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Abstract

This study aims at assessing the capability of comparing and combining different instrumental platforms in an untargeted approach with a view of detecting chemical contaminants in food matrices at low levels. A strategy based on liquid chromatography-high resolution mass spectrometry (LC-HRMS) and chemometrics has been applied on two different complex food contamination scenarios, with tea as study product. The first scenario aimed at mimic the presence of a dozen of contaminants at levels just above regulatory limits (i.e. 10 and 30 µg/kg); the second scenario, more complex, aimed at simulate the presence of several different contaminations at levels close to regulatory limits (10 µg/kg) in different samples. This work was carried on two LC-HRMS platforms (with respectively ToF and Orbitrap mass analyzer technologies), and a highly automated data treatment workflow was implemented to deal with data acquired on both platforms. The untargeted approach performed well on all scenarios (even the most complex) and analytical platforms. Performance comparison between LC-HRMS technologies was made possible thanks to a vendor-neutral data treatment process.

Keywords: Food safety; Independent Components Analysis; LC-HRMS; Orbitrap; ToF; XCMS

1. Introduction

Recent food safety crises like the presence of fipronil in European eggs (summer 2017) underline the limits of current targeted analytical approaches and the need for new untargeted methods able to point out such non-expected contaminants. To that end, mass spectrometry (MS)-based untargeted approaches were identified as having the strongest potential (Antignac et al., 2011; Castro-Puyana & Herrero, 2013; Lommen et al., 2007), giving promising results on relatively simple contamination scenarios: high levels of contaminants (around mg/kg) and rather simple matrices [orange juice (Tengstrand, Rosén, Hellenäs, & Åberg, 2013) or infant formulas (Inoue et al., 2015)]. Only a few recent studies have reported the detection of contaminants at levels down to 10 µg/kg (Cotton et al., 2014; Delaporte, Cladière, Jouan-Rimbaud Bouveresse, & Camel, 2019; Knolhoff, Zweigenbaum, & Croley, 2016; Kunzelmann, Winter, Åberg, Hellenäs, & Rosén, 2018) in more complex matrices such as honey (Cotton et al., 2014) or tea (Delaporte et al., 2019). Further developments are required, especially to assess the ruggedness of the proposed approaches (especially concerning the data treatment part) regarding both the instrument used and contamination scenarios studied. This research field is facing a huge interest worldwide (Antignac et al., 2011; Castro-Puyana, Pérez-Míguez, Montero, & Herrero, 2017).

The general workflow (see **Figure 1**) for untargeted food chemical safety assessment can be established based on reviews (Antignac et al., 2011; Castro-Puyana et al., 2017; Knolhoff & Croley, 2016). As a general rule, the first step consists in a broad-range sample treatment method followed by UHPLC-HRMS analysis (Cotton et al., 2014; Delaporte et al., 2019; Inoue et al., 2015; Knolhoff et al., 2016; Kunzelmann et al., 2018; Tengstrand et al., 2013). For the next step (data treatment), dedicated tools should be implemented. The use of in-line proprietary tools, often supplied with the instrument, has been reported (Inoue et al., 2015; Knolhoff et al., 2016), with the advantage of being user-friendly and to "fit-for-purpose" the data files generated by the instrument. However, such tools lack flexibility and versatility, making it impossible to analyze data from various instruments or to implement in-house data analysis methods. Other strategies are based on open-source tools (such as the XCMS R package) (Cotton et al., 2014; Delaporte et al., 2019). On top of that, easy-to-use user interfaces and free-to-use online calculation platforms were developed recently, such as web interfaces for the XCMS package, namely

Workflow4Metabolomics (Giacomoni et al., 2015) and XCMS-online (Tautenhahn, Patti, Rinehart, & Siuzdak, 2012). These tools are often designed to be versatile and modular, meaning that they may handle data from multiple instruments and be adapted to new fields and problematics.

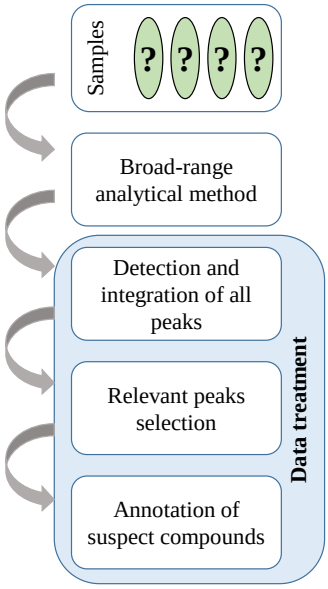

Figure 1: Generic workflow used for untargeted food safety assessment

Until now, the implementation of multiple LC-HRMS platforms has been assessed only on targeted food safety approach (Saito-Shida, Hamasaka, Nemoto, & Akiyama, 2018). Even though the comparison of multiple analytical platforms for untargeted approaches has been reported in metabolomics studies (Glauser, Veyrat, Rochat, Wolfender, & Turlings, 2013), it has never been done in the field of untargeted food safety analysis, existing untargeted approaches being developed on a single instrument. As a consequence, there is a need to test data treatment capability when considering different instrumental platforms since each data generated has its own characteristics (*m/z* resolution and accuracy, signal/noise ratio among others). In addition, untargeted methods were mostly developed on a single data set, with the notable exceptions of the method proposed by Tengstrand et al. (Tengstrand et al., 2013) later successfully applied on a more complex case (Kunzelmann et al., 2018), and of our developed method applied on two contamination scenarios (Delaporte et al., 2019). To assess ruggedness of those methods, several contamination scenarios should be considered that mimic more complex and realistic food safety applications (e.g. multi-class contaminations, levels and molecules involved, variable contamination between samples).

So, this work aims at assessing the implementation and ruggedness of an untargeted approach for detection of non-expected food contaminants. It is based on open-source tools and methods, previously developed on green tea samples using a single LC-HRMS platform (Delaporte et al., 2019). Here, two complex contamination scenarios are considered (with green and black tea leaves), and all analysis were conducted on two different platforms, i.e. UHPLC-Q-Orbitrap and UHPLC-ToF which are the most used HRMS technologies for untargeted contaminants detection thanks to their mass accuracy and resolution (Castro-Puyana et al., 2017; Knolhoff & Croley, 2016).

2. Material and methods

2.1 Reagents and sample collection

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 ToF mass spectrometer was calibrated using Leucine Enkephalin (LC/MS grade, Waters®) and the Orbitrap with the Pierce™ calibration solutions (Thermo Fisher Scientific, caffeine 2 $\mu g.mL^{-1}$, MRFA 1 $\mu g.mL^{-1}$, Ultramark 1621 0.001% and n-butylamine 0.0005% for positive mode; SDS 2.9 $\mu g.mL^{-1}$, taurocholate 5.4 $\mu g.mM^{-1}$ and Ultramark 1621 0.001% for negative mode).

Analytical standards solutions (100 µg/mL in ACN or MeOH, purity >97%) of malathion, ochratoxin A and bisphenol S or individual labelled compounds (acrylamide-d3, bisphenol A-d14, dimethoate-d6 or malathion-d6) were purchased from CIL Cluzeau France. All other standard solutions (100 µg/mL in ACN or MeOH), including herbicide mix (100 µg/kg in ethyl acetate, purity >98%) were provided by Sigma Aldrich (Saint-Quentin Fallavier, France). A pooled stock solution containing all labelled molecules (each at 1 $\mu g.mL^{-1}$) was prepared in ACN and stored in the fridge.

Loose tea samples (green and black) were purchased at local retailers. The green tea was an organic Bancha tea from Japan, and the black tea an organic Keemun tea from China.

A standard solution (called stability mix) containing 32 known contaminants from different families at 16 ng/mL (pesticides, mycotoxins, migrants from packaging and process-induced toxicants) was used to

check the stability of the instrument before each analytical sequence (Cladière, Delaporte, Le Roux, & Camel, 2018).

2.2 Study set-up

The study was carried out by two different persons: a study designer who set up the experimental design and spiked the samples, and an operator who did the chemical and data treatment analyses. In particular, the composition and levels of contaminants of the different sample groups were not known by the operator, from the beginning of sample extraction to final compounds annotation. In addition, most of compounds studied were analyzed for the very first time using our method and therefore nor the operator, neither the study designer had any background regarding the overall method efficiency for the analyzed compounds (only that they are LC-MS amenable). After all the analyses, the results were sent back by the operator to the study designer for detection performance assessment.

Two different contamination scenarios have been established (**Table 1**) using two spiking mix. Spiking mix n°1 is composed of 11 herbicides in ACN, and spiking mix n°2 of three food contaminants from three different classes (malathion for pesticides, ochratoxin A for mycotoxins and bisphenol S for migrants from packaging) in ACN.

TABLE 1 CONTAMINATION SCENARIOS CONSIDERED

Group number	Scenario n°1 *Green tea*	Scenario n°2 *Black tea*
1	10 µg/kg with mix n°1	10 µg/kg with mix n°1
2	Unspiked	10 µg/kg with mix n°2
3	30 µg/kg with mix n°1	Unspiked

Details on spiked contaminants such as raw formula, physico-chemical properties and respective European Maximum Residue Limits (MRLs) when applicable can be found in **Table A.1** of Supplementary material. Spiking levels were chosen in accordance with EU regulations n° 396/2005 (pesticides) and 1881/2006 (other contaminants). Each sample was spiked using the following procedure: 1 g of sample was firstly weighted in a centrifuge polypropylene tube (Corning, New York, USA) and the spiking mix added using the smallest volume possible (below 100 µL); 100 µL of ACN was added to the unspiked samples. For quality control purpose, 40 µL of the labelled spiking mix were added to all

samples (leading to a concentration of 40 µg/kg of labelled compounds in the samples). Each sample was then mixed using a vortex and left for equilibration during 2 hours at room temperature, and then put in the fridge overnight.

While the operator just had information about the group's number (group n°1, 2 or 3) for black and green teas (to ensure real blind analysis relative to the contaminants), two different contamination studies were designed as indicated in **Table 1**. The first scenario, considered as "simple", consists in three groups (of three samples each) from the same green tea; two of these groups were spiked with the mix n°1 (one at 10 µg/kg, which corresponds to the default safety MRL in the EU legislation, and one at 30 µg/kg), the third being used as a control group. The second scenario, considered more complex, consists in three groups (of three samples each) from the same black tea; one group was spiked at 10 µg/kg with the mix n°1, another one at the same level with the mix n°2, and the last one used as a control.

Here we aimed to test the capability of our untargeted approach to discriminate low levels of the same contamination, enabling detection of non-conformities (scenario n°1), and also to detect unexpected contaminants in samples that do comply with the regulation and face different contamination patterns (scenario n°2).

2.3 Sample treatment

Samples were extracted using a method based on previous work (Cladière et al., 2018): 5 mL of an ACN/MeOH (90/10 v/v) mixture acidified with 0.1% FA were added to each sample for extraction. Tubes were then agitated upside-down on an agitator plate for 1 hour, and centrifuged at 3,000 g for 10 minutes. Then 2 mL of the supernatant were collected and evaporated to dryness under a gentle nitrogen stream at 35°C. The extract was reconstituted in 0.2 mL of ACN acidified with 0.1% FA, and completed by 0.8 mL of H_2O acidified with 0.1% FA, leading to a total volume of 1 mL. The reconstituted extract was then centrifuged at 12,000 g for 10 minutes, 0.5 mL collected and filtered at 0.2 µm using a syringeless filter vial (mini-uniprep G2, Whatman) before analysis. For each type of tea (green and black), a quality control sample (QC) was made by pooling together in a glass tube 0.2 mL of each final extract considered in the analytical sequence, from which a 0.5 mL aliquot was sampled and filtered using a syringeless filter vial.

2.4 UHPLC-HRMS methods

Samples were analyzed on two UHPLC/HRMS platforms. The first is a Waters® Acquity UPLC® H-Class system, composed of a quaternary solvent manager pump, a refrigerated sample manager Flow-Through-Needle and a column oven, coupled to a Waters® high resolution Time-of-Flight mass spectrometer Xevo® G2-S ToF operated in centroid mode (resolution of 30,000 FWHM at *m/z* 200, *m/z* range from 60 to 800, 2 scans/s) using an electrospray ion source (ESI). The second is a Thermo Scientific UltiMate 3000 UHPLC system composed of quaternary pumps, refrigerated auto-sampler and a column oven coupled to a Q-Exactive Orbitrap mass spectrometer operated in centroid mode (resolution of 70,000 FWHM at *m/z* 200, *m/z* range from 60 to 800) with a heated electrospray ion source (HESI). For the Orbitrap MS method "AGC target" and "Maximum IT" parameters were set to 10^6 and 200 ms respectively.

For each platform, the injection volume was 10 µL. Separation was made on a C18-PFP column (150×2.1 mm, 2 µm particles diameter, ACE supplied by AIT France), and the same chromatographic gradients were used on both instruments. For ESI+, the mobile phase was composed of water (A) and ACN (B), both acidified with 0.1% FA, and MeOH (C), flowing at 0.4 $mL.min^{-1}$. Gradient started at 100% A and reached 100% B in 10 min, 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 at pH 6.45 with 10 mM of ammonium formate (A) and MeOH (B) flowing at 0.3 $mL.min^{-1}$. 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 temperature of the column oven was kept at 30°C. Parameters for electrospray ion sources can be found in **Table A.2.1 & A.2.2** of Supplementary material.

Each sequence started with 10 mobile phase injections and 5 stability mix injections to equilibrate the instrument (Dunn et al., 2011). Sample injection orders were randomized, and QC samples as well as blanks injected every 15 samples. For each ionization mode and instrument, each sample was analyzed in triplicate.

2.5 Data treatment workflow

Before any untargeted analysis, the overall method quality (extraction + UHPLC-MS analysis) was visually assessed for each injection replicate thanks to the Total Ion Current (TIC) and the peak intensity of each labelled molecule. This step only aimed at the early detection of analytical outliers and did not replace the quality control procedure that will be implemented afterwards.

A highly automated data treatment workflow based on previous work (Delaporte et al., 2019) has been implemented here (**Figure 2**). Vendors raw data files were first converted to an open-source format (.mzXML) with the help of ProteoWizard module "MSConvert" (Chambers et al., 2012) using a noise threshold of 100 for ToF data, and then uploaded onto the Workflow4Metabolomics (W4M) computation platform (Giacomoni et al., 2015). Data matrices were built using XCMS R package (Smith, Want, O'Maille, Abagyan, & Siuzdak, 2006). Parameters for XCMS algorithm were inspired by those suggested by Patti (Patti, Tautenhahn, & Siuzdak, 2013) for high resolution UHPLC-Q-ToF and UHPLC-Orbitrap instruments.

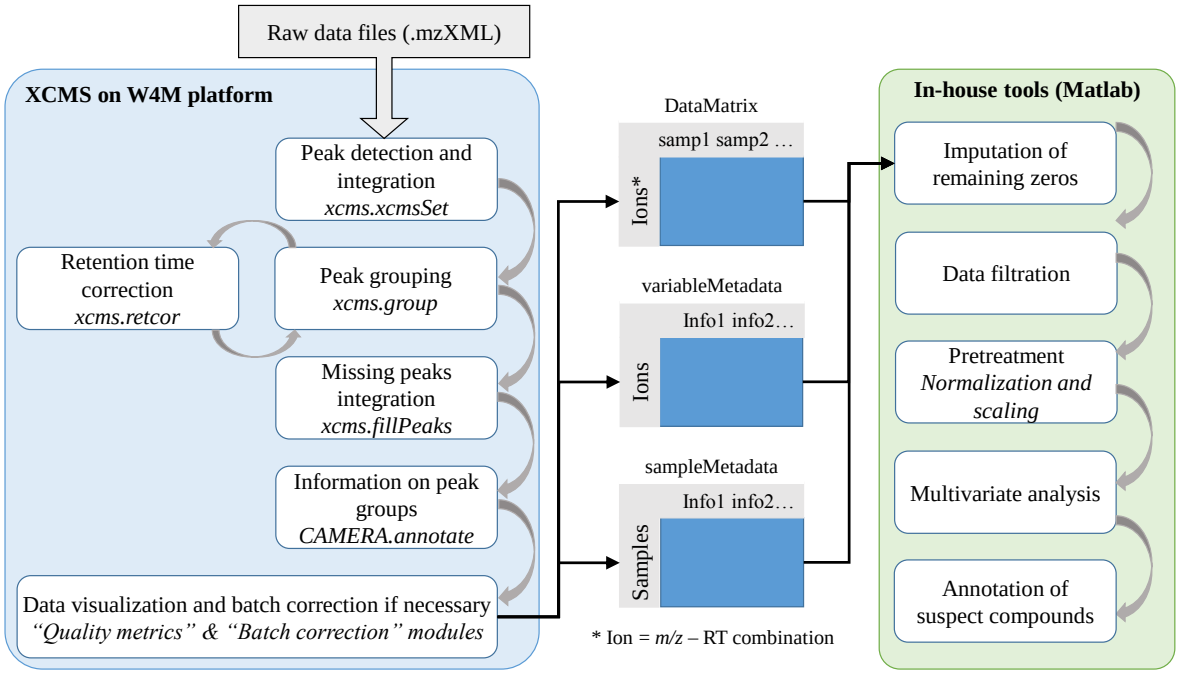

Figure 2: Untargeted data treatment workflow

The full list of parameters for both Orbitrap and ToF data files can be found in **Table A.3** of Supplementary material. In-line with XCMS, data sets were quickly visualized using the "Quality Metrics" module of W4M. A LOESS ("Locally Estimated Scatterplot Smoothing") analytical drift

correction on ion intensities (Van Der Kloet et al., 2009) was applied when needed as spotted in **Figure A.1** of Supplementary material.

In-house tools and methods were then implemented to achieve the detection of contaminants. As shown in **Figure 2**, remaining zero values were first imputed (especially for Orbitrap data) since some methods used afterwards are sensitive to zeros in the data matrix. A first missing value completion method, fillPeaks, was applied within XCMS as shown in **Figure 2**. It relies on the forced integration of missing ions. However, especially in low-noise data, we observed that this method tends to generate a lot of zero values when no peaks could be found. Therefore, we implemented a method to impute those remaining missing values, which appeared as zeros in our data matrices. The presence of injection replicates was used to impute them by the best value possible (Delaporte, Cladière, & Camel, submitted). Briefly, for each ion (identified in the data matrix by their combination "retention time-*m/z*"), if a zero is found in only one injection replicate, it is imputed by the mean of the replicates. If more than one replicate has a zero value, they are imputed by the estimated limit of detection of the instrument [i.e. mean of the 3% lowest values, zeros excluded (Libiseller et al., 2015)].

It was then necessary to reduce the number of ions present in the data matrix (several thousands). To that end, two complementary filtration strategies were implemented in parallel. The first (which will be called "t-test") relies on the implementation of univariate t-tests between sample groups and blank injections to remove ions already present in blanks, and then between groups to remove ions showing no significant differences between groups. These tests are followed by the calculation of the fold change (FC, calculated for each ion as the ratio of the medians of the highest group over the lowest). Fixed thresholds are used to filter the data matrix, respectively <0.05 for p-values (of "t-test") and >2 for FC. The alternative filtration strategy relies instead on the calculation, for each ion, of a relevant minimum FC (FC_{min}) from which one can assume that a significant signal difference is observed between groups (Ortmayr, Charwat, Kasper, Hann, & Koellensperger, 2017). FC_{min} calculation relies on the estimation of the uncertainty on FC based on the relative standard deviations observed for the considered samples. Only ions exhibiting a FC higher than the FC_{min} are kept.

Data matrices then go through several preprocessing methods. They were log- and pareto-scaled (Antignac et al., 2011; Delaporte et al., 2019) and normalized with a median-based Probabilistic Quotient Normalization (PQN) based on QC samples (Delaporte et al., 2019; Dieterle, Ross, Schlotterbeck, & Senn, 2006). PQN aims at limiting the influence of potential dilution effects occurring in the study, whereas the previously used LOESS method aims at correcting a signal intensity drift, caused for example by the fouling of the ion source during the study.

At this point, the data matrix is most often composed of a hundred to few thousands ions (with the notable exception of ToF data in positive ionization mode, for which only five ions were remaining). As a consequence, to visualize the data and assess the presence of trends and patterns, it is necessary to implement data analysis methods to reduce its dimensionality. In metabolomics studies, this step is typically done using multivariate methods (Gorrochategui et al., 2016). Independent Component Analysis (ICA) was the method selected here to visualize the data based on the remaining ions (Delaporte et al., 2019). This method was implemented using the JADE algorithm (Rutledge & Jouan-Rimbaud Bouveresse, 2015) and the optimal number of ICs to compute determined with Random-ICA method (Kassouf, Bouveresse, Rutledge, Jouan-Rimbaud Bouveresse, & Rutledge, 2017). ICA is a blind source signals decomposition method, that gives two main outputs, respectively signals (representing the pure source signals found in the data matrix) and scores (representing the weight of each sample in the different signals). The scores make it possible to visualize a potential separation of samples, and then the signals enable to link a sample group separation to specific ions. For example, if a sample group separation is observed along component n°1 (IC1) (with sample group n°1 having higher scores than sample group n°2), signals constituting IC1 will be sorted in descending order and the corresponding discriminating ions will be selected.

All discriminating ions were then annotated using automated in-house data-mining tools to detect isotopic patterns and adducts based on previous work (Cotton et al., 2014), and they were matched against a broad-range in-house database containing around 2,000 known toxicants and their most probable adducts in LC-HRMS (Delaporte et al., 2019). The presence of the spiked contaminants has not been assessed in the

database before the study to maintain their blind character. Finally, ions selected thanks to the multivariate analysis were manually curated to highlight suspect compounds.

2.6 Performance assessment

As already mentioned the operator carried out all the instrumental analysis and the data treatment process without any knowledge on the possible contamination of the samples (real blind procedure). At the end of the process, this operator issued a summary with two main information: which molecules are suspected to cause a group separation (with their respective *m/z*, retention time and putative annotation), and for each, in which group the concentration is the highest. Percentages of molecules detection for each experiment were then assessed, by returning back the results to the study designer who spiked the samples. Two detection percentages were computed from these results by the designer of the study as shown in. The first was calculated as the ratio between the number of blindly annotated contaminants and the number of contaminants actually detected in the spiked samples by a manual peak detection made by the study designer: it represents the success rate of the blind data treatment approach to detect and characterize a suspect signal in the data matrix. The second was calculated between the number of blindly annotated molecules against the number of LC-ESI-MS amenable molecules, defined as the number of molecules detected by a targeted analysis (conducted by the study designer) of a highly concentrated standard solution (500 ng/mL) with our method. This percentage characterizes the sensitivity of the global methodology (analytical and data treatment methods) to detect a contamination.

3. Results and discussion

Raw data sets have been deposited to the EMBL-EBI MetaboLights database (DOI: 10.1093/nar/gks1004. PubMed PMID: 23109552) with the respective identifiers MTBLS771 and MTBLS772 (Haug et al., 2013). The complete data sets can be accessed at https://www.ebi.ac.uk/metabolights/MTBLS771 and https://www.ebi.ac.uk/metabolights/MTBLS772.

3.1 Data sets metrics and pretreatment

After peak extraction from raw data files by XCMS, the main metrics (number of ions and percentage of zero values) were calculated for each data set (see **Table 2**). All data sets appeared to be rather similar in terms of number of ions or percentage of zeros, regardless of the instrument used. An analytical drift on

peak intensities was clearly visible on ToF data sets (an example is displayed in **Figure A.1** of Supplementary material) and was corrected using the LOESS method. No drift was observed on measured retention times and *m/z* whatever the data set considered.

TABLE 2 DATA SETS AVAILABLE AND THEIR RESPECTIVE METRICS

Matrix	UHPLC-HRMS platform	Ionization mode	Analytical drift correction	Number of ions *before filtering*	% zeros
Green tea *contamination mix n°1*	ToF	ESI+	Yes *(LOESS)*	15.548	6.45
		ESI-		28,275	4.76
	Q-Orbitrap	ESI+	No	16,053	8.33
		ESI-		17,646	9.69
Black tea *contamination mixes n°1 & 2*	ToF	ESI+	Yes *(LOESS)*	11,912	6.57
		ESI-		21,461	4.45
	Q-Orbitrap	ESI+	No	12,736	4.46
		ESI-		12,976	5.62

For ToF data, about twice as many ions were detected in negative ionization mode as compared to positive mode. However, it does not seem to have an impact on the percentage of zeros, which remains very similar between all data sets. A group-wise study of percentages of zeros reveals that, as expected, blank injections contain a higher proportion of zeros (between of 20 and 50% of values) than spiked tea samples (between 1 and 5 % of values). Still, the presence of zeros may lead to errors in the following steps of the process (especially for univariate statistics and log transformation), as a consequence they must be imputed. Zeros, i.e. missing values (see in 2.5) were first classified according to their nature thanks to the methodology described in 2.5 based on the presence of injection replicates, and then imputed either by the mean of replicates or by an estimated instrumental noise.

3.2 Contamination case n°1

Sample groups were successfully separated thanks to the multivariate analysis by ICA for both polarities and instruments. Results for ToF data are displayed in **Figure 3** (and in **Figure A.2** of Supplementary material for Orbitrap data).

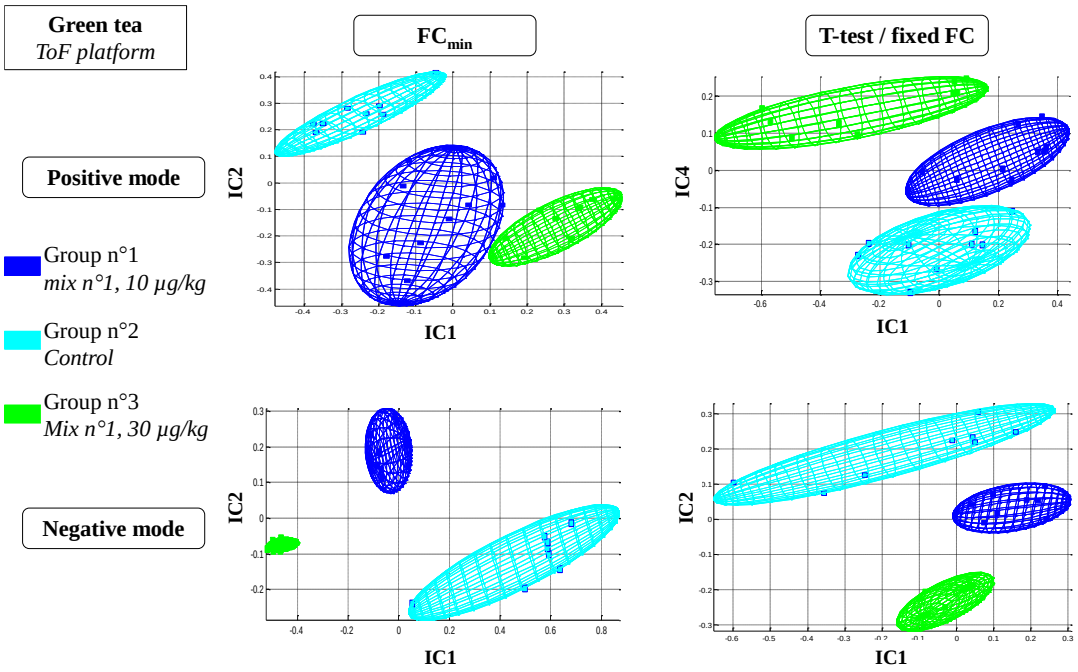

Figure 3: Score plot of ICA output for green tea samples analyzed on ToF platform

A clear concentration trend can be observed between sample groups on the discriminating component. For each filtration method applied, one or several discriminating components were found, the corresponding ions annotated and detection rates calculated (see **Table 3** for annotated contaminants and **Table 4** for detection rates). Hence, UHPLC-ToF platform enabled the successful detection of 4 contaminants (corresponding to 36% of the spiked molecules) in positive mode and of two others (corresponding to 18% of the spiked molecules) in negative mode. Similar results were obtained with t-test / fixed FC and FC_{min} filtration strategies. Results from both polarity modes and data filtration strategies were gathered and a global detection rate of 55% was calculated for ToF platform. A targeted search of spiked contaminants in the raw data files shows that only 7 of them can actually be seen (in which only 5 exhibit a signal/noise ratio above 3 for the 10 µg/kg level). It means that the main loss of information came from the analytical step, the data treatment being able to detect and annotate 6 contaminants out of 7 (i.e. 86%) actually visible in the raw chromatograms. A contaminant (dodemorph, see **Table 3**) was initially detected with the t-test based filtration approach, but appeared to be a false positive. This annotation was unsure from the start since it had not been reported with FC_{min} filtration.

TABLE 3 ANNOTATED CONTAMINANTS IN GREEN TEA SAMPLES

Measured mono-isotopic mass of adduct	Measured retention time (min)	Ionization mode	Most intense adduct	Proposed raw formula	Proposed mono-isotopic mass of compound	Mass error (ppm)	Proposed putative annotation	Detected in groups	Relative intensities	Filtration method
Orbitrap										
216.1009	7.83	POS	$[M+H]^+$	$C_8H_{14}ClN_5$	215.0938	-0.61	Atrazin	3 & 1	gp3 > gp1	FC_{min} / t-test
216.1414*	**10.22**	**POS**	**$[M+H]^+$**	**$C_{11}H_{21}NOS$**	**215.1344**	**-1.38**	**Cycloate**	**3 & 1**	**gp3 > gp1**	**FC_{min} / t-test**
253.1655	6.72	POS	$[M+H]^+$	$C_{12}H_{20}N_4O_2$	252.1586	-1.53	Hexazinone	3 & 1	gp3 > gp1	FC_{min} / t-test
215.0960	7.30	POS	$[M+H]^+$	$C_8H_{14}N_4OS$	214.0888	-0.37	Metribuzin	3 & 1	gp3 > gp1	FC_{min} / t-test
188.1105	8.94	POS	$[M+H]^+$	$C_9H_{17}NOS$	187.1031	0.50	Molinate	3 & 1	gp3 > gp1	FC_{min} / t-test
261.0229 / 259.0085**	7.01 / 12.34	POS / NEG	$[M+H]^+$ / $[M-H]^-$	$C_9H_{13}BrN_2O_2$	260.0160	-1.73 / 3.18	Bromacil	3 & 1	gp3 > gp1	FC_{min} / t-test
215.0587	12.69	NEG	$[M-H]^-$	$C_9H_{13}ClN_2O_2$	216.0666	2.37	Terbacil	3 & 1	gp3 > gp1	FC_{min} / t-test
ToF										
216.1016	6.52	POS	[M+H]+	$C_8H_{14}ClN_5$	215.0938	2.78	Atrazin	3 & 1	gp3 > gp1	FC_{min} / t-test
*282.2797****	*10.32*	*POS*	*[M+H]+*	*$C_{18}H_{35}NO$*	*281.2719*	*1.91*	*Dodemorph*	*1; 2 & 3*	*gp1 > gp2 > gp3*	*t-test*
253.1669	5.44	POS	[M+H]+	$C_{12}H_{20}N_4O_2$	252.1586	4.01	Hexazinone	3 & 1	gp3 > gp1	FC_{min} / t-test
215.0967	6.02	POS	[M+H]+	$C_8H_{14}N_4OS$	214.0888	2.90	Metribuzin	3 & 1	gp3 > gp1	FC_{min} / t-test
188.1119	7.57	POS	[M+H]+	$C_9H_{17}NOS$	187.1031	8.21	Molinate	3 & 1	gp3 > gp1	FC_{min} / t-test
259.0085	10.39	NEG	[M-H]-	$C_9H_{13}BrN_2O_2$	260.0160	3.13	Bromacil	3 & 1	gp3 > gp1	FC_{min} / t-test
215.0586	10.73	NEG	[M-H]-	$C_9H_{13}ClN_2O_2$	216.0666	1.99	Terbacil	3 & 1	gp3 > gp1	FC_{min} / t-test

* Detected only with Orbitrap mass analyzer

** Detected in both positive and negative ionization mode

*** False positive, not present in the spiking solution

TABLE 4 DETECTION RATES FOR CONTAMINATION CASE N°1.

UHPLC-HRMS platform	Mode	Detection rate [found compounds / added compounds]			Detection rate [found compounds / compounds visible in raw data]
		t-test / fixed FC	*FCmin*	**Global**	
ToF	ESI+	36%	36%	55%	86%
	ESI-	18%	18%		
Q-Orbitrap	ESI+	55%	55%	64%	100%
	ESI-	18%	18%		

On the UHPLC-Q-Orbitrap platform, 6 contaminants (i.e. 55% of the spiked molecules) were blindly detected in positive mode and 2 others in negative mode, leading to a total detection rate of 64%, which is slightly better than with the ToF platform. Interestingly, one contaminant could be annotated only in the Orbitrap data set as displayed in **Table 3**. All annotated spiked contaminants can be detected at levels as low as 10 µg/kg in samples, which is relevant regarding existing EU MRLs. Interestingly, the data treatment success rates rise to 100% for Orbitrap platform, meaning that all compounds present in the raw data files were successfully annotated by our methodology.

The sample diversity considered here is rather low since all samples come from the same brand and production batch. This topic has been discussed previously (Delaporte et al., 2019; Knolhoff et al., 2016), but the need for a control, unspiked sample for relevant signals selection is an issue that has not been solved yet despite of its critical aspect for untargeted food safety assessment. Moreover, this study relies on spiked samples, which also may lead to better detection rates than if natively contaminated (i.e. "real") samples were analyzed. In fact, the extraction of contaminants trapped within the sample matrix (and not solely present on its surface, as in the case of spiked samples) may induce more matrix effects and lower recoveries of compounds of interest that will probably make the implementation of the approach more difficult. The solving of these issues would surely imply further adjustments concerning the analytical method (namely the sample treatment step) and possibly the data treatment process. In a final step, ring studies will be necessary as it is done for traditional targeted approaches and in the field of metabolomics studies. Yet, our results are still highly promising since the detection rates obtained are within the same order of magnitude of the ones presented in similar studies (Kunzelmann et al., 2018). On top of that,

several contaminants studied here have never been analyzed with our method, so that their potential analytical responses and detectability were truly "unknown".

3.3 Contamination case n°2

In this more complex contamination scenario, group separation was also achieved. However, this time the group separation pattern observed in all cases suggests the presence of different contaminations among sample groups, and not a single contamination present at several levels as in scenario n°1. This highlights the interest of using ICA here, since it produces independent components, meaning that the ions combination used to build IC1 should be different from the combination used to build IC2. This is particularly visible on data from Orbitrap platform (in **Figure 4**).

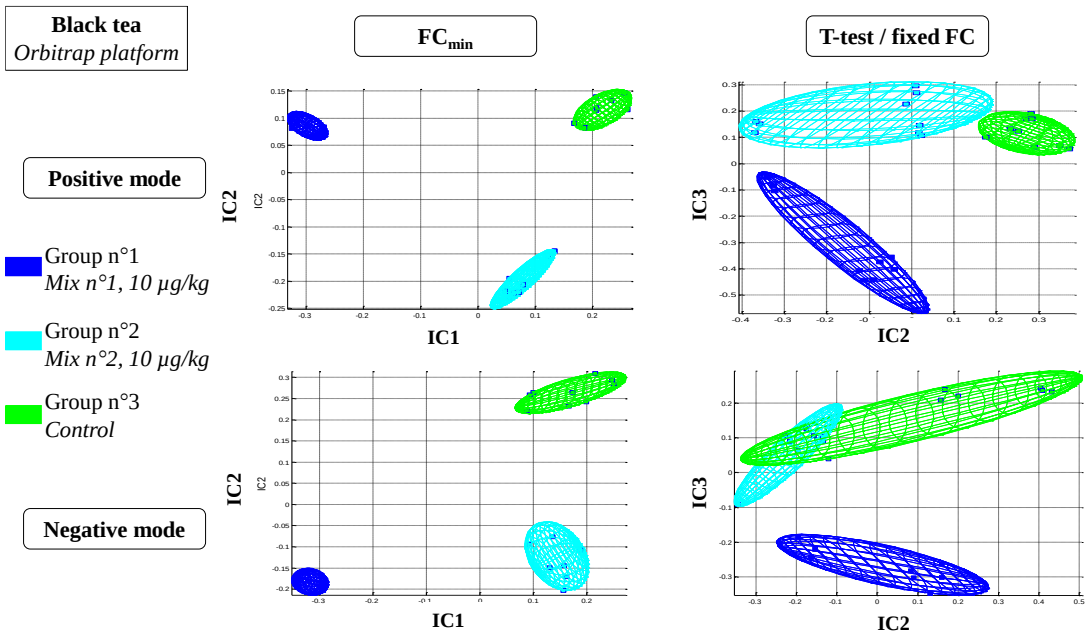

Figure 4: Score plot of ICA output for black tea samples analyzed on Orbitrap platform

Again, the two previously described filtration methods (t-test/fixed FC and FC_{min}) were implemented, but some combinations of instrument/polarity/filtration method do not enable a clear sample separation, and some even lead to the selection of too few ions to perform a multivariate analysis (see **Figure 4** for Orbitrap data and **Figure A.3** of Supplementary material for ToF data). For example, for positive ionization mode data acquired on the ToF platform, the FC_{min} filtration method leads to the selection of 5 ions, thus multivariate analysis was not needed and each ion was putatively annotated individually.

The annotation of discriminating ions is completed by the visualization of ion intensity differences among sample groups thanks to an in-house Matlab script to assess the relative levels of annotated contaminants. The final outcome of this process is presented in **Table 5**. A separate detection rate has been then calculated for each spiking mix (see **Table 6**).

The contamination pattern of group n°1 was first investigated thanks to ICA decomposition. On the UHPLC-ToF platform, 3 contaminants can be tentatively annotated in positive ionization mode, and 2 others in negative mode, corresponding to detection rates of 27% and 18% respectively, leading to 45% when considering both modes which is lower than previously observed on green tea. In fact, the detection rate in scenario n°1 is favored by the presence of a group with a "high" spiking level (30 µg/kg), not present in the scenario n°2. Consequently, one compound (molinate) is missed in case n°2 (but found in case n°1) since it exhibits a signal / noise ratio close to the limit of detection of the ToF platform. A similar decrease of detection rate was reported by Kunzelmann (Kunzelmann et al., 2018) from 89% detection at 25 $\mu g.kg^{-1}$ too less than 35% at 5 $\mu g.kg^{-1}$. Interestingly, more contaminants can be annotated using the UHPLC-Q-Orbitrap platform.

Overall, same performances were achieved as for contamination scenario n°1, with 6 contaminants annotated in positive mode (i.e. 55% of spiked molecules) and 2 others in negative mode (i.e. 18% of spiked molecules) in sample group n°1. Globally, 7 contaminants (out of 11, corresponding to a detection rate of 64%) were successfully annotated in this sample group, and 100% of compounds present in the raw data files were successfully annotated by our methodology.

TABLE 5 ANNOTATED CONTAMINANTS IN BLACK TEA SAMPLES

Measured mono-isotopic mass of adduct	Measured retention time (min)	Ionization mode	Most intense adduct	Proposed raw formula	Proposed mono-isotopic mass of compound	Mass error (ppm)	Proposed putative annotation	Detected in groups	Relative intensities	Filtration method
Orbitrap										
353.0243*	**9.54**	**POS**	**$[M+Na]^+$**	**$C_{10}H_{19}O_6PS_2$**	**330.0361**	**-2.96**	**Malathion**	**2**	**N/A****	**FCmin**
216.1009	7.83	POS	$[M+H]^+$	$C_8H_{14}ClN_5$	215.0938	-0.61	Atrazine	1	N/A	FCmin / t-test
216.1414*	**10.22**	**POS**	**$[M+H]^+$**	**$C_{11}H_{21}NOS$**	**215.1344**	**-1.38**	**Cycloate**	**1**	**N/A**	**FCmin / t-test**
253.1655	6.72	POS	$[M+H]^+$	$C_{12}H_{20}N_4O_2$	252.1586	-1.53	Hexazinone	1	N/A	FCmin / t-test
215.0960	7.30	POS	$[M+H]^+$	$C_8H_{14}N_4OS$	214.0888	-0.37	Metribuzin	1	N/A	FCmin
188.1105*	**8.94**	**POS**	**$[M+H]^+$**	**$C_9H_{17}NOS$**	**187.1031**	**0.50**	**Molinate**	**1**	**N/A**	**FCmin / t-test**
261.0229 / 259.0084***	7.01 / 12.34	POS / NEG	$[M+H]^+$ / $[M-H]^-$	$C_9H_{13}BrN_2O_2$	260.0160	-1.60 / -1.30	Bromacil	1	N/A	FCmin / t-test (POS) FCmin (NEG)
215.0587	12.69	NEG	$[M-H]^-$	$C_9H_{13}ClN_2O_2$	216.0666	-2.80	Terbacil	1	N/A	FCmin / t-test
ToF										
402.0733*	**11.03**	**NEG**	**$[M-H]^-$**	**$C_{20}H_{18}ClNO_6$**	**403.0823**	**-4.07**	**Ochratoxin A**	**2**	**N/A**	**FCmin**
249.0220*	**9.56**	**NEG**	**$[M-H]^-$**	**$C_{12}H_{10}O_4S$**	**250.0300**	**-2.80**	**Bisphenol S**	**2**	**N/A**	**FCmin**
216.1016	6.52	POS	$[M+H]^+$	$C_8H_{14}ClN_5$	215.0938	2.78	Atrazine	1	N/A	FCmin
253.1669	5.44	POS	$[M+H]^+$	$C_{12}H_{20}N_4O_2$	252.1586	4.01	Hexazinone	1	N/A	FCmin
215.0967	6.02	POS	$[M+H]^+$	$C_8H_{14}N_4OS$	214.0888	2.90	Metribuzin	1	N/A	FCmin
259.0084	10.39	NEG	$[M-H]^-$	$C_9H_{13}BrN_2O_2$	260.0160	-1.32	Bromacil	1	N/A	FCmin / t-test
215.0585	10.73	NEG	$[M-H]^-$	$C_9H_{13}ClN_2O_2$	216.0666	-3.27	Terbacil	1	N/A	FCmin / t-test

* Compounds detected on only one instrument

** N/A = Not applicable

*** Detected in both positive and negative ionization mode

TABLE 6 DETECTION RATES FOR CONTAMINATION CASE N°2.

A) CONTAMINANTS SPIKED IN GROUP N°1. OVERALL (TOF + ORBITRAP) DETECTION RATE: **64%**

UHPLC-HRMS platform	Mode	Detection rate [found compounds / added compounds]			Detection rate [found compounds / compounds visible in raw data]
		t-test / fixed FC	*FCmin*	**Global**	
ToF	ESI^+	0%	27%	45%	71%
	ESI^-	18%	18%		
Q-Orbitrap	ESI^+	36%	55%	64%	100%
	ESI^-	9%	18%		
	ESI^-	9%	18%		

B) CONTAMINANTS SPIKED IN GROUP N°2. OVERALL (TOF + ORBITRAP) DETECTION RATE: **100%**

UHPLC-HRMS platform	Mode	Detection rate [found compounds / added compounds]			Detection rate [found compounds / compounds visible in raw data]
		t-test / fixed FC	*FCmin*	**Global**	
ToF	ESI^+	0%	0%	67%	67%
	ESI^-	67%	67%		
Q-Orbitrap	ESI^+	33%	33%	33%	100%
	ESI^-	0%	0%		
	ESI^-	0%	0%		

Then group n°2 was proposing a completely different contamination pattern as shown in **Table 5**. On the ToF platform, this contamination has been detected only in negative mode, with two contaminants putatively annotated (ochratoxin A and bisphenol S). The missing contaminant (malathion, normally amenable in positive mode) was not detected due to a high analytical drift on signal intensity that cannot be corrected satisfyingly. Interestingly, both LC-MS platforms show a real complementarity since malathion can be annotated in positive mode using Orbitrap data. However, this time bisphenol S and ochratoxin A cannot be annotated, even using a targeted approach (see in **Table 5**). This is not due to an issue in the data treatment process or to an analytical drift as for ToF data, but to a lack of sensitivity of the method (the peak cannot be seen at all in the raw data files) maybe caused by strong matrix effect inherent to the tea matrix. These results indicate that the approach benefits from the presence of numerous contaminants since the detection of the contamination with 3 compounds was more difficult than with 11 compounds. With fewer contaminants, the outcome of the approach seems indeed more vulnerable to potential failures in the analytical process. It is the first time this fact is highlighted since it did not

occurred in previous studies focusing on few contaminants (Delaporte et al., 2019, Knolhoff et al., 2016). Ensuring their ruggedness seems to be one of the key stake of those approaches, especially since untargeted food safety studies could imply the detection of a single contaminant, for which the analytical response may not be optimal.

These results are very encouraging since despite the presence of two different contaminations at very low levels (only 10 $\mu g.kg^{-1}$), satisfactory detection rates were achieved on both analytical platforms. Even with this complex situation, annotation rates are similar with those found for already existing, simpler cases (Kunzelmann et al., 2018).

3.4 ToF / Orbitrap comparison

The two instruments possess different characteristics, both in terms of ionization source (geometry, ESI vs. HESI) and mass analyzer performances (resolution, accuracy, detection technology). Therefore, their performance in terms of sensitivity and scope are expected to be different. Interestingly, our developed methodology can be applied on data collected from both instruments with promising results as indicated above. Unexpected contamination could be detected and characterized by the use of different LC-HRMS platforms, even at levels as low as 10 µg/kg and with samples exhibiting different contaminations within the same analytical sequence. This is a major advance for the development and implementation of untargeted approach for chemical food safety applications, since existing approaches only focus on a single contamination scenario and one analytical platform.

The majority of contaminants were detected on both platforms, giving rather similar annotation success rates. Both platforms performed well on the simplest case (n°1), with close detection rates (55% for ToF analyzer vs. 64% for Orbitrap). The main difference observed came from the false detection of a contaminant (dodemorph, see **Table 3**) when using the t-test based filtration on the ToF data. This false positive cannot be explained since this peak was absent from Orbitrap data, as well as from the spiking mix. On the rest of the data (both ToF and Orbitrap), both filtration strategies gave similar results.

However, several contaminants were missed by both instruments for case n°1. Based on **Table 3** and **Table A.1** of Supplementary material, the missed contaminants are the following: butylate, EPTC, isopropalin and pebulate (plus cycloate for ToF platform). For cycloate, it is most likely due to the mass

spectrometry detection, and more particularly the geometrical and technological differences between the two ionization sources, two critical parameters in complex matrices such as tea. A closer look at the respective physico-chemical properties of the remaining compounds, and especially vapor pressures, enables to give hypothesis on their non-detection. First of all, two of them (butylate and EPTC) have vapor pressures over 100 mPa (see **Table A.1** of Supplementary material), which may lead to a loss during the evaporation/concentration step. However, other contaminants have similar vapor pressures (for example molinate) and are still detected. The explanation for the non-detection of butylate and EPTC therefore should be a combination between significant losses during sample treatment and strong matrix effects in mass spectrometry. For the two last ones (pebulate and isopropalin), only few papers were reported (Mayer-Helm et al., 2006; Liu et al., 2004) with levels analyzed rather high compared with our study (between 0.1 and 1 mg/kg), suggesting that those compounds may be difficult to analyzed using LC-ESI-MS. This hypothesis must be taken with care given the technological developments in LC-MS between the mid-2000s and late 2010s. Still, the signal/noise ratios obtained with our method for those two compounds are rather low, even in a clean solvent matrix, so that it is likely that even minor loss due to either the sample treatment or the ionization process would lead to their non-detection in a complex matrix such as tea.

Differences between the two instruments appeared more clearly during the analysis of the contamination case n°2, which is more complex. In fact, as displayed in **Table 5** (in bold), several contaminants were annotated in data from only one instrument, which clearly highlights the interest of implementing multiple LC-HRMS technologies for untargeted studies. As indicated in **Table 6**, for contamination mix n°1 (11 herbicides) same detection rates were achieved than in case n°1, and non-detects should then have the same causes. For contamination mix n°2 (mix of OTA, BPS and malathion), the detection rate difference along with the complementarity observed between the two instruments suggest that non-detects probably come from the mass spectrometer technology (including the ionization sources and ion paths) and not from the upstream analytical process (sample treatment and chromatography methods). In fact, the ToF instrument used appeared to be more sensitive to source fouling (and so to ion intensity drift), leading to the non-detection of malathion in positive mode.

Differences were also observed between the two filtration methods, since FC_{min} strategies gave overall better results than t-test strategy on our data. This observation may be explained by the computing, in the FC_{min} strategy, of the discarding criteria as a function of the repeatability of the measurement for each ion, whereas t-test strategy uses fixed thresholds for all ions.

However, the achievement of such performances required the development of a data process adjusted to specificities of both LC-HRMS platforms. In particular, the process already developed (Delaporte et al., 2019) for ToF platform was modified to handle zeros generated by the low noise of the data produced by the Orbitrap platform. Moreover, analytical drift correction was required for data from ToF platform only. These issues were easily handled using easy-to-use, open-source and freely available tools which highlight the advantages of using those tools to take full advantage of the use of multiple instruments.

In addition, some specificity of each instrument can be spotted. Hence malathion and cycloate were only detected on the Orbitrap platform, while ochratoxin A and bisphenol S were recovered using the ToF instrument. Thus, the future of food safety control using untargeted approaches will benefit from the implementation of multiple analytical platforms in the process, which will be eased by the use of open-source, flexible tools.

4. Conclusion

In this work, an untargeted strategy to detect trace contaminants at relevant levels in complex food samples has been blindly implemented on two complex contamination scenarios in a model food product (green and black tea leaves) using two LC-HRMS platforms (respectively LC-ToF and LC-Q-Orbitrap). To the best of our knowledge, this is the first time that two different platforms are compared for untargeted food chemical safety assessment. The comparison of the performances of the two instruments has been eased by the use of freely available and open-source tools which enabled the implementation of the same data treatment workflow for all data sets. Most contaminants have been detected with both instruments, despite the complexity of the scenarios investigated (unexpected contaminants, different contamination between samples of the same data set and low contamination levels, i.e. down to 10 µg/kg). Most contaminants spiked have never been analyzed with our analytical method before and so were truly unknown to the methodology, which is rather new in this kind of study. As far as we know, this is the first

time that several contamination patterns have been investigated simultaneously at such levels (down to 10 µg/kg). This constitutes a major step towards an implementation of untargeted approaches in routine analysis. Different information could be obtained between two LC-HRMS systems, and this is especially critical in complex matrices, in which strong signal suppression phenomenon are often observed. Our data treatment approach performed well on all data sets, which is highly promising with a view of its implementation on other studies and LC-HRMS platforms for blind contaminants detection. However, the cases investigated in this study differ from real food safety applications. First, the sample diversity considered here is much lower than a real case, which would involve samples from several production batches, geographical origins and processes. A first attempt has been made to increase the sample diversity in our previous work with promising results (Delaporte et al., 2019), but still with limited variability and applicability as an unspiked control sample was always needed to detect a contamination. No doubt that future works would imply the study of a greater, "real-life" sample variability, by taking into consideration the different factors mentioned above. Moreover, contaminants may be more difficult to recover from a natively contaminated sample than from a spiked one; a water soaking step might help to recover native contaminants in the case of dried tea samples (Martínez-Domínguez et al, 2015). The solving of these two issues (sample variability and the gap between spiking studies and real samples) would certainly constitute major scientific breakthrough in the field of chemical food safety assessment.

The development of untargeted approaches benefits from its application on multiple data sets and contamination scenarios. For that purpose, data sets used in this publication will be made publicly available on the MetaboLights data exchange platform.

5. Appendix A: Supplementary material

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8. Declaration of interest

None.

9. References

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TABLE A.1 MAIN CHARACTERISTICS OF STUDIED COMPOUNDS

Contaminant	Formula	Molecular weight	log Kow	pKa	Vapor pressure (mPa, 20°C)	Authorized substance for tea	MRL (mg/kg) in tea leaves
	Spiking mix n°1						
Atrazine *Pesticide*	$C_8H_{14}ClN_5$	215.0938	2.7	1.7	3.90×10^{-2}	No	0.1
Bromacil *Pesticide*	$C_9H_{13}BrN_2O_2$	260.0160	1.88	9.27	4.10×10^{-2}	No	0.01
Butylate *Pesticide*	$C_{11}H_{23}NOS$	217.1500	4.1	N/A[1]	1.70×10^{2}	No	0.05
Cycloate *Pesticide*	$C_{11}H_{21}NOS$	215.1344	4.11	N/A	8.30×10^{2}	No	0.01
EPTC *Pesticide*	$C_9H_{19}NOS$	189.1187	3.2	N/A	4.50×10^{3}	No	0.05
Hexazinone *Pesticide*	$C_{12}H_{20}N_4O_2$	252.1586	1.17	2.2	3.00×10^{-2}	No	0.01
Isopropalin *Pesticide*	$C_{15}H_{23}N_3O_4$	309.1689	5.29	N/A	1.17	No	0.0.1
Metribuzin *Pesticide*	$C_8H_{14}N_4OS$	214.0888	1.26	0.99	1.21×10^{-1}	Yes	0.1
Molinate *Pesticide*	$C_9H_{17}NOS$	187.1031	2.86	N/A	5.00×10^{2}	No	0.05
Pebulate *Pesticide*	$C_{10}H_{21}NOS$	203.1344	4	N/A	9.00×10^{-3}	No	0.01
Terbacil *Pesticide*	$C_9H_{13}ClN_2O_2$	216.0666	1.89	9.5	6.25×10^{-2}	No	0.01
	Spiking mix n°2						
Malathion *Pesticide*	$C_{10}H_{19}O_6PS_2$	330.0361	2.75	N/A	3.10	Yes	0.5

Bisphenol S *Migrant from packaging*	$C_{12}H_{10}O_4S$	250.0300	2.91	N/A	6.00×10^{-5}	N/A	N/A
Ochratoxin A *Mycotoxin*	$C_{20}H_{18}ClNO_6$	403.0823	4.74	3.2 / 7.9	4.15×10^{-9}	N/A	N/A

[1] N/A = Not Applicable

TABLE A.2.1 SOURCE PARAMETERS FOR TOF PLATFORM

Parameter	ESI^+	ESI^-
Capillary voltage (kV)	1.5	1.0
Sample cone (V)	20	35
Source offset (V)	20	80
Source temperature (°C)	130	130
Desolvatation temperature (°C)	500	500
Cone gas flow (L/h)	50	20
Desolvatation gas flow (L/h)	1,200	600

TABLE A.2.2 SOURCE PARAMETERS FOR ORBITRAP PLATFORM

Parameter	ESI^+	ESI^-
Capillary voltage (V)	3,000	2,500
Capillary temperature (°C)	350	300
Desolvatation gas flow (A.U)	60	35
Auxiliary gas flow (A.U)	20	10

A.U = Arbitrary Unit

TABLE A.3 PARAMETERS FOR PEAK EXTRACTION BY XCMS

Module	Parameter	ToF	Orbitrap
xcmsSet	scanrange	NEG: 180-2400 POS: 120-2060	All
	nSlaves	1	1
	method	centWave	centWave
	ppm	15	3
	peakwidth	5-60	5-20
	mzdiff	-0.001	-0.001
	snthresh	10	10
	integrate	1	1
	noise	0	0
	prefilter	0	3,5000
group	method	density	density
	minfrac	0.5	0.5
	bw	2	2
	mzwid	0.015	0.015
	sleep	0.001	0.001
retcor	method	peakgroups	peakgroups
	smooth	loess	loess
	extra	1	1
	missing	1	1
	span	0.2	0.2
	family	gaussian	gaussian
	plottype	mdevden	mdevden
fillPeaks	method	chrom	chrom
CAMERA.annotate	nSlaves	4	4
	sigma	6	6
	perfwhm	0.6	0.6
	ppm	15	3
	mzabs	0.015	0.015
	maxcharge	1	1
	maxiso	4	4
	minfrac	0.5	0.5
	quick	TRUE	TRUE
	convertRTMinute	FALSE	FALSE
	numDigitsMZ	4	4
	numDigitsRT	0	0
	intval	into	into

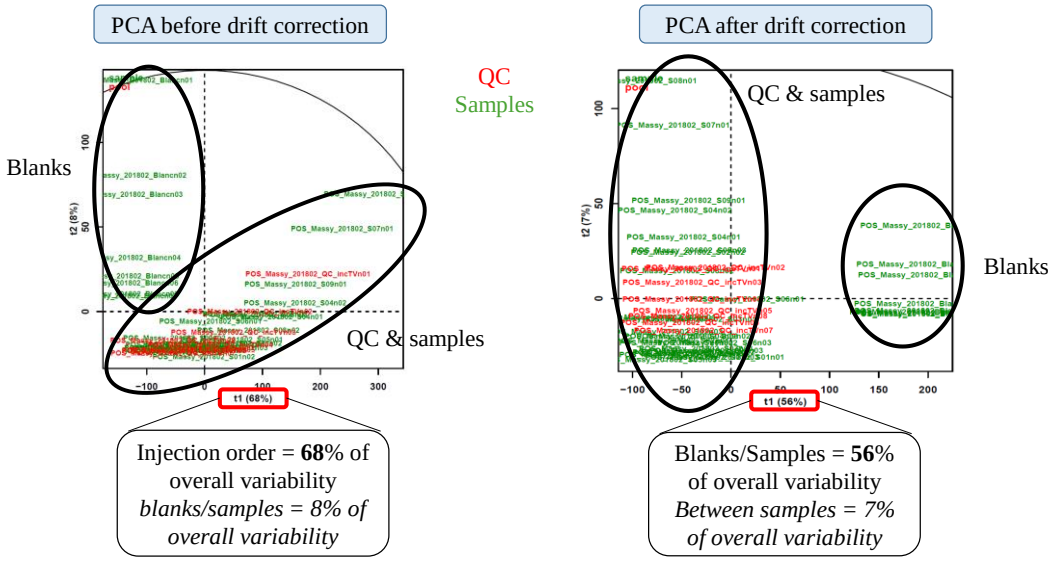

FIGURE A.1 PCA OF RAW DATA BEFORE AND AFTER DRIFT CORRECTION (GREEN TEA, TOF DATA ACQUIRED IN POSITIVE IONIZATION MODE)

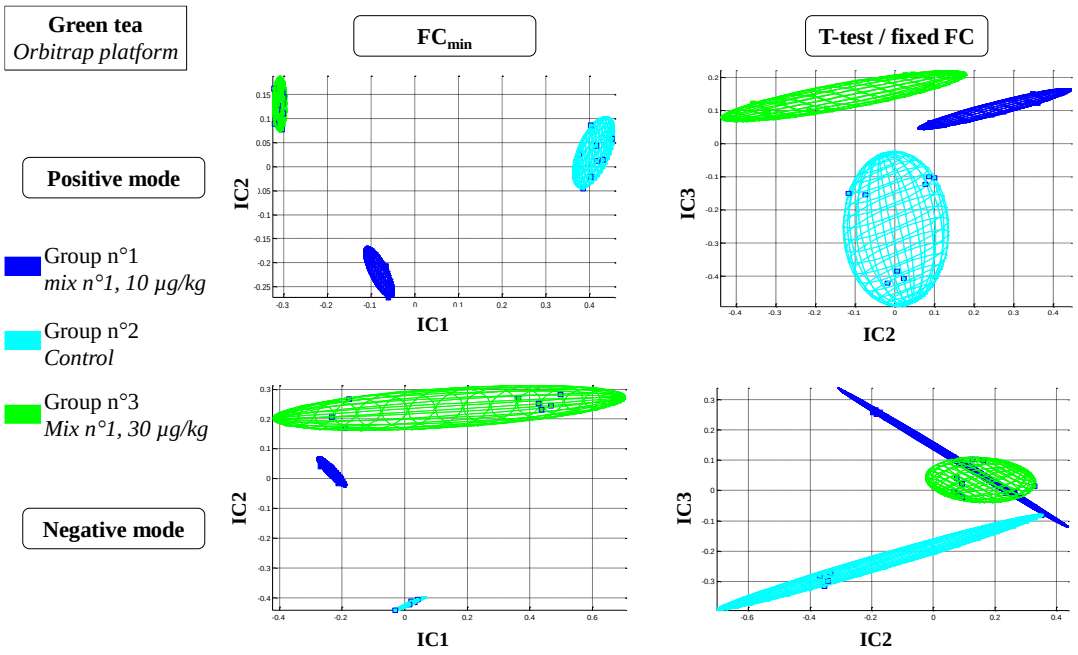

FIGURE A.2 SCORE PLOTS OF ICA OUTPUT ON GREEN TEA SAMPLES ANALYZED ON ORBITRAP PLATFORM

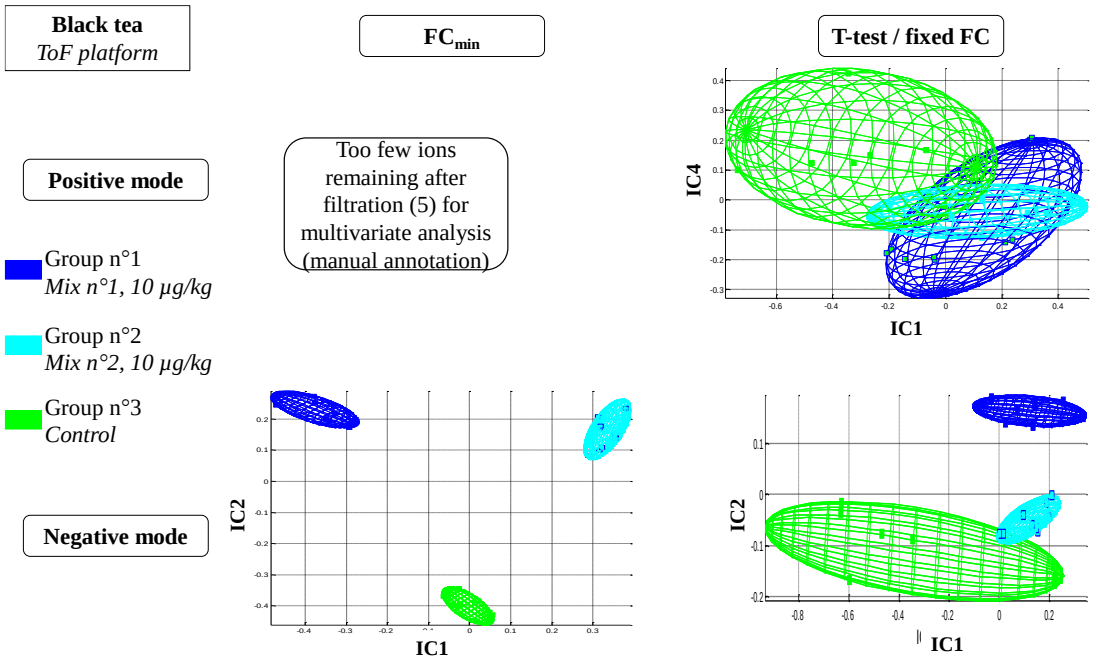

FIGURE A.3 SCORE PLOTS OF ICA OUTPUT ON BLACK TEA SAMPLES ANALYZED ON TOF PLATFORM