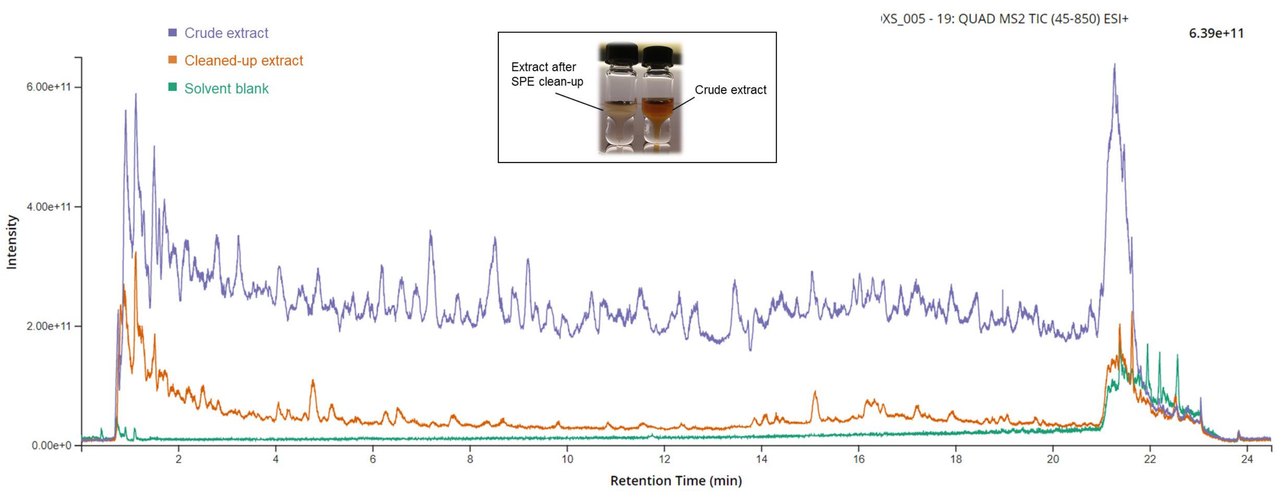
Optimized LC conditions are listed in the experimental section of the full publication, which can be accessed using the QR Code below.

The Oasis™ MCX SPE cartridge was used for eliminating a portion of the matrix co-extractives that can compromise method performance and can contaminate the system. The SPE protocol adopted seen in Figure 2 is based on a typical load-wash-elute approach, making use of the cation-exchange mechanism for protonated basic compounds (i.e. alkaloids) under acidic conditions.

To illustrate the benefits of the SPE clean-up, we acquired chromatograms of a tea sample spiked with 35 PAs, extracted with and without applying the SPE clean-up prior to concentration and reconstitution. For this study, RADAR was used, an acquisition mode that acquires both MRM and full scan MS simultaneously.

In Figure 3, a crude extract full scan chromatogram is overlayed with a cleaned-up full scan chromatogram. It is evident the drastic decrease in signal for the total ion current (TIC) of the purified extract compared to the crude extract. This has the impact of significantly reducing the amount of matrix co-extractives being introduced into the LC-MS/MS, reducing the potential for isobaric interference, and reducing contamination of the system.

Figure 3. RADAR chromatograms of a rooibos tea extract (full scan *m/z* 45-850, 0.1 s scan time). Insert: picture of a clean-up extract (left) and crude extract (right).



CONCLUSION

A suitable UHPLC-MS/MS method was developed and validated for the quantitative determination of all 35 pyrrolizidine alkaloids in six matrices, across a range of plant-based food commodities and honey.

The listing of multiple specific isomers within this regulation makes separation within a complete method very challenging under conventional chromatographic conditions. We achieved baseline separation for 27 of the 35 analytes, whilst four pairs of coeluting isomers were quantified as a sum.

The optimized sample preparation procedure using Oasis™ MCX SPE cartridge for clean-up was found to be very effective in reducing the interferences which can coelute with PAs.

The method performance was established by assessing trueness, repeatability, linearity, and limits of detection and quantification. LOD/LOQ across all matrices investigated were found to be significantly below the maximum limits set by the EU Regulation, allowing the method to be employed for testing of food intended for infants and young children.

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