

EUROPEAN UNION REFERENCE LABORATORY

PESTICIDE RESIDUES IN CEREALS & FEEDING STUFF



Appendix 4

EURL for Cereals and Feeding stuff National Food Institute Technical University of Denmark

Screening Validation Report 1

Screening of pesticide residues in cereals by UPLC-TOF

(QuEChERS method)

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1. Introduction

Qualitative multi residue methods, especially those involving automated MS-based detection, offer laboratories a cost-effective means to extend their analytical scope to analytes which potentially have low probability to be present in the samples. The more commonly occurring analytes should continue to be sought and measured using quantitative MRMs.

This report describes the screening validation of the QuEChERS method combined with UPLC-TOF MS. The method was validated for 35 pesticides in cereals at the screening detection limit, 0.01 mg/kg.

The QuEChERS method has an extraction and clean-up step, which has been developed to be Quick, Easy, Cheap, Efficient, Rugged and Safe. The method is most commonly used on fruit and vegetables¹.

The method validated here is based on the procedure for dry matrixes (<30% water content) according to the document CEN/TC 275/WG 4 N 0204 (CEN document). Even though cereals have a fat content of about $2\%^2$ no attempt has been made to remove the fat from the extract, e.g. freezing out as proposed in the CEN document, since no problems caused by fat has been observed.

2. Principle of analysis

Cold water/ice water, acetonitrile and an internal standard are added to the milled sample. The sample is shaken and a salt and buffer mixture is added and the sample is shaken again. After centrifugation the supernatant is transferred to a tube with PSA and MgSO₄. After shaking and an additional centrifugation step the final extract is obtained.

Screening:

Different cereal samples were spiked at 0.01 mg/kg with a mix of pesticide standards (Dr. Ehrenstorfer), extracted by and analysed by UPLC-QTOF (UPLC: Dionex RSLC; TOF: Bruker Daltronics, MaXis).

LC conditions:

Column: Acclaim RSLC C18 2.2m 2.1x100mm (Dionex) Eluents: ESI(+): A: H₂O/MeOH 90/10 (v/v), B: MeOH (both contain 5mM

NH₄formate, 0.01% HCOOH)

Gradient and flowrate: see figure 1

Injection volume: 1µl

Runtime: 20 min

Temperature: 30°C

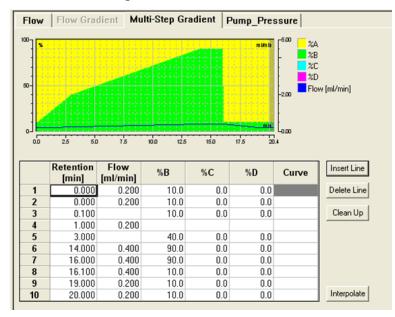


Figure 1: Figure and table of the multistep gradient used

TOF conditions:

TOF instrument: Bruker Maxis QTOF (only operated as TOF)

Ionisation mode: ESI positiv

Calibration was performed externally prior to a sample series with a sodium formate solution, and additionally internally for each chromatogram by injecting the calibrant at the beginning of each run.

Database:

Automated target detection for the pesticide standard mix spiked to cereal extracts can be achieved by automated peak detection on the EICs expected for the $[M+H]^+$, $[M+NH_4]^+$ or $[M+Na]^+$ ions of each compound in a database. A database containing names, sum formulas, exact masses and retention times for 100 pesticides was set up. Based on accurate mass and known retention times the compounds present in the sample are identified. For each identification candidate additionally the theoretical isotope abundance pattern is compared to the experimental obtained isotopic pattern.

3. Validation design

The method was sought validated for 38 pesticides in cereals. The validation was performed on 20 replicates, and blank material was spiked at the expected screening reporting limit (SDL), which in this case was 0.01 mg/kg. The validation was performed on wheat, rye, oat, barley (5 replicates each).

4. Chromatograms

Examples of a chromatogram obtained when analysing the extracts by UPLC-TOF are presented in figure 2. The chromatogram show the base peak chromatogram (BPC) overlaid with extracted ion chromatograms (EIC) of the compounds detected from the database. Below the chromatogram all detected traces with accurate mass measurements are shown (the list was cut off for fitting purposes).

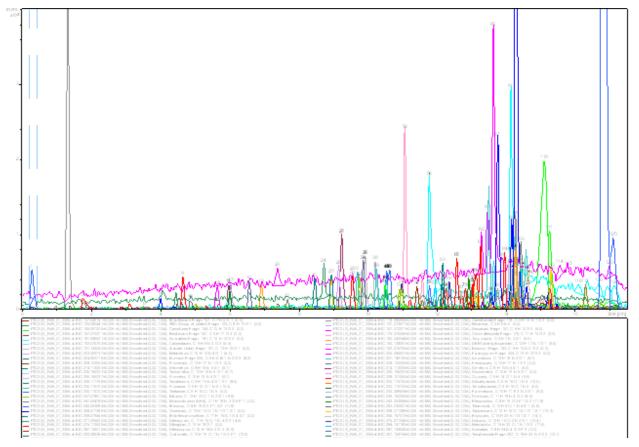


Figure 2: Chromatogram showing both base peak chromatogram overlaid with extracted ion chromatograms of the compounds detected from the database. The accurate mass of all the peaks detected.

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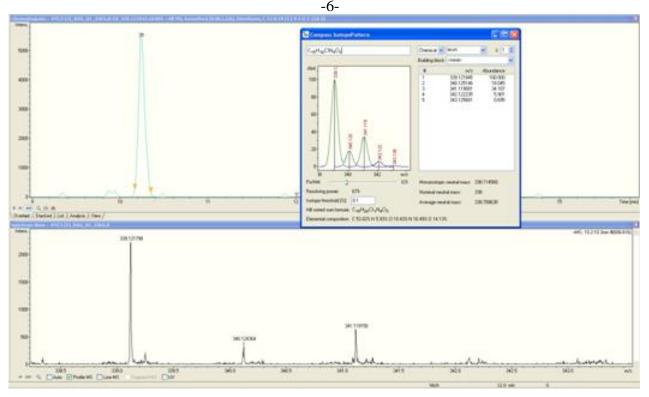


Figure 3: Top: Extracted ion chromatogram of the selected peak Bottom: Measured isotope pattern from the selected peak Small box right upper corner: Theoretical isotope pattern (from molecular formula)

For each extracted ion chromatogram a peak can be isolated and the measured isotopic pattern can be investigated, as shown in figure 3. The measured isotopic pattern may be compared/matched to the theoretical isotopic pattern for the given pesticide. The match is measured and if the given criteria are fulfilled, the probability for the correct formula is high.

5. Criteria for the acceptance of validation results

Screening Detection limit, LDL

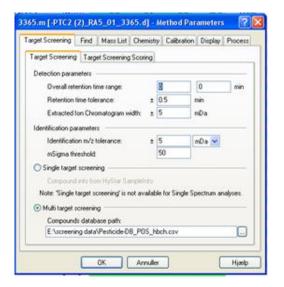
The screening detection limit (SDL) of a qualitative screening method is the lowest concentration for which it has been demonstrated that a certain analyte can be detected in at least 95% of the samples (i.e. a false negative rate of 5% is accepted)³.

Criteria for searching the database

For identification the database set up for pesticides was used. The probability of correct identification depend on which criteria's that are selected relating to the database search. Figure 4 is screen shot of the parameters selected for this validation. The mSigma value indicates a high probability of unequivocal identification. If the identification tolerance value is below 50 it indicates a high probability of correct formula. If the values from the screening report are above 50

and 5mDa respectively, the inputs are checked manually. Retention time is required for the attribution of the individual isomers.

Retention time tolerance: 0.5 min Identification tolerance: 10 mDa. mSigma threshold < 100



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Figure 4: Parameters for the detection with database

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Figure 5: List of detected compunds form the database listed according to the degree of identification

When the software has finish the database searching, a table with identified compounds is shown, see figure 5. The table includes how well the compounds matches the measured accurate mass,

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retention time and isotopic pattern compared to the theoretical, and rates the degree of unequivocal identification of the compound.

6. Results and discussion

The 81 pesticides sought validated presented in table 1. Hereof 35 pesticides were accepted for validation at 0.01 mg/kg, here the compounds were detected in at least 95% of the samples, see table 1.

It was tested whether the criteria were set to strict, and if more pesticides would be accepted with less strict criteria. Changing the criteria as intensity and area threshold did not affect the identification, however setting the accurate mass and msigma value less strict allowed for a few additional pesticides to be validated.

Compounds	No. detected	% detected
3-hydroxy carbofuran	12	60%
Bensulfuron-methyl	17	85%
Bromacil	1	5%
Butralin	7	35%
Buturon	20	100%
Carbaryl	5	25%
Carbendazim	20	100%
Chlorbromuron	17	85%
Chlorotoluron	19	95%
Chlorsulfuron	7	35%
Chromafenozide	1	5%
Crufomate	20	100%
Cyanazine	1	5%
Cyprazin	19	95%
DEET (diethyltoluamide)	19	95%
Desmetryn	20	100%
Difenoxuron	20	100%
Diflubenzuron	9	45%
Dimefuron	20	100%
Dimethachlor	9	45%
Dinotefuran	7	35%
Dioxacarb	5	25%
Esprocarb	18	90%
Ethoprop (Ethoprophos)	20	100%
Etofenprox	13	65%
Fenhexamid	11	55%
Fenothiocarb	17	85%
Flonicamid	20	100%
Flumetsulam	19	95%
Fluometuron	20	100%
Forchlorfenuron	11	55%
Formetanate	1	5%
Furalaxyl	19	95%
Furathiocarb	20	100%
Imazalil	20	100%
Imazamox	13	65%
Imazapyr	9	45%
Imazaquin	17	85%
Imidacloprid	9	45%

Table 1: Compounds sought validated for screening in cereals. The pesticides accepted are marked with pale green.

_	9	_

Isopropalin	2	10%
Isoproturon	17	85%
Isoxathion	19	95%
Linuron	17	85%
Malaoxon	20	100%
Mefenacet	19	95%
Mepronil	18	90%
Metacriphos	1	5%
Metamitron	19	95%
Methoprene	11	55%
Metolachlor	18	90%
Metosulam	3	15%
Monuron TCA	19	95%
Neburon	19	95%
Nitenpyram	13	65%
Novaluron	3	15%
Orbencarb	12	60%
Oxadiargyl	8	40%
Oxadiazon	11	55%
Oxydemeton-methyl	20	100%
Paraoxon-methyl	20	100%
Profoxydim	14	70%
Prometon	19	95%
Propazine	20	100%
Pyraclostrobin	17	85%
Pyributicarb	20	100%
Pyrimidifen	14	70%
Quinoxyfen	20	100%
Sebuthylazine	20	100%
Simetryn	20	100%
Spiroxamine	20	100%
Tebuthiuron	20	100%
Terbumeton	20	100%
Thiacloprid	18	90%
Thidiazuron	9	45%
Thiobencarb	14	70%
Thiodicarb	2	10%
Thiophanate-methyl	13	65%
Tricyclazole	20	100%
Trifloxysulfuron	19	95%
Triflumizole	3	15%
Warfarin	10	50%

7. Conclusions

Only 35 compounds were validated at the expected screening detection limit at the criteria set for the database identification. It should be noted that the expected screening detection limit at 0.01 mg/kg may be too low for cereals, and should be 0.02 mg/kg instead. For future validation both levels should be tested. Similar validation was performed on fruit and vegetables, with spiking at SDL (0.01 mg/kg), here many more compounds were validated. This indicates that it should be taken into consideration if the SDL for cereals should be 0.02mg/kg.

For routine analysis non detects should be reported as <SDL mg/kg. If detected, a result can only be reported after confirmatory analysis using a quantitative method.

8. References

1 http://www.quechers.com/

2 The Composition of Foods – fourth edition by Erling Saxholt, Gyldendals, 1996.

3 Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and

Feed, Document N° SANCO/12495/2011, European Commission, Brussels, 2012.