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Standard substances free quantification makes LC/ESI/MS non-targeted screening of pesticides in cereals comparable between labs

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ABSTRACT

LC/ESI/MS is the technique of choice for qualitative and quantitative food monitoring; however, analysis of a large number of compounds is challenged by the availability of standard substances. The impediment of detection of food contaminants has been overcome by the suspect and non-targeted screening. Still, the results from one laboratory cannot be compared with the results of another laboratory as quantitative results are required for this purpose. Here we show that the results of the suspect and non-targeted screening for pesticides can be made quantitative with the aid of *in silico* predicted electrospray ionization efficiencies and this allows direct comparison of the results obtained in two different laboratories. For this purpose, six cereal matrices were spiked with 134 pesticides and analysed in two independent labs; a high correlation for the results with the R^2 of 0.85.

KEYWORDS: Quantification without standards; Pesticides; LC/ESI/MS; Food monitoring; Cereal

1. INTRODUCTION

LC/ESI/MS is widely used for qualitative and quantitative analysis in food monitoring. This technique allows for the detection of a wide range of compounds such as pesticides, mycotoxins, veterinary drugs, other contaminants, and their metabolites in various food products (Dzuman, Zbynek, Zachariasova, Milena, Veprikova, Godula, & Hajslova, 2015; Jamin, Bonvallot, & Tremblay-franco, 2014; Mol et al., 2008). In ESI source, different compounds possess vastly different ionization efficiencies, the number of gas-phase ions produced at the same molar concentration; therefore, the quantification of these compounds requires corresponding standard substances to establish the relation between MS response and analyte concentration (Hermans, Ongay, Markov, & Bischoff, 2017; Kruve, Kaupmees, Liigand, & Leito, 2014). For example, more than 1000 different compounds against pests are in use worldwide (EU - Pesticides database; Li & Jennings, 2017). Thus, it is common that pesticide residue analysis includes 500 or more analytes in the routine scope and the same number of standard substances needs to be purchased and included in the sequence. Unfortunately, standard substances are often unavailable for various reasons and it is even more of a predicament when new active substances are put on the market, residue definitions are changed to include metabolites of compounds or when compounds that are newly discovered or unstable.

Non-targeted analysis and suspect screening earn much attention in food safety monitoring in these years, as these allow identifying compounds present in the sample from MS data without the need for authentic standard substances (Fraser et al., 2014; Hird, Lau, Schuhmacher, & Krska, 2014). However, for quantification purposes, standard substances are still required. As numerous tentatively identified compounds are often found it becomes either impossible or too expensive to purchase all these tentatively identified compounds. Furthermore, the peak areas obtained for different samples can be compared only if run in the same sequence and no matrix effect is expected. Comparison between different days, instruments or laboratories is impossible. Therefore, the ability to obtain quantitatively comparable results that do not depend on the instrument and lab is of utmost interest and importance (Kruve & Kaupmees, 2017).

To overcome this problem some semi-quantification approaches have been proposed. An approach based on chemical similarity was proposed by, eg., Kalogiouri et al., (Kalogiouri, Aalizadeh, &

Thomaidis, 2017) who selected several most appropriate standard substances for semi-quantification according to the similarity calculation of molecular descriptors between tested molecules and quantification standard substances (14 phenolic compounds). Pieke et al., (Pieke, Granby, Trier, & Smedsgaard, 2017) demonstrated a proof of concept strategy for semi-quantification using LC/ESI/MS combining generic ESI ion source optimization using 18 quantification markers where the marker with the smallest difference in retention time was chosen to give the best average prediction of the concentration of the analytes. However, due to the limited number of test compounds and lack of validation with more compounds, the prediction quality is still unclear.

The prediction of ionization efficiency (IE) has the potential to offer higher accuracy and a wider application range. Recent attempts to predict ESI response have considered multiple physiochemical parameters simultaneously, and a correlation between ionization efficiency and different physicochemical properties (e.g., gas-phase basicity, $\log P$, polarity, pK_a , surface area, molar volume, etc.) has been observed (Alymatiri, Kouskoura, & Markopoulou, 2015; Cramer, Johnson, & Kamel, 2017; Fusaro, Mani, Mesirov, & Carr, 2009; Golubović, Birkemeyer, Protić, Otašević, & M., 2016; Hermans et al., 2017; Huffma, Poltash, & Hughey, 2012; Oss, Kruve, Herodes, & Leito, 2010; Wang et al., 2019; Wu et al., 2013). The application scopes have been confined to the specific type of compounds such as drug-like molecules (Cramer et al., 2017), amino acids (Hermans et al., 2017), peptides (Raji et al., 2009), steroids (Alymatiri et al., 2015), or organic acid et al (Wu et al., 2013). Unfortunately, these approaches have been hardly used for quantification. The solvent also has an important effect on ionization efficiency and needs to be taken into account, which is often neglected.

Our group (Kruve & Kaupmees, 2017; Kruve et al., 2014; P. Liigand, Liigand, Cuyckens, Vreeken, & Kruve, 2018; Oss et al., 2010) has previously proposed a more generic model to predict ionization efficiencies and the ionization efficiency predictions have been used to obtain the concentrations of small molecules analysis with gradient elution in ESI negative mode (Kruve & Kaupmees, 2017) for the analysis of standard substances. Additionally, we have shown that ionization efficiencies can be predicted for compounds in case of the analysis of complicated matrices (blood, urine, etc.) with flow injection analysis (P. Liigand et al., 2018). Very recently we have developed a fast and easy Quantem approach to predict the ionization efficiencies for a wide range of compounds and to use it for estimating the concentration of the compounds in LC/HRMS non-targeted screening (J. Liigand et al., 2019).

Based on the previously obtained promising results we aim to evaluate whether ionization efficiency prediction model can be used to reach quantitatively comparable results for suspect screening between labs for the analysis in food samples. Here we focus on the analysis of pesticides in cereals as cereals are one of the largest foods commodity and consumed worldwide (González-Curbelo, Herrera-Herrera, Ravelo-Pérez, & Hernández-Borges, 2012), there is an intense attention to the occurrence of pesticides and mycotoxins exposure from cereal to maintain a high food safety standard. We, firstly, apply the Quantem model to predict the ionization efficiencies of detected pesticides to obtain the concentrations (using 4 orders of magnitudes spiking levels) in six common cereal matrices (oat, barley, rice, rye, wheat, and maize) (J. Liigand et al., 2019). Secondly, we evaluate the robustness of the concentration predictions of 134 common pesticides by comparing the results obtained from two laboratories in Denmark and Estonia on two different mass spectrometric setups (Agilent 6495 triple quadrupole and Bruker Daltonics micro-TOFq).

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

Milli-Q grade water was used for all analyses (18.2 M Ω ·cm, TOC 1–2 ppb, prepared by a Millipore Milli-Q Advantage A10 water-purification system). Acetonitrile (LC-MS grade), and formic acid (LC-MS grade) were purchased from Sigma-Aldrich (Steinheim, Germany). Magnesium sulfate (purity > 99%) was purchased from Lach:ner (Neratovice, Czech Republic), and absorbent Bondesil-C18 was purchased from Agilent Technologies (Santa Clara, CA, USA). All other solvents used in this study were of analytical grade.

Thirty standard substances that were used to transfer the model from one lab to another were purchased from Sigma-Aldrich (Steinheim, Germany) or synthesized in-house (J. Liigand et al., 2019). All the pesticide standard substances (all with a purity > 96%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). The pesticide standard substances stock solutions of 1 mg/mL were prepared in toluene and stored at –18 °C. A standard mixture of 10 μ g/mL in 80% acetonitrile was prepared from these stock solutions. Working solutions in the concentration range of 4 nM to 35 μ M on 6 different concentration levels were prepared by diluting the standard mixture with the extracts of blank cereal as a matrix (free of pesticide residues). Due to the instability of some of the compounds the same spiked

samples could not be analyzed in two labs. To overcome this obstacle, the same sample preparation was carried out in both labs and the extracts were spiked. The small differences in the spiking experiments were determined by weighting and corrected thereafter. A full list of all the pesticides and compounds used for transferring the model (structures, retention time, isotope distribution, concentration, et al.) are shown in Table S1 in supporting information. The extracts used for the matrix matching were obtained by the extraction and clean-up procedure described below.

2.2 Samples collection and sample extraction

The blank samples (free of pesticides) of cereals specifically grown for preparation of European Union proficiency test material (EUP) for the six EUPs: EUP-CF8 (wheat), EUP-C3 (oat), EUP-CF10 (rye) and EUP-C6 (barley), EUP-CF9 (maize), EUP-SRM6 (rice).

The extraction was based on the generic QuEChERS method developed by Dzuman's group (Dzuman, Zbynek et al., 2015). Two grams of homogenized cereal samples (wheat, oat, rye, rice, barley, maize) were weighed into a 50 mL polypropylene centrifugation tube, and 10 mL acidified Milli-Q water containing 0.2% formic acid was added and left to soak the matrix for 0.5 h. Then, 10 mL acetonitrile was added, and the samples were extracted for 0.5 h using a laboratory shaker (Type 358S, Lubawa, Poland). Thereafter 4 g of magnesium sulfate and 1 g of sodium chloride were added, and the tube was shaken for 1 min followed by centrifugation (10 min, 4500 RPM; Centrifuge 5430R, Eppendorf AG, Hamburg, Germany). The organic upper layer (2 mL) was collected into a clean tube and shaken with 0.1 g of Bondesil-C18 and 0.3 g of magnesium sulfate for 2 min followed by centrifugation (10 min, 4500 RPM). Then 1.5 mL of purified supernatant was transferred into an autosampler vial with insert and spiked with different concentration of tested compounds prior to injection on LC-MS system.

2.3 Instrumental parameters

2.3.1 Agilent 6495 triple quadrupole

Samples were analyzed on an Agilent 1290 ultrahigh performance liquid chromatograph (Agilent Technologies, CA, U.S.) coupled to an Agilent 6495 triple quadrupole instrument (QQQ) in University of Tartu (UT). Samples were injected onto an Agilent Zorbax RRHD SB-C18 reversed-phase column (1.8 μ m, 2.1 $\times$ 50 mm). The mobile phase consisted of water (A) containing 0.1% formic acid and acetonitrile (B). The analysis was done using a gradient elution at a flow rate of 0.3 mL/min at 30 $^{\circ}$ C. The gradient was from 5% to 100% of acetonitrile in 7 min, then acetonitrile was maintained at 100%

for 2 min and returned back to 5% of acetonitrile in 2 min, and maintaining starting conditions at 5% of B for 2 min equilibration with 5% of B to giving a total runtime of 13 min.

An Agilent's Jet Stream electrospray ionization (ESI) interface was used in positive ion mode (ESI+) with the following settings: capillary voltage 3 kV, nebulizer pressure 20 psi, sheath gas flow rate 11 L/min, sheath gas temperature 350 °C, dry gas temperature 250 °C, and dry gas flow rate 14 L/min. Spectra were collected from m/z 100 to 1100 Da. The injection volume was 1 µL. Peaks were manually integrated, and peak areas were used for all calculations.

2.3.2 Bruker Daltonics micro-TOFq

Samples were also analyzed on an Agilent 1200 HPLC (Agilent Technologies, CA, U.S.) coupled to a Bruker Daltonics micro-TOFq (QToF) mass spectrometer (Bruker Daltonics, Bremen, Germany) in the lab of Technical University of Denmark (DTU). Samples were injected onto a Nucleoshell C18 reversed-phase column (2.7 µm, 100 × 2 mm, MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). The mobile phase consisted of water (A) containing 2.5 mM ammonium formate at pH = 3.0, and acetonitrile (B). The analysis was done using a gradient elution at a flow rate of 0.3 mL/min at 40 °C: isocratic at 2% of B for 1.0 min, then increased to 100% of B at 22.0 min, and maintained to 25 min, whereafter the gradient returned to starting conditions 2% of B at 25.1 min followed by 2.9 min equilibration with 2% of B to giving a total runtime of 28 min.

ESI+ was used in the following settings: capillary voltage 3 kV, nebulizer pressure 20 psi, dry gas temperature 200 °C, and dry gas flow rate 8 L/min. Funnel radio frequency (RF) 400 Vpp, multipole RF 200 Vpp. Spectra were collected from m/z 100 to 1100 Da at a rate of 2 Hz.

2.4 Matrix effects, LoD, LoQ

The matrix effect (ME) was calculated with the following formula in Eq. 1:

$$\%ME = \left(\frac{Slope_{matrices}}{Slope_{solvent}} \right) \times 100\% \quad \text{Eq. 1}$$

The calibration curves were prepared as the peak area versus the concentration of each analyte. $Slope_{matrices}$ is the slope of the calibration curves of analytes spiked into the cereal samples, $Slope_{solvent}$ is the slope of the calibration curves of analytes into the solvent.

$$LoD = 3.3 \times \frac{S_{residuals}}{slope} \quad \text{Eq. 2}$$

$$LoQ = 10 \times \frac{S_{residuals}}{slope} \quad \text{Eq. 3}$$

Detection of limit (LoD) and detection of quantification (LoQ) were calculated based formula Eq. 2 and Eq. 3 (Evard, Kruve, & Leito, 2016), $S_{residuals}$ is the residual standard deviation of the calibration curve of each compound in the LoD region, and the *slope* is the slope value of the calibration curve of the analytes.

2.5 Model application.

Previously developed Quantem model for predicting electrospray ionization efficiencies ($\log IE$, predictions are made in the logarithmic scale) together with the transformation approach (J. Liigand et al., 2019) was used to estimate the concentration of the compounds for the results of both labs. For this empirical solvent descriptors (viscosity, surface tension, polarity index, pH, NH_4 presence) and 1444 PaDEL molecular descriptors were used for the model development (Yap, 2011). Solvent descriptors were calculated based on the mobile phase used in the study and the retention time of each compound. PaDEL descriptor calculator (Dong et al., 2015) was used to calculate the molecular descriptors of all the compounds (http://www.scbdd.com/padel_desc/index/). For the transformation, the same compounds as described previously (J. Liigand et al., 2019) were used.

For transferring the predicted ionization efficiency values, a set of compounds (either spiked to the matrix or as a standard solution) was measured in the linear range at the beginning and the end of the sequence with the same method as analytes. From the analysis results, logarithmic response factors ($\log RF$) were calculated for transformation compounds as Eq. 4:

$$\log RF = \log \frac{Signal \cdot IC}{C_{spiked}} \quad \text{Eq. 4}$$

where *Signal* is the MS1 peak area and *IC* is an isotopic correction, which is the sum of the abundances of all isotopomers for each compound. Molar concentrations are used for calculation of response factor.

Next ionization efficiencies were predicted using the Quantem model (J. Liigand et al., 2019). The generic ionization efficiency values predicted with Quantem model need to be transferred to the instrument and method-specific response factors. To obtain this the logarithmic response factors of transformation compounds were correlated with predicted ionization efficiencies of the same compounds. The predicted logarithmic response factors of analytes were obtained using Eq. 5:

$$\log RF_{pred} = Slope \times \log IE_{pred} + intercept \quad \text{Eq. 5}$$

where slope and intercept values are determined from the abovementioned correlation graph of logarithmic response factors of transformation compounds vs their predicted ionization efficiencies. As analysts are mainly interested in concentrations, the concentrations are calculated from predicted response factors of analytes according to Eq. 6:

$$C_{pred} = \frac{Signal \cdot IC}{10^{\log RF_{pred}}} \quad \text{Eq. 6}$$

where *Signal* is MS1 peak area, and *IC* is an isotopic correction, which is the sum of the abundances of all isotopomers for each compound. As a result, molar concentrations are obtained.

To estimate the accuracy of prediction the absolute times differences of spiked concentration and predicted concentration in both cereal matrices as well as in pure solvent were calculated as Eq 7.

$$\text{Prediction error} = \max \left\{ \begin{array}{l} \frac{C_{pred}}{C_{spiked}} \\ \frac{C_{spiked}}{C_{pred}} \end{array} \right\} \quad \text{Eq. 7}$$

a

3. RESULTS AND DISCUSSION

3.1 Predicting Ionization Efficiency Values

The Quantem model (J. Liigand et al., 2019) developed previously, was used to predict the ionization efficiencies of the studied compounds. These values were transferred to instrument-specific response factors ($\log RF$) with the aid of Eq 4 and good transformation accuracy was observed both on QQQ in UT ($R^2 = 0.77$) and QToF in DTU ($R^2 = 0.66$).

The transformation compounds are used for transferring the ionization efficiency values into instrument-specific values and should be measured under conditions as close as possible to the actual sample analysis. Therefore, it is of interest if the transformation compounds need to be spiked into the sample or can be analyzed as a separate mixture within the same analysis sequence. To test this transformation compounds were spiked into two matrices (barley and oat) and were run as a separate standard solution. The concentration prediction accuracy for 109 randomly selected pesticides were compared. It was seen that if the model transferring compounds were spiked into the matrices, the mean errors changed only slightly from 3.8 to 3.9 times, thus there is hardly any difference (Table S2). Therefore, it is feasible to transform the predicted ionization efficiency values by measuring the transformation compounds in the standard solution instead of spiking into specific matrices.

Figure 1 Correlation between the predicted and measured concentrations for the pesticides in solvent (QQQ in UT).

3.2 Ionization Efficiency and concentration prediction in solvent and matrices.

3.2.1 Concentration prediction in a solvent on QQQ

In order to determine the applicability of this Quantem model for a wider range of compounds, a set of pesticides and mycotoxins with varying chemical nature was chosen and measured. The studied set on QQQ includes 139 common pesticides from different compound classes, such as amide, anilide, ether, pyrimidine, ketone. Each pesticide was studied at 6 concentration levels covering more than 4 orders of magnitude in both matrices and solvent. The spiked concentrations ranged from 10 nM to 35 μ M. The MRL for pesticides in cereal samples is 0.01 to 0.02 ppm. This corresponds to a pesticide concentration of $\sim$ 100 nM, which is in the lower end of the studied concentration range.

The predicted $\log IE$ values (Table S1) for all the tested compounds varied from 1.3 to 4.5. From the prediction of concentration in pure solvents, we found that for 48% of compounds, the concentration prediction error is better than 2 times, for 87% of compounds better than 5 times, and for 98% of studied compounds better than 10 times. The highest prediction error of concentration was 16 times. The 3 best-performing pesticides were imazamox, tetraconazole, and metrafenone (1.1 times for all). It is evident that the model is universal for both nitrogen as well as for oxygen bases, as among the top performers are both nitrogen and oxygen bases. Unsurprisingly, compounds (altogether 4 compounds, e.g., propiconazole) included previously in developing the $\log IE$ prediction model show high performance (J. Liigand et al., 2019). The correlation between measured and predicted concentration values in the solvent is presented in Figure 1; it can be seen that the agreement between measured and predicted concentration of test compounds by the model was good, the average accuracy was 3.0 times.

Comparing the different concentration levels, the accuracy was consistent except for the lowest concentration level, where it is only slightly lower (4.1 times).

3.2.2 Concentration Prediction in Matrices on QQQ.

The performance of Quantem model for prediction of $\log IE$ values for 6 cereal matrices was evaluated for the analyses of the same compounds at the same concentration levels as in solvent. Altogether, 6 concentration levels from 10 nM to 35 μ M were used. The detail prediction error results in different matrices were shown in Table S3. It was found the mean errors of concentration mismatch between predicted and measured values in all cereal matrices was 3.8 times (Figure 2), the average concentration prediction error for each cereal matrix varied from 3.3 times to 4.6 times. In general, higher prediction accuracy was observed for wheat than for the other matrices (prediction error of 3.3 times), oat performed worst (4.6 times). Comparing all different concentration levels (Table S5), the prediction

error of the lowest concentration level was slightly higher (4.4 times). For 46% of compounds, the concentration prediction error was less than 2 times, for 81% of compounds less than 5 times, and for 94% of compounds less than 10 times.

Figure 2 Correlation between the predicted and measured concentrations for pesticides in 6 cereal matrix extracts. The measurements were performed on the QQQ instrument in UT.

The concentration prediction accuracy of carbosulfan was most severely influence by matrices (prediction error 64.6 times), which had the highest difference while compared to prediction accuracy in solvent (3.9 times). The reason of carbosulfan's poor performance could be that it contains the N-S-N moiety which was not present in the training set of the Quantem model. On the other hand, carbosulfan is unstable, it decomposes slowly at room temperature especially in acidic organic solvents (Barros & Barros, 1995; Leppert, Markle, Helt, & Fujie, 1983). The QuEChERS sample preparation used in this study uses 0.2% formic acid in the sample extraction procedure. We also observed that the degradation of carbosulfan results in poor linearity in matrices. It can be found that carbosulfan in oat matrix results in the largest concentration prediction error of 173.8 times. Carbosulfan was followed by fluometuron (15.9 times), pirimicarb (15.7 times), pyridaben (15.4 times), and pyridate (14.1 times) on average in all matrices.

3.2.3 Matrix effect

The results are influenced by the matrix effect: the ionization suppression due to the matrix compounds co-eluting with the analyte. The most severe matrix effect was observed in oat matrix, the matrix effect was in the range of 2% (suppressed by 98%) to 109% (enhanced by 9%) for all compounds relative to the pure solvent (Table S7). In comparison to other cereals, oat is a rich source of polar lipids and free fatty acids. It has been discussed that phospholipids and free fatty acids will cause matrix effects in ESI (Krasilnikov, Batalova, Popov, & Sergeyeva, 2018; Pucci, Di, Alfieri, Bonelli, & Monteagudo, 2009), which can explain the severe matrix effect found in oat. The matrix effect may vary with analyte concentration (Furey, Moriarty, Bane, Kinsella, & Lehane, 2013; Kruve, Auling, Herodes, & Leito, 2011).

The concentration prediction errors were in general consistent for most compounds in both solvent and matrices, which means that the matrix effect does not influence the prediction accuracy significantly and the transformation step help to account for the matrix effects. For example, fluometuron, pyridaben, pirimicarb, pirimicarb-desmethyl, EPN, and thiamethoxam, had low prediction accuracy in both solvent and matrices. For some compounds, specific matrices caused a notable decrease in prediction accuracy. Carbosulfan and pyridate suffered the most severe matrix effects, carbosulfan was suppressed 98% and 95% in oat and maize matrices (concentration prediction error of 173.8 times and 55.5 times), respectively. Signal of pyridate was suppressed 87% and 73% in oat and maize matrices (concentration prediction error of 20.3 times and 24.1 times), respectively. Signal of benfuracarb was suppressed 58% in the oat matrix, which results in 5.9 times concentration prediction error. Spirodiclofen and pyridaben were also suppressed 53% and 47% in the matrices, resulted in concentration prediction error of 10.3 times and 15.4 times on average.

3.2.4 Concentration Prediction in Matrices on QToF

In order to test the robustness of the Quantem model and the transferability of the ionization efficiency prediction model between labs, the same matrices spiked at the 6 concentration levels from 4 nM to 11 μ M with QToF in DTU. The ionization efficiency prediction and transformation approach were the same as in the case of QQQ instrument. There were 134 pesticides in common that could be

271 detected and quantified both in DTU and UT. After model transformation, the predicted $\log IE$ values
272 (Table S4) for all the tested compounds on QToF varied from 1.6 to 4.5.

273 As shown in Figure 3, the concentration prediction accuracy was even better on QToF in DTU. The
274 average concentration prediction errors for all compounds was 2.4 times. For 61% of the compounds, the
275 prediction error was better than 2 times and for 94% of compounds better than 5 times. These values are
276 reasonably close to the ones obtained on the QQQ instrument in UT and described above. The average
277 concentration prediction errors were 2.3 times in solvent and 2.6 times in all matrices. The prediction
278 errors in different matrices were in the range from 2.5 to 3 times, which is quite closed. Rice and rye
279 performed slightly better than other matrices. The concentration prediction errors were still in general
280 consistent for most compounds in solvent and matrices. As the QToF instrument used for analysis in
281 DTU is high-resolution mass spectrometry, the ability to distinguish the molecules with close MS weight
282 is better compared to QQQ; also, the total running time for each sample in DTU is longer than the running
283 time in UT which leads to a better separation on HPLC part. As a result, the integration of the compounds
284 on QToF was influenced less by other compounds and the matrices background than QQQ. This might
285 explain the slightly better performance of the concentration prediction for the data in DTU than UT.

286 On the whole, the quantitation of pesticides in cereal matrices with LC/ESI/MS without standard
287 substances is feasible to achieve the prediction error of less than 4 times on average both on QToF in
288 DTU and QQQ in UT.

Figure 3 Correlation between the predicted and measured concentrations for pesticides in 6 cereal matrix extracts. The measurements were performed on QToF instrument in DTU.

3.2.5 Comparison of the predicted concentrations between labs.

Moreover, after applying Quantem model we can also directly compare the concentrations obtained both in UT and DTU. The methods applied in UT and DTU are intrinsically different. These use slightly different chromatography, different mobile phase, different ESI source as well as different mass analyzers and vendors. As a result, the chromatograms obtained in both labs are not comparable. However, as the Quantem model translate these chromatograms into quantitative data for pesticides and the results can be numerically compared. Here we observed a very high correlation for the results from UT and DTU ($R^2 = 0.85$). Also, the slope and intercept of the correlation line were very close to the ideal fit, the slope was 0.95 while the ideal would be 1. The results are shown in Figure 4. From this, it is obvious that for the first time the measurement results from two different laboratories can be made quantitatively comparable for suspect screening without the necessity of standard substances. This finding is extremely valuable for applications in both food monitoring and environmental areas.

Figure 4 Correlation between the predicted concentrations for pesticides in 6 cereal matrices measured on QQQ at UT and on QToF at DTU.

3.2.6 LoD and LoQ

In spite of the generally good correlation between the predicted concentrations for the analysis of two different labs (UT and DTU), some of the compounds were detected only by one of the labs. In the case of interlaboratory comparisons, such differences often occur and commonly indicate different LoD and LoQ values. Therefore, we compared the estimated concentrations of the compounds detected by both labs with the concentrations of the compounds detected only in DTU with QToF instrument. The comparison revealed that most of the compounds not detected by UT were having among some of the lowest concentrations (Figure 5). Therefore, the comparison for the concentration determined from different labs has the potential to bring clarity to the discrepancies often observed in collaborative trials of non-targeted screening.

To further validate the conclusion, we determine the LoD values of all of the studied compounds. This was possible as the concentrations of the pesticides were known from the spiking experiments. A conservative LoD determination strategy was used (Evard et al., 2016). It was observed that the LoD values ranged between 0.1 nM and 1 μ M, while most values were between 1 nM and 0.1 μ M. The LoD and LoQ values are brought in Table S9. The comparison of the determined LoD values for UT and DTU, in Figure S1, revealed that different compounds had varying LoD values between different labs. Sometimes the LoD was lower in UT, sometimes in DTU.

Figure 5 The predicted concentrations of pesticides for compounds detected in both laboratories vs detected only in DTU. The results are summarized over all studied matrices.

Conclusion

Here we have shown, for the first time that results obtained from suspect screening can be made quantitatively comparable between different laboratories using different instruments as well as different analysis methods. To achieve this, we use the ionization efficiency prediction with Quantem model to transfer the obtained signals into concentrations. In contrast to signals, the concentrations are comparable between different instruments and labs. The consistency between the results obtained independently in two labs was very high ($R^2 = 0.85$) in spite of the differences in the instrumentation and methods. Also, the independent results show high accuracy. The quantitation of pesticides in different cereals matrices without standard substances is feasible with the accuracy of less than 4 times on average for chromatographic analyses. It can serve as a useful tool for preliminary quantitation in various fields such as food control, metabolomics, and environmental research, and there is a significant improvement compared to the conventional approach. Also, the obtained accuracy is sufficient for using the standard substance-free quantitation as the first step in food monitoring.

SUPPORTING INFORMATION DESCRIPTION

Supplementary data related to this article can be found in the Supporting Information file.

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T.W. carried out all the measurements, planned the experiments and wrote a big part of the manuscript. J.L. carried out all the ionization efficiency modelling and concentration prediction and helped writing the manuscript. J.S. and H.L.F. helped to plan and analyze data related to pesticide analysis. A.K. designed the project and helped to plan the experiments and modelling and helped writing the manuscript.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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468 Highlights

469 Six cereal matrices containing 134 pesticides (10 nM to 35 μ M) were analysed

470 Measurements were done on QQQ and ToF instruments in two independent laboratories

471 Pesticide are quantified with predicted electrospray ionization efficiencies

472 Quantitative results obtained in two labs were consistent with the R^2 of 0.85

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