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Multi-residue analysis of pesticides, plant hormones, veterinary drugs and mycotoxins using HILIC chromatography – MS/MS in various food matrices

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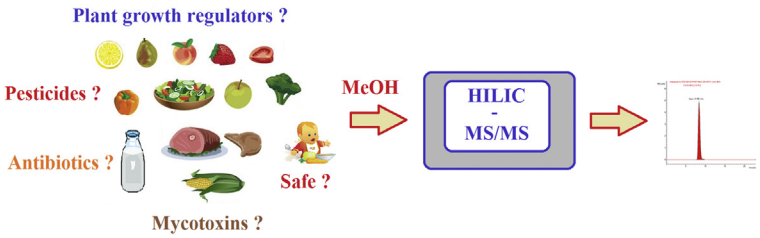
HIGHLIGHTS

- Development and validation of a multi-residue HILIC-MS/MS method.
- Simultaneous determination of 28 xenobiotics (polar and hydrophilic compounds).
- Isolation of the analytes from the matrix was achieved with a fast and effective technique.
- Nine different representative substrates: fruits-vegetables, cereals and pulses, animal products and cereal based baby foods.
- Combined and expanded uncertainty estimation for each analyte per substrate.

GRAPHICAL ABSTRACT



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ABSTRACT

One of the recent trends in Analytical Chemistry is the development of economic, quick and easy hyphenated methods to be used in a field that includes analytes of different classes and physico-chemical properties. In this work a multi-residue method was developed for the simultaneous determination of 28 xenobiotics (polar and hydrophilic) using hydrophilic interaction liquid chromatography technique (HILIC) coupled with triple quadrupole mass spectrometry (LC-MS/MS) technology. The scope of the method includes plant growth regulators (chlormequat, daminozide, diquat, maleic hydrazide, mepiquat, paraquat), pesticides (cyromazine, the metabolite of the fungicide propineb PTU (propylenethiourea), amitrole), various multiclass antibiotics (tetracyclines, sulfonamides quinolones, kasugamycin and mycotoxins (aflatoxin B1, B2, fumonisin B1 and ochratoxin A). Isolation of the analytes from the matrix was achieved with a fast and effective technique. The validation of the multi-residue method was performed at the levels: 10 μg/kg and 100 μg/kg in the following representative substrates: fruits-vegetables (apples, apricots, lettuce and onions), cereals and pulses (flour and chickpeas), animal products (milk and meat) and cereal based baby foods. The method was validated taking into consideration EU guidelines and showed acceptable linearity ($r \geq 0.99$), accuracy with recoveries between 70 and 120% and precision with RSD $\leq$ 20% for the majority of the analytes studied. For the analytes that presented accuracy and precision values outside the acceptable limits the method still is able to serve as a semi-quantitative method. The matrix effect, the limits of detection and quantification were also estimated and compared with the current EU MRLs (Maximum

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Residue Levels) and FAO/WHO MLs (Maximum Levels) or CXLs (Codex Maximum Residue Limits). The combined and expanded uncertainty of the method for each analyte per substrate, was also estimated.

1. Introduction

Food safety is a critical parameter as to ensure public health. During plant cultivation, food processing, storage and transport, a variety of contaminants such as herbicides, plant growth regulators (PGR), mycotoxins and antibiotics may enter the food chain. Crops are treated with herbicides as to control weeds under indoor or outdoor conditions. Plant growth regulators are used widely either to improve the appearance of a product (increasing the size of the fruit or giving a better color and shape) or to influence the growth of the plant, the production of roots, flowers or to assist fruit formation. The raising of animal for food, depends heavily on the use of antibiotics as to ensure the animal health and well-being under farming conditions. Except contaminants that are introduced in the food chain from humans, nature itself can produce compounds that are associated with health risk. A significant member of this category are mycotoxins, mold secondary metabolites can be produced in the field, during harvesting and transportation or storage [1].

The possibility of the presence of more than one of these contaminants in the final step of the food chain, the final product that reaches the consumer, is high. For example, *chlormequat* is authorized is Southern and Northern EU as a plant growth regulator in cereals for stem stabilization [2]. Though metabolism studies conducted in lactating goats using ^{14}C-labelled *chlormequat chloride*, a 76% and 4% of the total radioactive residue was found in meat and milk, respectively [3]. Residues of the analyte were detected in meat samples in Beijing (2011) [4]. The active substance *enrofloxacin* (a synthetic fluoroquinolone antimicrobial agent) is authorized as a veterinary drug for cattle, pigs, turkeys and chickens, for the treatment of infections of the respiratory and alimentary tract [5–9]. Its residues were found to be present in pork [10], cattle and chicken meat [11] and according to the EU national residue monitoring plans of 2010, 11 bovine meat, 2 sheep/goat, 3 poultry, 19 pork and 5 egg non-compliant samples were reported by two Member states (Italy, Spain) [12]. Additionally the presence of natural origin toxins like *fumonisin B1* [13] and *aflatoxin* M_1 are common in milk [14,15] and *ochratoxin A* in meat products [16–18] though animal nutrition.

For monitoring of the residue levels of the undesirable compounds in food a variety of analytical methods need to be employed as, despite their origin and scope, these compounds belong to different chemical classes. The use of multi-residue methods for the simultaneous determination of different classes of mycotoxins, antibiotics and pesticides individually, is widely spreading from analytical laboratories that perform official controls, research facilities or laboratories of the private sector.

When it comes for mycotoxin multi-residue analysis, the sample preparation steps mostly involve liquid-liquid extraction (LLE) with organic solvents and the requirement of the presence of water (for polar metabolites, such as fumonisins) and hexane and cyclohexane as to remove non-polar contaminants, supercritical fluid extraction (SFE) and solid phase extraction (SPE) containing different bonding phases, ranging from silica gel, C-18 (octadecylsilane), florisil, phenyl, amino-propyl, ion exchange materials, both anionic and cationic, to affinity materials, such as immune-adsorbents and molecular imprinted polymers (MIPs). Anion exchange bonded phases provide good clean-up of extracts containing FUMs. The separation of the contaminants is performed with several types of chromatographic methods with thin layer chromatography (TLC), high performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrophoresis (CE) being the most common techniques [19].

The use of multi-residue methods is also becoming wildly common in routine laboratories, for veterinary drugs. Most published veterinary drug residue methods focus on one class of compounds (e.g. sulfonamides, quinolones, tetracyclines etc.). However there are a few papers describing approaches which cover several chemical classes [20,21]. Concerning sample preparation, as in the case of mycotoxins, liquid partitioning (or, with liquid samples, LLE) is used for analyte isolation, with subsequent clean-up and analyte enrichment by means of SPE. As a rule, non-selective SPE on a conventional C_{18}-bonded silica or a hydrophilic/lipophilic-balanced co-polymer is used, with IASPE (immunoaffinity solid phase extraction) - type selectivity being applied only for well-defined target-analyte procedures [22]. For determination, GC chromatography is scarcely used due to the nature of these contaminants, LC based methods combined with mass spectrometry (MS) are more common and the last years the use of HRMS systems (TOF, orbitrap) are gradual introduced [23].

On the other hand, although the use of multi-residue methods are common to pesticide residue analysis [24–26], the determination of specific classes of herbicides, which also act as plant growth regulators (PGR) [27], like quaternary ammoniums, *daminozide*, *maleic hydrazine*, *amitrole* and others is performed using specific single residue methods (SRM). At EU level, as to expand the knowledge between the official control laboratories, the EURL for SRM methods communicated a collection of methods concerning this contaminants [28]. The presence of multi-residue methods for the simultaneous determination of PGR is scarce [29–31].

The use of LC-ESI-MS/MS as a confirmation and quantification tool in mycotoxin [16,32,33], veterinary drug [34–36] and pesticide residue analysis, is growing rapidly in the last years replacing other more selective detectors or GC based operations. In most LC-MS or MS/MS methods observed, the separation is based on reverse phase chromatography and especially the use of C_{18} or C_8 columns using, in some cases ion pairing reagents for better LC separation depending on the analyte [37–39].

Hydrophilic Interaction Liquid Chromatography (HILIC) is a technique mostly used for the analysis of highly hydrophilic, ionic, and polar compounds [40]. The number of references in the use of HILIC as a separation technique is increasing rapidly in the last 6 years. From a keyword search preformed in Scopus, as for the number of references cited using HILIC chromatography, more than 75% of all articles were published during the period 2010–2015 with 30% of the total, published in the years 2014 and 2015. Although the use of this technique is applied in the analysis