Food Chemistry 242 (2018) 113–121

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Multi-class analysis for simultaneous determination of pesticides, mycotoxins, process-induced toxicants and packaging contaminants in tea

Mathieu Cladière*, Grégoire Delaporte, Even Le Roux, Valérie Camel

UMR Ingénierie Procédés Aliments, AgroParisTech, Inra, Université Paris-Saclay, 91300 Massy, France

ARTICLE INFO

Keywords:
Acrylamide
UHPLC-TOF-MS
C18-PFP
Food safety
Multi-residue analysis

ABSTRACT

This study attempts at uniting the analysis of four different classes of contaminants for both liquid and solid tea samples. A total of 32 compounds, classified as pesticides, mycotoxins, process-induced toxicants or packaging contaminants, were carefully chosen for their diversity of structures and physicochemical properties. The proposed method combines a sample treatment strategy coming from metabolomics with liquid chromatography analysis using a silica bonded C18-pentafluorophenyl column coupled to high resolution mass spectrometry. For tea brew, dilute and shoot method provides good quantification (70–120% recoveries and < 20% RSD) for more than 80% of compounds. For tea leaves, strong matrix effects are observed, thus, matrix-matched calibration is required to reach good performances, i.e. 63% of compounds quantified and 81% detected at 10 µg/kg. Finally, method performances were evaluated against existing regulations, and it appears that 69% of contaminants are quantified and 91% detected at levels lower than their respective European regulation limits.

1. Introduction

Food safety remains an everyday challenge toward the globalization of production and sometimes the lack of traceability of products. There are numerous sources of chemical contaminants in food products, ranging from the raw material itself (pesticides, mycotoxins and veterinary drugs in case of food of animal origin), its transportation, its processing (process-induced toxicants), and finally its packaging (migrants). Most analytical strategies, developed by laboratories and food safety authorities around the world, rely on the carrying out of several targeted analyzes in order to cover this broad range of contamination sources and quantify as many contaminants as possible. Such a multi-analysis approach is both very costly and time consuming; on top of that, the environmental footprint of the analysis is increased by the use of larger amounts of solvent and reagent compared with the single-analysis approach. As a result, recent reviews have underlined the need for multi-class methods capable of analyzing a large number of compounds in a single analysis (Antignac et al., 2011; Castro-Puyana & Herrero, 2013).

Indeed, the current technology based on liquid chromatography coupled to mass spectrometry (LC-MS) offers the feasibility of developing new approaches thanks to the increasing sensibility and possibility of full scan analysis using high resolution apparatus. As a consequence, few multi-class methods have emerged in recent years (Danezis, Anagnostopoulos, Liapis, & Koupparis, 2016; Jin et al., 2017; Petrarca, Fernandes, Godoy, & Cunha, 2016). Yet, although these methods are able to quantify hundreds of contaminants simultaneously, they generally focus on only one or two classes of contaminants with close physicochemical properties. To the best of our knowledge, only one study deals with the analysis of various classes of contaminants including migrants from packaging and process-induced toxicants, but it focuses on LC-MS optimization without assessing efficiency of sample treatment (Pérez-Ortega et al., 2016). The authors pointed out the difficulties of this approach since the structural diversity of targeted compounds leads to heterogeneous behaviors during the LC-MS analysis, both in terms of retention and matrix effects. Consequently, developing a multi-class method capable of analyzing contaminants having a wide-range of chemical structures remains a challenge, particularly in real food matrices due to their complexity.

To reach this objective, tea has been chosen as development matrix for four main reasons. Firstly, this is the most consumed manufactured beverage in the world with 4.8 million tons (of tea leaves) produced in 2013, increasing by 5% per year since 2008 (Chang, 2015); medium term outlooks suggest a slightly higher increasing of tea consumption (black or green) until 2023. Secondly, tea is produced in remote countries where contamination risks may be difficult to manage; as a consequence, monitoring and regulatory control analyzes regularly show the presence of chemical contaminants (especially pesticide residues) exceeding their European maximum limits (EFSA, 2016). Thirdly, tea can be analyzed in both solid and liquid states, through tea

* Corresponding author.
E-mail address: mathieu.cladiere@agroparistech.fr (M. Cladière).

http://dx.doi.org/10.1016/j.foodchem.2017.08.108
Received 23 May 2017; Received in revised form 30 August 2017; Accepted 30 August 2017
Available online 01 September 2017

leaves or brew, which is an interesting feature when developing analytical methods for food matrices. Finally, tea is a complex food product regarding its organic composition with a wide variety of phytochemicals (such as antioxidants, aroma compounds, xanthins and alkaloids) which commonly bring strong matrix effects during analysis, therefore making it an interesting real complex food matrix to consider.

This study reports the development and validation of a method for simultaneous extraction and quantification of multi-class contaminants (covering a wide range of chemical structures and properties) for both liquid and solid tea matrices. Until now, this is the first multi-class method covering four classes of food contaminants including pesticides, mycotoxins, process-induced toxicants and migrants from packaging. A total of 32 target compounds were carefully chosen in order to cover the diversity of classes and physicochemical properties encountered. As an illustration, for pesticides, organochlorinated, organophosphates, organosulfites, triazines, auxinic herbicides, neonicotinoids, benzoylureas, pyrazoles, dinitrophenols and carbamates have been considered; as far as we know, all these pesticide families are considered together for the very first time (Eitzer, Hammack, & Filigenzi, 2014; Hou et al., 2014). In addition, 4 mycotoxins, 2 process-induced toxicants (including acrylamide which has never been considered in any multi-residue method before) and 5 migrants from packaging have been examined. The challenge of our method lies in the different behaviors of these 32 compounds regarding extraction and chromatographic separation, in order to propose the best compromise and provide analytical performances in compliance with the European regulation on food contaminants (European Commission, 2005, 2006).

2. Materials and methods

2.1. Targeted contaminants

For developing our multi-class method, several target food contaminants were selected from different classes, namely process-induced toxicants (n = 2), migrants from packaging (n = 5), mycotoxins (n = 4), herbicides (n = 11), insecticides (n = 6) and acaricides (n = 4). The selection of these 32 compounds was based on two main criteria; their physicochemical properties to offer a broad range and a large diversity (i.e. hydrophobicity, aromaticity, functional groups) so as to be representative of other contaminants not considered here, and their relevance for tea. As an example, propargite (acaricide) has been quoted 11 times by the European Rapid Alert System for Food and Feed (RASFF) in 2016, leading to border rejections of black and green teas, while acetamiprid and imidacloprid (insecticides) were cited more than 40 times between 2012 and 2015.

The 32 compounds as well as their analytical information are listed in Table 1.

Analytical standards (100 µg/mL) for pesticides, mycotoxins, process-induced toxicants and labelled compounds acrylamide-d3, dimethoate-d6 and malathion-d6 (purity > 99%) were supplied by CIL Cluzeau (France). Ochratoxin-d5, bisphenol A, F and S, BADGE, BFDGE and bisphenol A-d14 (purity > 99%) were provided by Sigma Aldrich (France).

2.2. Materials and reagents

Acetonitrile (ACN) (HPLC plus gradient, LC-MS), water, methanol (MeOH) and formic acid (FA) (all LC-MS grade) were purchased from Carlo Erba. Ultrapure water (Milli-Q®) was produced by an Integral 3 water purification system from Millipore®. The compound used for MS calibration was Leucine Enkephalin (LC-MS grade), purchased from Waters®. Magnesium sulphate ($MgSO_4$) and sodium citrate (NaCit) salts (analytical grade) were provided by VWR France.

Analyzes of trace contaminants have been performed on a Waters® Acquity H-Class UPLC® system, composed of a quaternary solvent manager pump (QSM), a refrigerated sample manager Flow-Through-Needle (SM-FTN) and a column oven, coupled to a Waters® high resolution mass spectrometer with a Time of Flight analyzer Xevo® G2-S ToF (UHPLC/MS-ToF). An electrospray ionization source was used in both positive (ESI^+) and negative (ESI^-) modes.

2.3. Analysis conditions

2.3.1. Chromatographic conditions

Chromatographic separation was done on a column made of silica based particles bonded with C18-pentafluorophenyl functions (C18-PFP) (dimensions were 150 × 2.1 mm; 2 µm particles diameter, from ACE, provided by AIT, France). In addition to conventional hydrophobic interactions (provided by C18 chains), the PFP groups enable hydrogen bonds, π-π and dipole-dipole interactions, affording a higher capacity for retaining the highly polar compounds (such as acrylamide or acidic herbicides) than a regular C18-silica column.

2.3.2. MS analysis

Since different mobile phases were used for positive and negative ionization, analyzes were performed separately for both modes. All analyzes were done using the resolution mode (30,000 FWHM at 200 *m/z*) for a scan time of 0.5 s, with mass range between 60 and 800 *m/z* and data acquired in centroid. Internal calibration of ToF analyzer was performed with a continuous flow at 5 µL/min of Leucine Enkephalin for one scan every 30 s during 0.1 s.

For ESI^+ the mobile phase was composed of water (A), ACN (B), both acidified with 0.1% FA, and MeOH (C), flowing at 0.4 mL/min. The gradient started at 100% A and reached 100% B in 10 min, this composition being kept for 6 min before switching to 100% C to rinse the system in 1 min, being hold for 5 min, returning back to 100% A in 1 min and finally equilibrating for 3 min, with a total run duration of 26 min. For ESI^-, the mobile phase was composed of water buffered with 10 mM of ammonium formate (A) and MeOH (B) flowing at 0.3 mL/min. The gradient started at 100% A and reached 100% B in 13 min, holding this condition for 7 min before turning back to 100% A in 1 min and finally equilibrating for 3 min, with a total run duration of 24 min. For both chromatographic methods the column was heated at 30 °C.

The optimized parameters for ESI^+ and ESI^- are presented in Supplementary data, Table S1.

Based on the instrument factory settings, detection of acrylamide remained unsuccessful. Indeed, the ion path dedicated to discard neutral molecules before analysis (called “StepWave” by Waters®, which is the ionic path between the source and the mass analyzer) proved to be the limiting step for small molecules (m < 90 *m/z*). Therefore, its settings were set according to Waters® instructions for extending the range of mass detected in a single run (60–800 *m/z*) and ensuring acrylamide detection.

2.3.3. Quantification and quality controls

In order to select the best compromise for quantification of targeted contaminants in tea samples, two main quantification methods were considered: solvent calibration and matrix-matched calibration. Each time, classical external calibration was considered and compared with labelled molecules correction.

For external calibration, 11 standard solutions were prepared in a ACN/water mix (20/80 v/v) acidified with 0.1% FA, with concentrations ranging from 0.1 to 120 ng/mL for most compounds, except for a few molecules exhibiting lower sensitivity (namely hydroxymethylfurfural (HMF), deoxynivalenol (DON), bisphenol A (BPA) and bisphenol F (BPF)) with concentrations five times higher (from 0.5 to 600 ng/mL), as well as acrylamide (AA) with concentrations 10 times higher (1–1,200 ng/mL).

For labelled molecules correction, deuterated isotopes were used in addition to external calibration, either in positive mode (acrylamide-d3, dimethoate-d6, ochratoxin A-d5 and malathion-d6) and/or in negative