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# Multi-analyte high performance liquid chromatography coupled to high resolution tandem mass spectrometry method for control of pesticide residues, mycotoxins, and pyrrolizidine alkaloids

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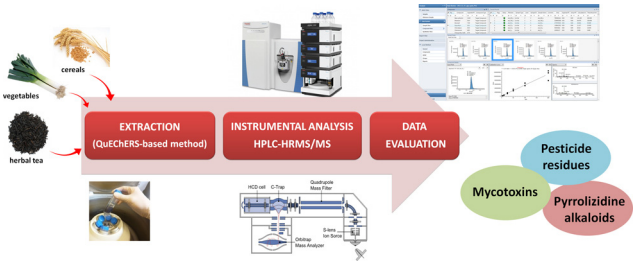
## HIGHLIGHTS

- HPLC–HRMS/MS method for analysis of 389 multi-class food contaminants was developed.
- The employed core–shell analytical column showed very good separation efficiency.
- Validation for matrices wheat, leek, and tea was performed.
- Recoveries and limits of quantification complied with the EU legislation.
- The mass spectral library of fragment ions in high resolution was created.

## GRAPHICAL ABSTRACT

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## ABSTRACT

A new reliable and highly sensitive method based on high performance liquid chromatographic (HPLC) separation and high resolution tandem mass spectrometric detection (HRMS/MS) has been developed and validated for determination of 323 pesticide residues, 55 mycotoxins, and 11 plant toxins represented by pyrrolizidine alkaloids. The method was validated for three matrices, leek, wheat, and tea differing in nature/amount of co-extracts that may cause various matrix effects. For target analytes isolation, optimized QuEChERS-based (quick, easy, cheap, effective, rugged, and safe) extraction procedure was employed. Spectral HRMS/MS library has been established providing an entire spectrum of fragment ions for each analyte, which allows unbiased identification and confirmation of target compounds. The limits of quantification (LOQs) of target analytes were below 10 $\mu g\,kg^{-1}$ for 82%, 81%, and 61% for matrices leek, wheat, and tea, respectively. Recoveries were in the acceptable range (70–120%) according to SANCO/12571/2013 for most of target analytes, except for highly polar 'masked' mycotoxin deoxynivalenol-

*Abbreviations:* AGC, automatic gain control; AIF, all ion fragmentation; C-trap, curved linear trap; CRM, certified reference material; dd, data dependent; dSPE, dispersive solid phase extraction; EFSA, European Food Safety Authority; ESI, electrospray ionization; EU, European Union; EUPT, European Union proficiency test; EURL, European Union reference laboratory; FAPAS, Food Analysis Performance Assessment Scheme; FWHM, full width at half maximum; HCD, higher collision dissociation; HESI, heated electrospray ionization; HPLC, high performance liquid chromatography; HRMS, high resolution mass spectrometry; IT, maximum inject time; LC, liquid chromatography; LOQ, limit of quantification; ML, maximum limit; MRL, maximum residue limit; MS, mass spectrometry; MS/MS, tandem mass spectrometry; NCE, normalized collision energy; PT, proficiency test; PTFE, polytetrafluoroethylene; Q-orbitrap, quadrupole-orbitrap; RPM, revolutions per minute; RSD, relative standard deviation; SANCO, Health and Consumers (Sante et Consommateurs); SIM, selected ion monitoring; U-HPLC, ultra-high performance liquid chromatography; TOF, time of flight.

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3-glucoside with recoveries 35%, 47%, and 42% for matrices leek, wheat, and tea, respectively. The linearities of calibration curves expressed as coefficients of determination were in the range of 0.9661–1.000, and repeatabilities expressed as relative standard deviations (RSDs) at LOQs lied in the range of 0.25–13.51%. The trueness of the method was verified using several certified reference materials (CRMs) and proficiency test samples.



## 1. Introduction

Food crops and products thereof may contain various potentially hazardous compounds, e.g. residues of plant protection products, or mycotoxins, secondary metabolites of parasitic fungi. Under certain conditions, also natural toxins such as pyrrolizidine alkaloids, which occur in some plants as a part of their defense system against pests, may enter the food chain [1–7]. The comprehensive control of these contaminants is not an easy task since, currently, there are over 1000 active pesticide substances registered in the European Union, and mycotoxins represent a large group of diverse compounds. Up to now, more than 400 toxic metabolites of filamentous fungi of the *Fusarium*,*Penicillium*, *Aspergillus*, and *Claviceps* genera have been reported [1,7]. Although only several mycotoxins are currently regulated [9–13], many of them are on the list of 'emerging' mycotoxins, for which occurrence data are required, since, they are subjects of health risk assessment process performed by EFSA [14].

A wide range of methods, nowadays mostly based on LC–MS, with up to hundreds analytes on the target list, has been developed for the above mentioned contaminants [15–18], nevertheless, simultaneous determination of their various groups has been only rarely considered [19–22]. The current 'gold' standard in routine laboratories concerned with food safety control is represented by unit resolution tandem mass spectrometric detectors (MS/MS) such as triple quadrupole thanks to high specificity, as well as sensitivity of target analytes detection [23–25]. However, the key limitation of MS/MS based methods is that, due to the monitoring of only specific ion transitions, neither post acquisition data re-interrogation, nor screening of unidentified unknowns, is possible. In this context, the growing interest in employing of high-resolution mass analyzers is not surprising. In the recent decade, full-scanning high resolution mass analyzers represented mainly by the time-of-flight (TOF) and orbital ion trap (orbitrap) mass analyzers have offered new challenges in food contaminants analysis. Especially reliable separation of isobaric compounds, thus, enabling reliable analysis of even very complex matrices represents the key benefit. In this context, achievable mass resolution of measurement is a very important parameter related to the mass accuracy obtained. While TOF analyzers available at the market provide mass resolution in order of thousands up to tens of thousands of full width at half maximum (FWHM) units, ultra-high resolution power values higher than hundreds of thousands of FWHM are enabled even by benchtop orbitrap mass spectrometers. Besides the quantitative analysis and targeted screening, identification of 'unknowns' and retrospective data evaluation are the main advantages of these mass analyzers.

In the recent years, application potential of high resolution mass spectrometry has been expanded by introduction of hybrid tandem mass spectrometric systems that combine quadrupole ion analyzer, the collision cell, and either TOF or orbitrap as high resolution mass analyzers. Such MS set-up allows acquiring of high resolution MS/MS spectra of specific precursor ions, thus providing additional information on their structure. The availability of hybrid tandem mass analyzers has obviously introduced new possibilities in the target analysis of pesticides, mycotoxins, and other food/feed contaminants [26–30]. As shown in several recent studies, the requirements of SANCO/12571/2013 regarding the number of identification points necessary to achieve for a satisfactory analysis (i.e., gaining of two product ions with $\Delta m/z < 5$ ppm) can be fulfilled even at very low concentration level [28–31].

Within this study, we focused on a comprehensive critical assessment of performance characteristics of newly developed multi-analyte/multi-group method employing high performance liquid chromatography coupled with tandem high resolution mass spectrometry with Q-orbitrap mass analyzer. To enable confirmation and demonstration of non-target screening potential, MS/MS spectral library was created using pure standards of 323 pesticides, 55 mycotoxins, and 11 pyrrolizidine alkaloids. Validation of the method was realized in QuEChERS-based extracts obtained from matrices leek, wheat, and tea.

## 2. Materials and methods

### 2.1. Materials and reagents

Ammonium acetate and ammonium formate (both LC–MS grade), MS grade formic acid (98%), anhydrous magnesium sulfate (>99.5%), sodium chloride (>99.5%), and HPLC grade acetonitrile were obtained from Sigma–Aldrich (Prague, Czech Republic). LC–MS grade methanol was purchased from Merck (Darmstadt, Germany). Absorbent Bondesil-C18 was purchased from Agilent Technologies (Santa Clara, CA, USA). Deionized water was produced by a Milli-Q system (Millipore, Bedford, MA, USA). Analytical standards of 323 pesticides, 55 mycotoxins, and 11 pyrrolizidine alkaloids were purchased from Sigma–Aldrich (Prague, Czech Republic), Merck (Prague, Czech Republic), PhytoLab (Vestenbergsgreuth, Germany), GeneTiCa (Prague, Czech Republic) Dynex Technologies (Prague, Czech Republic), and Chromservis (Prague, Czech Republic) and stored at −20 °C. For the validation purposes, fresh composite mixture at 1000 ng $mL^{-1}$ was prepared in amber volumetric flask from stock solutions of particular analytical standards.

### 2.2. QuEChERS-based method

With regards to the outcomes of study performed by Lacina et al., where various 'generic' extraction strategies dedicated for pesticides/mycotoxins analysis were critically assessed, QuEChERS-based method was chosen as a best compromise in terms of analytes recoveries and quantification limits achieved. Aiming to achieve the best recoveries of analytes from 'dry' matrices (wheat and tea in our case), optimized extraction protocol with prolonged extraction time described in Dzuman et al. was used [22,32]. To support efficient extraction of acidic analytes, and at the same time, to protect the base sensitive pesticides, acidified water was utilized. 10 g, 2 g, and 1 g of well homogenized leek, wheat, and tea, respectively, was weighed into a polypropylene centrifugation tube, and acidified Milli-Q water (10 mL, 0.2% formic acid) was added and left to soak the matrix for 30 min. Then, acetonitrile was added (10 mL), and sample was extracted for 30 min using a laboratory shaker (IKA Labortechnik, Staufen, Germany). The different sample weights for individual matrices were used in order to find the best sample/solvent ration in relation to the LOQs

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