Analysis of "Amitraz (sum)" in samples with incurred residues - Comparison of the approach covering the individual metabolites via LC-MS/MS with the approach involving cleavage to DMA

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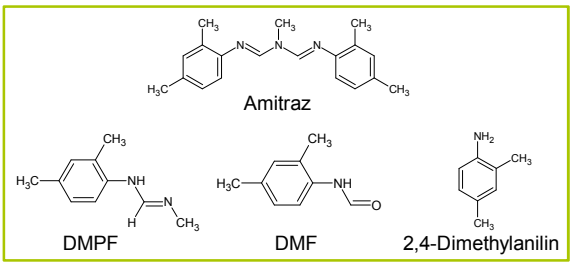
Introduction

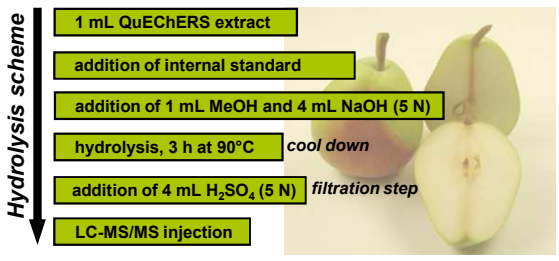
The current MRL residue definition (RD) for amitraz ("*Sum of amitraz plus all its metabolites containing the 2,4-dimethylanilin moiety*") requires methods that involve hydrolysis to 2,4-dimethylanilin (DMA). To calculate total amitraz from DMA a 1:2 stoichiometry was typically assumed reflecting the presence of two DMA moieties in the amitraz molecule. The strong hydrolysis conditions required and the difficulties in the analysis of DMA complicate the analytical methods based on this principle. Analysis of amitraz (sum) via DMA is thus rarely conducted routinely by residue control labs.



In 2007 the EURL-SRM distributed a QuEChERS-based method where amitraz parent and its most important MRM-amenable metabolites containing the DMA-moiety, namely N-2,4-Dimethylphenyl-N'-methyl-formamidine (DMPF) and N-2,4-Dimethylphenyl-formamide (DMF) and DMA are analyzed separately via LC-MS/MS [1]. Many labs follow this MRM-approach and calculate amitraz (sum) from there using a 1:1 stoichiometry between DMPF and amitraz. However, as amitraz degradation pathways are quite complex and branched, calculation of amitraz (sum) using the results of the metabolites can be quite tricky. Aim of this study was to compare the results for amitraz (sum) obtained by the two approaches using samples with incurred residues. To enable this, a simple QuEChERS-based method involving quantitative hydrolysis of amitraz and its metabolites into DMA was developed.

Hydrolysis of Amitraz and Metabolites

Samples were extracted using the QuEChERS method without d-SPE step. Alkaline hydrolysis to DMA was performed following addition of aqueous NaOH. Methanol was also added to prevent separation of water. Hydrolysis was shown to be complete for amitraz, DMF and DMPF, whereas DMA proved to be stable during hydrolysis. DMA D6 was used as ISTD.

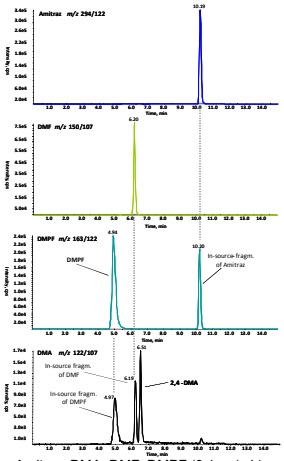


LC-MS/MS Analysis

- Column: Phenomenex Synergi Hydro RP (2.5 µm, 2.1 x 100 mm)
- Guard-column: Phenomenex AQ C18 (4 x 2.0 mm)
- Mobile Phase:
  - A: 5 mmol NH_4-Formate in water + 5% methanol
  - B: 5 mmol NH_4-Formatein methanol
- Flow: 0.2 mL min^{-1}

Mass transitions used for quantification:
- 294→122 (amitraz)
- 150→107 (DMF)
- 163→ 122 (DMPF)
- 122→107 (DMA)
- 128→110 (DMA D6)

Chromatographic separation of amitraz, DMF, DMPF and DMA was essential to avoid interferences caused by the in-source fragmentation of amitraz into DMPF and of DMF and DMPF into DMA.

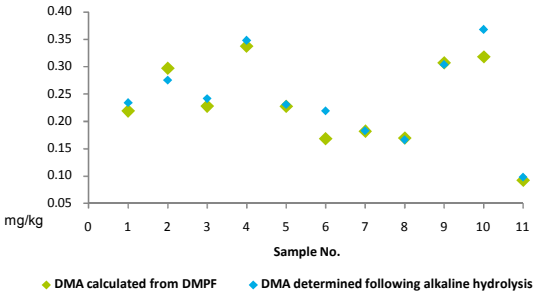


Amitraz, DMA, DMF, DMPF (0.1 µg/mL) spiked on QuEChERS extracts of blank pears

Experiments and Results

11 pear samples with amitraz treatment history were analyzed using both approaches: (I) QuEChERS followed by direct LC-MS/MS analysis of amitraz, DMF, DMPF and DMA; (II) QuEChERS followed by hydrolysis and analysis of DMA by LC-MS/MS.

Using approach (I) only DMPF was detected. When mathematically converting the DMPF results to DMA (using 1:1 stoichiometry) the results obtained were comparable to the DMA results obtained following approach (II) involving chemical conversion to DMA (see Figure below). This suggest that for the samples tested and the hydrolysis conditions used, DMPF is virtually the only source of DMA.



Sample No.	Ratio of DMA calc. from DMPF vs. DMA determined following hydrolysis
1	1.07
2	0.93
3	1.06
4	1.03
5	1.01
6	1.30
7	1.00
8	0.98
9	0.99
10	1.16
11	1.06
Average	**1.05**

Summary and Conclusion

- Amitraz and its metabolites DMF and DMPF were successfully hydrolyzed to DMA in QuEChERS extracts from pears.
- DMPF was the only component detected in pear samples and turned out to be the only DMA-source.
- Our results support the use of a 1:1 stoichiometry to calculate amitraz from DMPF. The alternative approach involving chemical (or mathematic) conversion of DMPF to DMA (with 1:1 stoichiometry) and a subsequent calculation of total amitraz based on a 1:1/2 stoichiometry underestimates total amitraz results by a factor of 2.

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References

[1] http://www.crl-pesticides.eu/library/docs/srm/meth_Amitraz_CrlSrm.pdf

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