Screening Validation on Pesticide Residues in Eggs Using LC-Q-TOF in ESI Positive Mode

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Abstract

The Agilent 6540 LC-Q-TOF has been successfully used by EURL-AO for the qualitative screening of pesticide residues in food of animal origin over the last years. Validation studies in honey, milk, meat or egg with established sample preparation methods like QuEChERS demonstrated the broad application area as well as the limits of the method.

For this study the Agilent 6545 LC-Q-TOF was used, which is a mid-range Quadrupole Time-of-Flight instrument, offering higher sensitivity and resolution compared to the legacy 6540 LC-Q-TOF. The instrument was used for a validation study including 158 selected LC-amenable pesticides spiked into different blank egg samples from the local market.

The Q-EMR-method developed in 2018 by EURL-AO was used for sample preparation. The Q-EMR was derived from the citrate buffered QuEChERS-AO method by including an optimized clean-up procedure using EMR lipid®. Especially the fat content of the extract is significant lower than in the QuEChERS-AO extracts. Thus, the benefit of the cleaner Q-EMR extract provides the opportunity to screen for LC and GC amenable pesticides using the same extract. This saves time and money.

The validation of the method was performed according to the document SANTE/11813/2017.

LC-Q-TOF Parameter

System:	Quadrupole Time of Flight 6545 LC/Q-TOF (Agilent Technologies)
Column:	Zorbax Eclipse Plus C18 2.1 x 150mm, 3.5µm
Flow rate:	0.3 mL/min
Injection volume:	5 µL
Ion source:	Dual AJS ESI in positve mode
High resoultion mode:	2 GHz Ext Dyn Range
Software:	Qualitative Analysis

Sample treatment for the validation of egg

158 analytes spiked at 5 different levels

Blank matrices
10 different samples of egg (barn eggs, eggs from organic farming, whole egg and free-range eggs)

5 spiking levels
0.001 mg/kg / 0.002 mg/kg / 0.005 mg/kg / 0.010 mg/kg / 0.020 mg/kg

Modified QuEChERS-AO extraction and clean up (Q-EMR)

Weigh in 5.0 g ± 0.1 g matrix

Spike with spiking solution and internal standards; wait for 10 min

Add 10 mL of water and shake for 10 min

Add 10 mL of acetonitrile and shake for 10 min

Add 3 g NaCl + 1 g trisodium citrate · 2 H_2O + 0.5 g disodium citrate · 6 H_2O, shake for 10 min

Centrifuge at 1500 g for 5 min

Clean 4 ml of supernatant using dSPE EMR Lipid, activated with 4 mL of water, shake for 10 min

Centrifuge at 1500 g for 5 min and transfer into Polish tube filled with $MgSO_4$ + NaCl

Shake for 10 min and centrifuge at 1500 g for 5 min

Analyze aliquote of supernatant by LC-Q-TOF

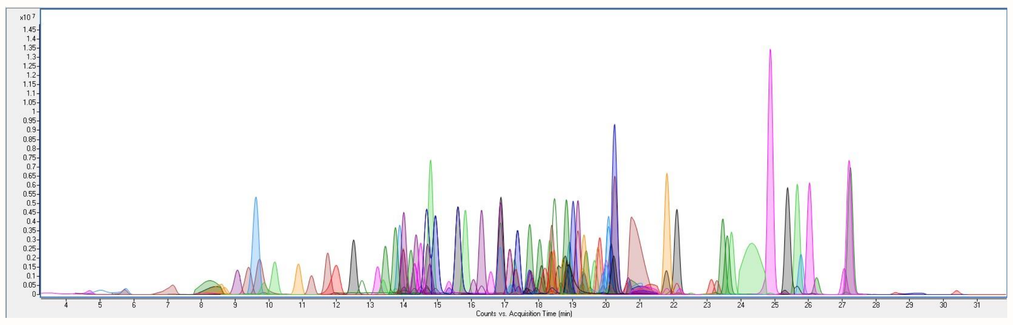

Figure 1: LC-MS-Q-TOF chromatogram of egg spiked with 158 analytes at 0.005 mg/kg

Analytes included in the validation study: 144

87 Pesticides with an obtained Screening detection limit of 0.001 mg/kg:
Avermectin, Acetamiprid, Acibenzolar-S-methyl, Ametryn, Amitraz, Azoxystrobin, Benzoximate, Boscalid, Bromuconazole, Buprofezin, Butafenacil, Carbetamide, Carbofuran, Carboxin, Chlorantraniliprole, Chloroxuron, Chlortoluron, Clofentezine, Clothianidin, Cycluron, Cyproconazole, Diclobutrazol, Diethofencarb, Difenoconazole, Dimoxystrobin, Diniconazole, Diuron, Doramectin, Epoxiconazole, Etaconazole, Ethiprole, Etoxazole, Fenamidone, Fenazaquin, Fenbuconazole, Fenhexamid, Fenoxycarb, Fenpyroximate, Flubendiamide, Flufenacet (Fluthiamide), Fluometuron, Fluoxastrobin, Forchlorfenuron, Fuberidazole, Furalaxyl, Hexaconazole, Imazalil, Imidacloprid, Indoxacarb, Ipconazole, Iprovalicarb, Isoproturon, Ivermectin, Mandipropamid, Mefenacet, Mepronil, Metconazole, Methoprotryne, Methoxyfenozide, Metobromuron, Metribuzin, Neburon, Nitenpyram, Nuarimol, Oxadixyl, Picoxystrobin, Promecarb, Propiconazole (Tilt), Propoxur (Baygon), Pyracarbolid, Pyraclostrobin, Quinoxyfen, Secbumeton, Siduron, Simetryn, Spiroxamine, Sulfentrazone, Tebufenozide, Terbumeton, Terbutryn, Tetraconazole, Thiacloprid, Thiamethoxam, Thiobencarb, Trifloxystrobin, Triticonazole, Zoxamide

35 Pesticides with an obtained Screening detection limit of 0.002 mg/kg:
3-Hydroxycarbofuran, Bendiocarb, Bupirimate, Carfentrazone-ethyl, Cyprodinil, Dioxacarb, Ethirimol, Fenarimol, Fenuron, Fluquinconazole, Flusilazole, Flutolanil, Flutriafol, Halofenozide, Hexythiazox, Kresoxim-methyl, Linuron, Mepanipyrim, Metalaxyl, Monolinuron, Myclobutanil, Paclobutrazol, Penconazole, Piperonyl butoxide, Prochloraz, Propargite, Pyridaben, Pyrimethanil, Pyriproxyfen, Tebuconazole, Tebufenpyrad, Thiabendazole, Triadimefon, Tricyclazole (Beam), Triflumizole

10 Pesticides with an obtained Screening detection limit of 0.005 mg/kg:
Chlorfluazuron, Clethodim, Cyazofamid, Flufenoxuron, Isoprocarb, Moxidectin, Pirimicarb, Pymetrozine, Rotenone, Thiofanox

3 Pesticides with an obtained Limit of Quantification/ Screening detection limit of 0.010 mg/kg:
Baycor (Bitertanol), Fludioxonil, Novaluron

3 Pesticides with an obtained Screening detection limit of 0.020 mg/kg:
Dinotefuran, Flonicamid, Prothioconazole

6 Pesticides with no detects under the conditions of the validation study:
Cymoxanil, Ethofumesate, Fenobucarb (BPMC), Hexaflumuron, Metaflumizone, Teflubenzuron

14 Pesticides with no detects under the conditions of the instrument method (excluded of the validation):
Bifenazate, Cyromazine, Diflubenzuron, Eprinomectin, Famoxadon, Fipronil, Fluazinam, Lufenuron, Mesotrione, Propham , Prometon, Prometryne, Triadimenol, Triflumuron

Results and Discussion

For most of the pesticides included in this validation study, MRL values are set by default at levels between 0.01 mg/kg and 0.05 mg/kg. 94% of the pesticides were identified at or below the MRL-level of 0.01 mg/kg. Thus, the presented validation of the screening method (cleanup: Q-EMR, detection: LC-Q-TOF) shows, that it is an appropriate tool to monitor LC-amenable pesticides in eggs.

114 analytes were part of the present validation as well as of a validation study in 2014 by EURL-AO using QuEChERS preparation and 6540 LC/Q-TOF detection (4 GHz mode). 6 analytes had the same screening detection limit (SDL, detection capability - ccß) with both methods, while 103 analytes (90%) showed a lower SDL in the present study. Just 3 analytes showed higher SDL (Flonicamid 0.01 mg/kg -> 0.02 mg/kg; Baycor 0.005 mg/kg -> 0.01 mg/kg and Dinotefuran 0.005 mg/kg -> 0.02 mg/kg) in the present study. Metaflumizone was not detectable under the conditions of the present study but validated in 2014 at a SDL of 0.01 mg/kg. On the other side Prothioconazole was validated at a SDL of 0.02 mg/kg applying the new method, only.

Under the perspective that pesticide analysis should be as fast, easy and cheap as possible and limit of detection/quantification should continuously be decreased, the shown method is a good alternative to the established methods.

	Number of pesticides validated at the SDL (Ratio of pesticides validated)						
Spiked concentration [mg/kg]	**0.001**	**0.002**	**0.005**	**0.01**	**0.02**	**0.04**	**Not validated**
Agilent 6540 and QuEChERS (Validation 2014 by EURL-AO)	-	-	179 (86%)	193 (92%)	201 (96%)	202 (97%)	7 (3%)
Agilent 6545 and Q-EMR	87 (60%)	122 (85%)	132 (92%)	135 (94%)	138 (96%)	138 (96%)	6 (4%)
	Pesticides included in both validation studies: 114						
Agilent 6540 and QuEChERS (Validation 2014 by EURL-AO)	-	-	105 (92%)	113 (99%)	113 (99%)	113 (99%)	1 (1%)
Agilent 6545 and Q-EMR	70 (61%)	101 (89%)	109 (96%)	110 (96%)	113 (99%)	113 (99%)	1 (1%)

References

- Agilent „QuEChERS Enhanced Matrix Removal – Lipid" https://www.agilent.com/en/products/sample-preparation/sample-preparation-methods/quechers/enhanced-matrix-removal-lipid#promotions
- SANTE/11813/2017. Analytical quality control and method validation procedures for pesticide residues analysis in food and feed. https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2017-11813.pdf.
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- Q-EMR-method by EURL AO (2018). https://circabc.europa.eu/w/browse/01c0939b-bd9f-4962-b034-4ba797522b1a, available on request
- Pictures: © Pixabay

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