

Improvements in acetone based extraction method for pesticide residues analysis

Ana Lozano^{1,2}, Barbara Kiedrowska², Jos Scholten², André de Kok² and Amadeo R. Fernández-Alba¹.

¹ Agrifood Campus of International Excellence (ceiA3), European Union Reference Laboratory for Pesticide Residues in Fruit & Vegetables, University of Almería, Almería, Spain.

² NVWA, Netherlands Food and Consumer Product Safety Authority, Laboratory of Food and Feed Safety. NRL for Pesticides in Food and Feed, Wageningen, The Netherlands.

INTRODUCTION

In the presented work, the improvement of the mini-Luke method in order to reduce solvent cost to the laboratories and trying to reduce the use of chlorinated solvent was performed. To that aim, recovery experiments (0.02 mg/kg level in lettuce) with different sample/extraction solvent ratios, different amounts of dichloromethane, Na₂SO₄ and MgSO₄ were performed with subsequent detection by LC-MS/MS-TQ. Fifty representative pesticides with different physicochemical characteristics were selected, belonging to different groups, with a special focus on the more polar compounds. The results obtained, using the same sample weight (15 g), showed that the total volume of organic solvents could be reduced by a factor of almost 2 (from 90 mL to 50 mL), and the volume of dichloromethane could be reduced to 10 mL, one third of the original.

Validation of the optimised method according to the EU QC and validation guidelines was carried out, using GC- and LC-MS/MS TQ detection, for 175 GC- and LC-amenable pesticides in lettuce and orange. Recovery studies performed with lettuce and orange matrix spiked at 0.005, 0.01 and 0.02 mg/kg yielded average recoveries in the range 70-120% for at least 167 analytes with relative standard deviation values below 20%. The linearity over three orders of magnitude was demonstrated ($r^2 > 0.99$). The matrix effect could be considered as not significant. The limit of quantification was 0.005 mg/kg for 96% of the compounds. The new NL-method was applied successfully in routine analysis and the EUPT FV-16 sample.

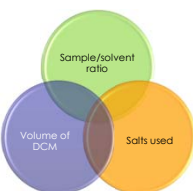
An orange blank sample was extracted by using both Citrate buffered QuEChERS and the proposed method. The number and intensity of the coextracted natural compounds from both methods were compared injecting the samples by LC-QToF-MS. It can be concluded that the optimized method contains less number coextracted natural compounds and that the intensity is lower.

EXPERIMENTAL

Matrices



Parameters to check



NL-method +(original mini-Luke)

- 15 g sample
- Add 20 mL (30 mL) of acetone + 15 g Na₂SO₄ + Procedure Internal Standard (P.I.S.)
- Mix 30 s by turrax at 15000 rpm
- Add 20 mL (30 mL) petroleum ether + 10 mL (30 mL) dichloromethane
- Mix 30 s by turrax at 15000 rpm
- Centrifuge 3 min at 3300 rpm
- LC: Evaporate 0.666 mL (1.2 mL) extract to dryness and reconstitute with 1 mL MeOH + I.L.S. (0.2 g sample/mL)
- GC: Evaporate 5 mL extract to dryness and reconstitute with 1.5 mL (0.83 mL) isooctane-toluene (9:1) + I.L.S. (1 g sample/mL)

LC-MS/MS

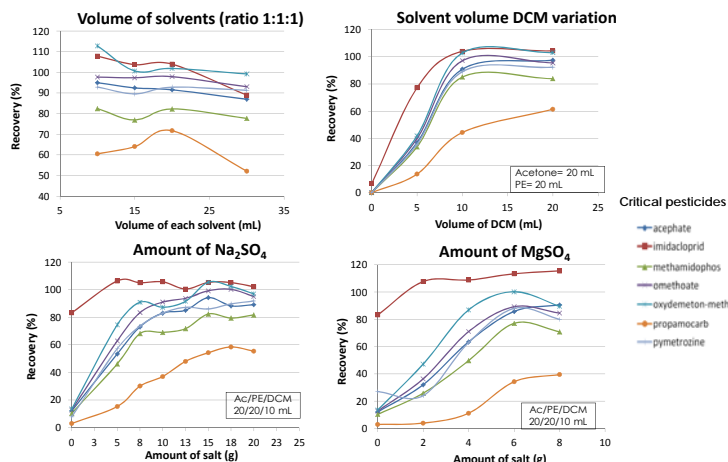
- System: Acquity UPLC + XEVO TQ-S (Waters)
- UPLC parameters:
- Injection volume: 1 µL
 - Flow rate: 0.45 mL/min
 - Column: Acquity UPLC BEH-C18; 2.1 x 100 mm, 1.7 µm (Waters)
 - Column temperature: 40°C
 - Mobile phases and gradient:
-
- 10 min run time
- MS parameters:
- Ion source mode: ESI (+)

GC-MS/MS

- System: Bruker 456 GC+ Scion-TQ MS/MS (Bruker)
- GC parameters:
- Injection volume: 5 µL
 - Injection mode: LVI-PTV, solvent vent
 - PTV Temperature program: 80 °C for 0.1 min then 200 °C/min to 300 °C. (19.5 min)
 - Column: VF-5ms 30 m x 0.25 mm ID and 0.25 µm (Bruker)
 - Constant flow: 1 mL/min
 - Oven program:
- | | °C/min | FINAL (°C) | HHLD (min) |
|---------|--------|------------|------------|
| Initial | | 80 | 1 |
| Ramp1 | 25 | 180 | 0 |
| Ramp2 | 8 | 280 | 0 |
| Ramp3 | 30 | 300 | 3.17 |
- 22 min run time
- MS parameters:
- Ionisation mode: EI
-

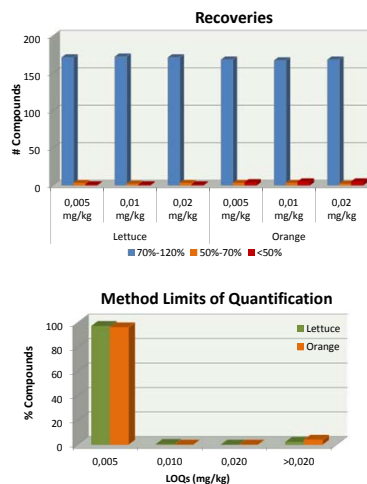
RESULTS

Optimisation and miniaturisation of the method for lettuce

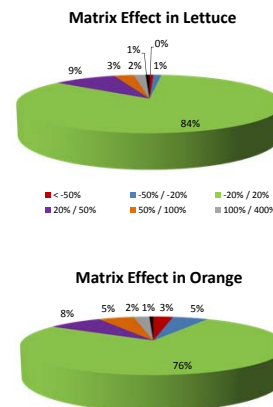


The results obtained showed that it is possible to decrease each solvent used to 20 mL (acetone, PE and DCM) or even further in case of DCM (10 mL), when maintaining the same sample weight (15 g). When a salt is used, the optimum amount for sodium sulphate is 15 g and for magnesium sulphate 6 g, showing very similar recoveries whatever salt is applied (except for propamocarb). For validation studies, Na₂SO₄ was used.

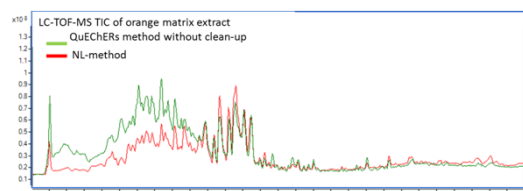
Validation of the method



Almost all the recovery results are within the range 70-120%, except biphenyl, nitenpyram, propamocarb in lettuce and aminopyrad and aminopyradophos, nitenpyram and propamocarb in orange. However, these mostly had RSDs < 20%.

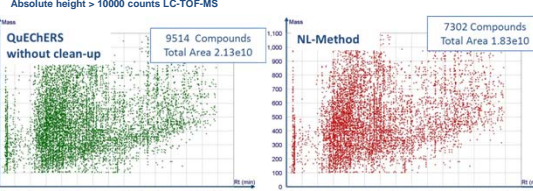


Coextracted natural compounds



As it can be seen at the upper graphs, the number of coextractives component and their intensity from orange by using QuEChERS without clean-up is higher than when the proposed method is used.

Co-extracted matrix components of orange Dilution 1/5



CONCLUSIONS

- The results obtained showed that the total volume of organic solvents could be further reduced by a factor of 2, when maintaining the same sample weight (15 g) and the volume of dichloromethane could be reduced to one third of the original. Regarding to recoveries, there is no significant difference between the old and new version.
- Recoveries for all of the analytes (except the six mentioned above) were achieved in the range 70-120%, with RSD(%) values below 20%. Linearity of response over two orders of magnitude was demonstrated ($r^2 > 0.99$). The linear range started for most of the pesticides at 0.0005 µg/mL. Matrix effect was considered as not significant for at least 76% of the pesticides. LOQs were for almost all the compounds 0.005 mg/kg.
- The differences between results for the old and the new version of the Dutch mini-Luke method, when analysing the EUPT FV-16 sample, were less than a factor of 1.6.
- A comparison in terms of number of coextracted compounds and their intensity was made between QuEChERS without clean-up and the NL method. Cleaner extracts were obtained with the optimised method.

| Pesticides | Recovery (%) of 0.050 mg/kg | NL-method results (mg/kg) | Z-Score NL-method | Mini-Luke results* (mg/kg) | Z-Score mini-Luke |
|--------------------|-----------------------------|---------------------------|-------------------|----------------------------|-------------------|
| Acetamidopiprid | 94 | 0.630 | 0.0 | 0.684 | 0.3 |
| Acinualthrin | 123 | 0.264 | 0.0 | 0.254 | 0.3 |
| Buprofezin | 102 | 0.565 | 0.8 | 0.617 | 1.3 |
| Chlorothalonil | 74 | 3.442 | 2.3 | 2.140 | -0.1 |
| Chlorpyrifos-ethyl | 79 | 3.695 | 0.0 | 3.160 | -0.6 |
| Cypermethrin | 81 | 0.594 | 0.5 | 0.597 | 0.5 |
| Cyprodinil | 105 | 0.669 | 0.1 | 0.775 | 1.3 |
| Dazomet | 105 | 0.079 | -0.2 | 0.088 | 0.2 |
| Difenoconazole | 98 | 0.928 | -0.1 | 0.987 | 0.2 |
| Endosulfan-alpha | 83 | 1.153 | 0.0 | 1.220 | 0.2 |
| Endosulfan-beta | 81 | 0.910 | -0.2 | 1.010 | 0.2 |
| Fenhexamid | 82 | 1.024 | 0.8 | 0.926 | 0.3 |
| Fludioxonil | 112 | 0.296 | 0.8 | 0.255 | 0.2 |
| Iambda-Cyhalothrin | 90 | 0.069 | -0.5 | 0.079 | 0.1 |
| Methoxyfenozide | 103 | 0.193 | 0.5 | 0.179 | 0.2 |
| Pyrimicarb | 82 | 0.778 | 0.3 | 0.840 | 0.6 |
| Pyridaben | 93 | 0.153 | 0.1 | 0.163 | 0.4 |
| Spinosad | 88 | 0.076 | -0.9 | 0.098 | 0.0 |
| Tetraconazole | 95 | 0.110 | 0.2 | 0.122 | 0.6 |

* Submitted results
The sample was injected using a dilution factor of 4 and 30.