Validation of a multi-residue LC-MS/MS method for the determination of pesticide residues in cereals and feeding stuff

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INTRODUCTION

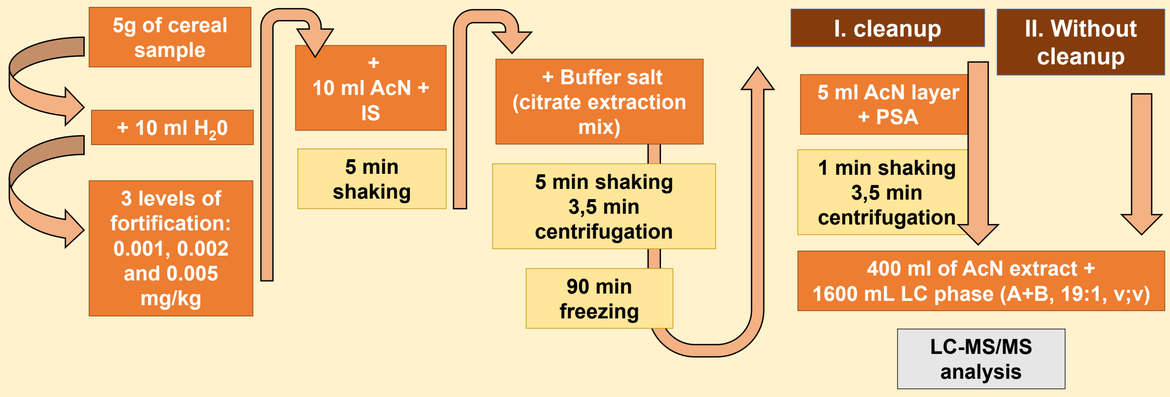
Monitoring of plant protection products residues in food of plant origin and feeding stuffs is important in ensuring food safety.

The aim of the research was to validate a multi-residue method for simultaneous determination residues of hundreds pesticides as well as their metabolites at low levels - 0.001 mg/kg and 0.002 mg/kg in cereals and oil seeds, taking into account various sample cleanup, with the use of liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).

MATERIALS AND METHODS

The method validation was performed on blank samples of wheat and barley grains and soybeans. The appropriate volume of the reference mixture of 428 pesticides was used to spike blank samples at 2 fortification levels: 0.001 mg/kg and 0.002 mg/kg. Experiments were performed in 6 replicates for each level.

Samples were extracted by QuEChERs method [1]. The validation was carried out according to the SANTE/12682/2019 guideline [2].



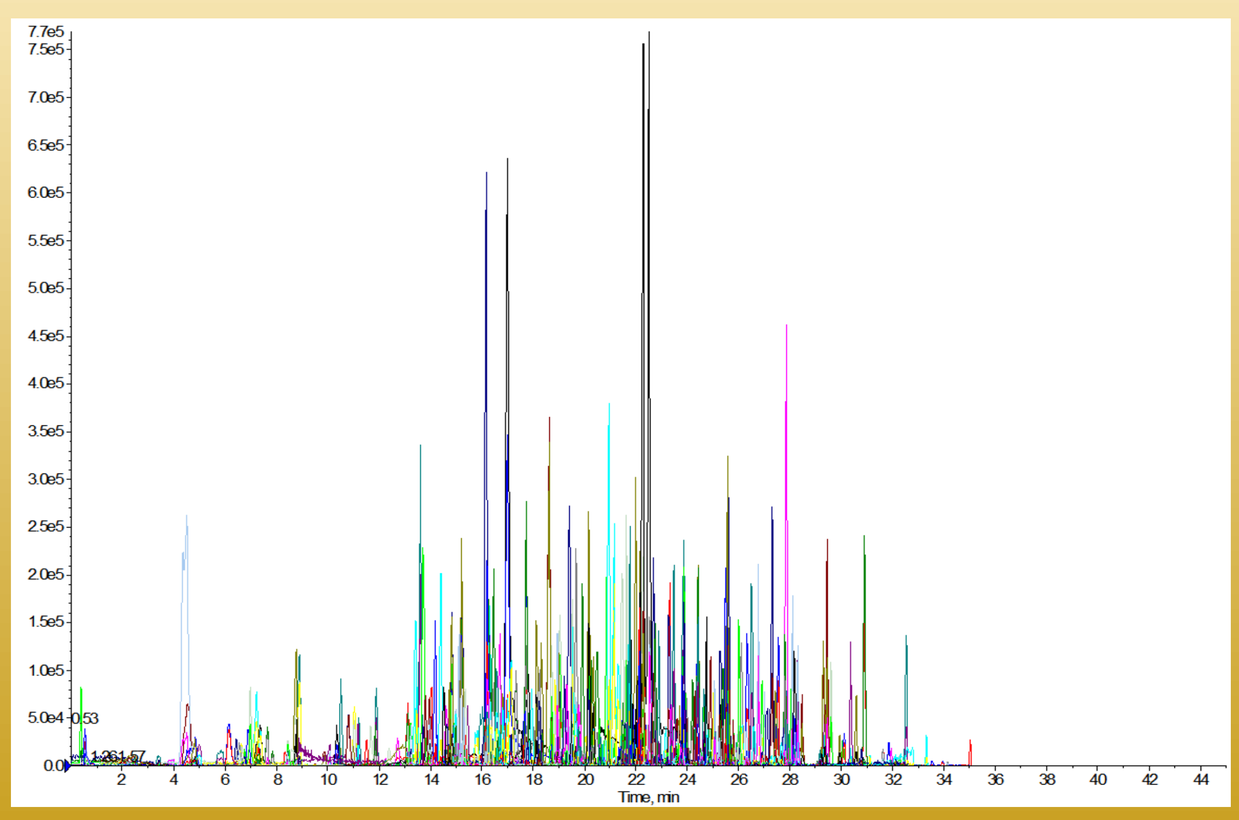
Graph 1. Procedure od QueChERs extraction

LC-MS/MS technique using multiple reaction monitoring (MRM) method enabling the detection of 428 compounds at the same time was used to measure the content of pesticide residues samples. The set consisting of Eksigent liquid chromatograph expert ultraLC 100-XL coupled with a quadrupole mass spectrometer Qtrap 6500 (Sciex) was used for qualitative and quantitative measurements. Separation of analytes was carried out using a mobile phase gradient system on a Kinetex C18 chromatography column (100 mm x 2.1 mm, 2.6 µm). The multi-point calibration in solvent in the range of 0.05-5 ng/mL was used for quantitative determinations.

INSTRUMENTAL METHOD PARAMETERS

Mass spectrometer (AB SCIEX QTRAP 6500)
Detection Type: ESI+, ESI-
Curtain gas: 30 L/min
5000V ion source voltage
Evaporation temperature: 400°C
Injection volume: 10 µl.
Software: Analyst Software version 1.6.2, MultiQuant Software version 3.0.2

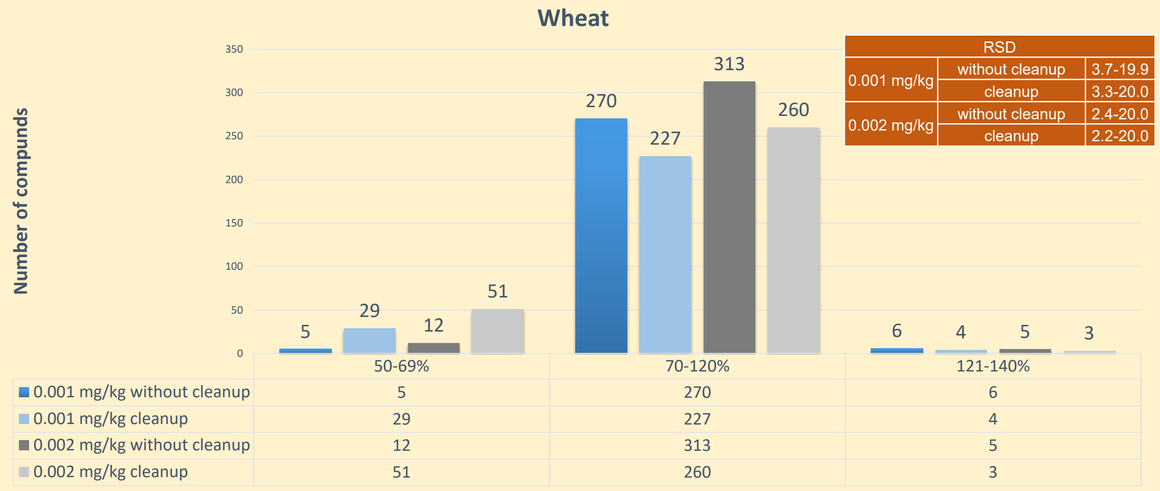
Liquid chromatograph (Eksigent expert ultraLC 100-XL)
Column: Kinetex C18 (100 mm x 2.1 mm, 2.6 µm),
temp. 40°C
Mobile phase:
Phase A: 0.1% solution of formic acid in water with the addition of 5 mmol of ammonium formate;
Phase B: 0.1% solution of formic acid in methanol with the addition of 5 mmol of ammonium formate.
Mobile phase flow 0.5 ml/min, gradient system.
Analysis time: 45 min

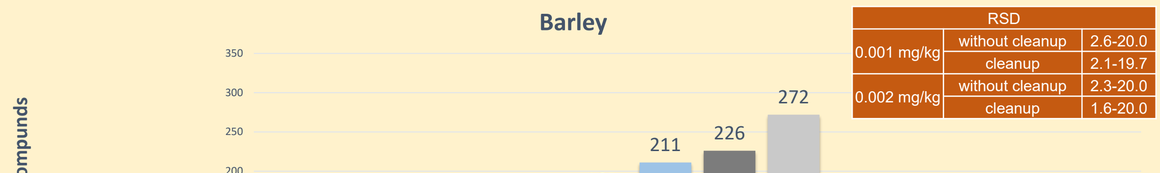


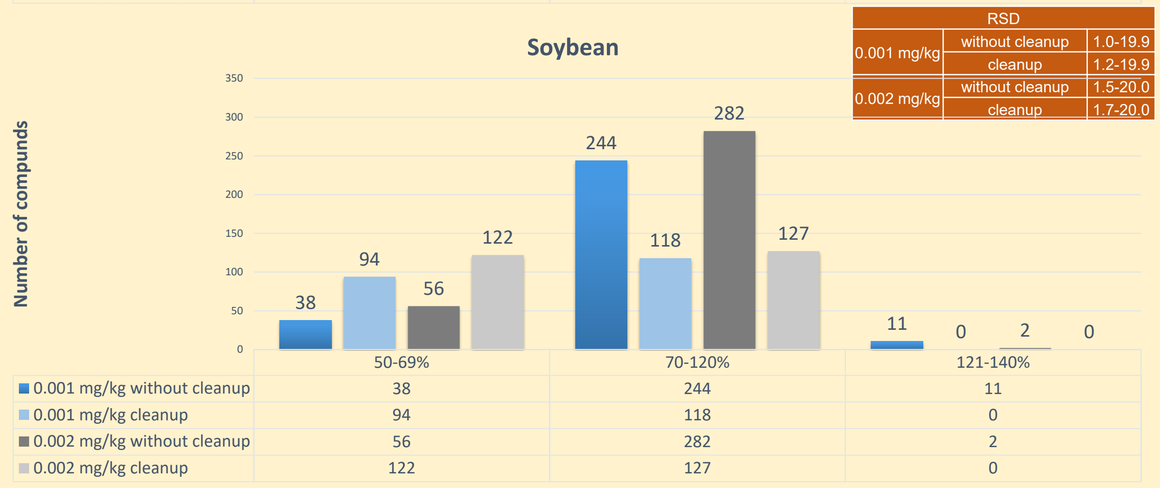
Graph 2. Extracted ion chromatogram.(MRM transitions in positive polarity)

RESULTS

The method validation parameters were assessed in accordance with the requirements of the SANTE/12682/2019 guide [2].
The majority of compounds were in the 70–120% recovery range and were characterised by precision lower than 20%. All calibration curves were linear with a correlation coefficient greater than 0.995.







Graph 3. Number of compounds validated at specific recovery range and fortified level

Of all pesticides tested, in the wheat samples without cleanup fortified at 0.001 mg/kg the recoveries for 270 compounds were in the range of 70-120%, while in the cleanup samples for 227. At the level of 0.002 mg/kg the number of compounds with recoveries in the above range was 313 and 260 respectively.

In the barley samples without cleanup fortified at the level of 0.001 mg/kg recoveries for 165 compounds were in the range of 70-120%, while in the cleanup samples for 211. At the level of 0.002 mg/kg the number of compounds with recoveries in that range was 226 and 272 respectively.

In the soybean samples without cleanup that were fortified at the level of 0.001 mg/kg recoveries for 244 compounds were in the range of 70-120%, while in the cleanup samples for 118. At the level of 0.002 mg/kg the number of compounds with recoveries in the above range was 282 and 127 respectively.

In all of the above tests for the individual fortification level the precision never exceeded 20%.

CONCLUSIONS

- For wheat as well for soybean the higher number of recoveries in the range of 70-120% were found for samples without cleanup comparing to cleanup for both tested fortification levels and the significantly big difference was observed for soybean (> 50%). The recoveries of pesticides in the soya bean were in the range of 50-60% for lots of compounds. In barley fortified at the levels of 0.001 mg/kg and 0.002 mg/kg, the number of recoveries in the range of 70-120% was lower for samples without cleanup than for cleanup samples. The high number of recoveries over 120% was found for samples not treated with PSA.
- Cleanup with PSA resulted in losses in the recoveries of the pesticides in wheat and soybean, while in barley not used of PSA resulted in increasing number of recoveries above the range of 120%.
- The limit of quantification (LOQ) was set up at the level of 0.001 mg/kg in wheat for 270 or 227 compounds, in barley for 165 or 211 compounds and in soybean for 244 or 118 compounds depending used procedure.

REFERENCES

[1] EN 15662:2018. Foods of plant origin – Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE – QuEChERS-method.
[2] SANTE/12682/2019 guidance document on analytical quality control and method validation procedures for pesticide residues analysis in food and feed.