Detecting and quantifying foreign substances in plant protection products by GC-MS: Lessons learned from four rounds of proficiency testing

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Overview
What to expect

- Foreign substances
- GC-MS as a useful tool for analysing foreign substances
- Proficiency tests for foreign substances: concept
- Qualitative and quantitative results of four rounds of proficiency testing
- Comparison of different analytical approaches / method parameters
- Surprises...and lessons learned
- Conclusions on laboratory competence and outlook
Foreign substances

Definition

- Plant protection products (ppps) consist of active substance(s), co-formulants and known impurities of both
- Composition of a ppp is fixed in the authorisation process
- Foreign substance = compound not listed in the authorisation
- Thus, foreign substances should not be present in a ppp
- A foreign substance may be “anything” in terms of chemistry / functionality (e.g. another active substance, additional co-formulant, something completely unrelated)

Sources, limits

- Foreign substances may enter a ppp by:
  - Accident (e.g. contamination from previous production batch of a different ppp, mistake during production)
  - Deliberate action (e.g. exchange of one co-formulant against another with the same function)
  - Neglect of duties (modification of formulation without notification of authority)
- Tolerance limit applied within EU: 0.1% (relative to ppp) stated in reference document for formulation analysis of market control samples
- Lower limits may apply in some cases (highly toxic substances, exceedance of residue limit)
An analytical method for foreign substances needs to cover a broad range and be suitable for routine application.

Gas chromatography coupled to mass spectrometry (GC-MS) is highly suited in that respect.

First step: untargeted screening
- all volatile compounds that can be extracted into an organic solvent are analysed by GC-MS in full scan mode
- library search for the mass spectra of the chromatographic peaks → tentative identification of compounds
- Comparison of compounds with composition of ppp, considering chemical/technological knowledge

Second step in case a foreign substance is tentatively identified: targeted analysis and quantification
- Purchase of analytical standard
- Confirmation of substance identity by comparison of retention time and mass spectrum
- Quantification of foreign substance by targeted GC-MS method (selected ion monitoring)
- Assessment of compliance of ppp sample
GC-MS analysis of foreign substances

Advantages and disadvantages

- Fast sample preparation and generic measurement method
- Commercial GC-MS library contain 100,000s of compounds (wide range covered)
- GC-MS instrumentation widely available
- Evaluation of screening data can be quite tedious and requires experience
- Substances need to be volatile
- In Austria we have been applying the GC-MS screening approach with subsequent quantification of found foreign substances successfully for many years
- Method also applied in many other countries

Proficiency tests for foreign substances

Assessing laboratory competence

- How competent are the laboratories applying this approach? Are the results comparable? Possibility of external quality assurance of one's laboratory's method?
- Proficiency testing is the "gold standard" in that respect
- No (commercial) proficiency tests (PTs) available
- Austrian official laboratory started organising PTs in 2017 for all official laboratories within EU
- To date four rounds of PTs: 2017, 2018, 2020, 2023
- Analyses of relevant impurities & co-formulants have been added from 2018 onwards
Two-step PT design:

- Step 1: Compound identification on the basis of the composition of ppp as submitted during authorisation in anonymised form (GC-MS mandatory)
- Interim report on compound identification – compound to be quantified in second step specified (avoidance of laboratories quantifying wrong substance)
- Step 2: Compound quantification (no analytical technique prescribed)
- Final report with assessment of quantitative results and method comparison

- Test samples: “real life” ppps from Austrian market control
- Samples used “as is”, no spiking
- Design reflecting every-day situation in laboratories applying this analytical approach

Over the four rounds in total laboratories from 15 EU countries participated:

* laboratories not using GC-MS for identification were excluded
Results of the proficiency tests

Substance identification - Overview

- Correct identifications of foreign substances:

<table>
<thead>
<tr>
<th>Year</th>
<th>Samples</th>
<th>Foreign substances</th>
<th>Substance(s)</th>
<th>Correct identifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017</td>
<td>1</td>
<td>1</td>
<td>Diacetone alcohol</td>
<td>4 (+ 4) / 12</td>
</tr>
<tr>
<td>2018</td>
<td>1</td>
<td>1</td>
<td>N,N-Dimethyl decanamide</td>
<td>6 / 8</td>
</tr>
<tr>
<td>2020</td>
<td>1</td>
<td>4</td>
<td>Dimethyl succinate</td>
<td>4 correct: 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dimethyl glutarate</td>
<td>3 correct: 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dimethyl adipate</td>
<td>2 correct: 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Butylated hydroxytoluene</td>
<td>Flexisolv®</td>
</tr>
<tr>
<td>2023</td>
<td>2</td>
<td>Sample A: 1, Sample B: 1</td>
<td>N-methyl-2-pyrrolidone, propylene glycol</td>
<td>3 (+ 5) / 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4 / 9</td>
</tr>
</tbody>
</table>

Reported foreign substances:

<table>
<thead>
<tr>
<th>Lab code</th>
<th>Foreign substance</th>
<th>Extraction solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diacetone alcohol</td>
<td>Methanol</td>
</tr>
<tr>
<td>3</td>
<td>Diacetone alcohol</td>
<td>Methanol</td>
</tr>
<tr>
<td>4</td>
<td>Spiroxamine</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>5</td>
<td>Spiroxamine</td>
<td>Acetone</td>
</tr>
<tr>
<td>6</td>
<td>Spiroxamine</td>
<td>Acetone</td>
</tr>
<tr>
<td>7</td>
<td>Decanoic acid</td>
<td>Acetone</td>
</tr>
<tr>
<td>8</td>
<td>Diacetone alcohol</td>
<td>Sample analysed as is</td>
</tr>
<tr>
<td>11</td>
<td>Spiroxamine</td>
<td>Ethyl acetate</td>
</tr>
<tr>
<td>12</td>
<td>Spiroxamine</td>
<td>Acetone/tetrahydrofuran</td>
</tr>
<tr>
<td>13</td>
<td>Spiroxamine</td>
<td>Acetone</td>
</tr>
<tr>
<td>14</td>
<td>Metconazole</td>
<td>Not stated</td>
</tr>
<tr>
<td>15</td>
<td>Diacetone alcohol</td>
<td>Dichloromethane</td>
</tr>
</tbody>
</table>

Diacetone alcohol is a contaminant of the solvent acetone, even in high-purity quality.
### Results of the proficiency tests

**PT 2017 – substance identification**

#### Reported foreign substances:

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<thead>
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- Diacetone alcohol had been the foreign substance targeted by the organiser (extraction with acetonitrile)
- Re-analysis showed that spiroxamine was also present, although approx. 100x lower than diacetone alcohol
- Participants extracting with acetone were unable to identify diacetone alcohol as foreign substance

#### Results of the proficiency tests

**PT 2017 – substance identification**

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<td>4</td>
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<td>Acetonitrile</td>
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<td>Spiroxamine</td>
<td>Acetone</td>
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<td>7</td>
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<tr>
<td>8</td>
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</tr>
<tr>
<td>13</td>
<td>Spiroxamine</td>
<td>Acetone</td>
</tr>
<tr>
<td>14</td>
<td>Metconazole</td>
<td>Not stated</td>
</tr>
<tr>
<td>15</td>
<td>Diacetone alcohol</td>
<td>Dichloromethane</td>
</tr>
</tbody>
</table>

- Besides diacetone alcohol results also spiroxamine results upon extraction with acetone were evaluated as being correct
- All other results were evaluated as wrong
- Choice of extraction solvent is critical, solvent contaminants may mask foreign substances!
Results of the proficiency tests
PT 2017 – substance quantification

❖ Statistical data and z-score graph:
❖ Number of results: 11 (9*)
❖ Outliers: 2
❖ Mean*: 10.2 g/l
❖ Median*: 9.43 g/l
❖ Standard deviation*: 1.25 g/l
❖ RSD*: 12.3%
* after removal of outliers

❖ 8 satisfactory results, 1 questionable result, 2 unsatisfactory results (possibly calculation errors)

Results of the proficiency tests
PT 2018 – substance identification

❖ Reported foreign substances:

<table>
<thead>
<tr>
<th>Lab code</th>
<th>Foreign substance</th>
<th>✓</th>
<th>❌</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>N,N-dimethyldecanamide</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>N,N-dimethyloctanamide</td>
<td>❌</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>N,N-dimethyldecanamide</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>N,N-dimethyldecanamide</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Decanoic acid methyl ester</td>
<td>❌</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>N,N-dimethyldecanamide</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>N,N-dimethyldecanamide</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>N,N-dimethyldecanamide</td>
<td>✓</td>
<td></td>
</tr>
</tbody>
</table>

❖ N,N-dimethyldecanamide had been the foreign substance targeted by the organiser
❖ N,N-dimethyloctanamide and some other small peaks had also been noted in the analysis but all had significantly lower intensities than N,N-dimethyldecanamide
❖ All reported compounds were present in the sample but only the most intense foreign substance was evaluated as correct result
Results of the proficiency tests
PT 2018 – substance quantification

❖ Statistical data and z-score graph:
   ❖ Number of results: 8 (7*)
   ❖ Outliers: 1
   ❖ Mean*: 2.63 g/l
   ❖ Median*: 2.58 g/l
   ❖ Standard deviation*: 0.20 g/l
   ❖ RSD*: 7.6%
   * after removal of outliers

❖ 6 satisfactory results, 1 questionable result, 1 unsatisfactory result
❖ Results better (lower RSD) than in first round despite 4-fold lower concentration

Results of the proficiency tests
PT 2020 – substance identification

❖ The sample contained four foreign substances, with three belonging to a mixed solvent (Flexisolv®), and one antioxidant at low concentration
❖ Despite Flexisolv® constituents being “hidden” in an aromatic solvent cluster, eight laboratories correctly identified all three constituents of it
❖ Low-concentrated BHT proved more challenging but was still found by most laboratories

<table>
<thead>
<tr>
<th>Lab code</th>
<th>DMS</th>
<th>DMG</th>
<th>DMA</th>
<th>BHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>2</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>3</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>4</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>5</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>6</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>7</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>8</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>10</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
</tbody>
</table>

Overall 8 / 9 8 / 9 9 / 9 7 / 9

DMS: dimethyl succinate, DMG: dimethyl glutarate, DMA: dimethyl adipate, BHT: butylated hydroxytoluene
Results of the proficiency tests
PT 2020 – substance quantification

- Selected foreign substance for quantification: dimethyl adipate

- Statistical data and z-score graph:
  - Number of results: 10
  - Mean: 21.9 g/kg
  - Median: 22.2 g/kg
  - Standard deviation: 1.88 g/kg
  - RSD: 8.6%
  - Assigned value (median): 22.2 g/kg
  - Target standard deviation: 1.66 g/kg (corresponding to 7.5%)
Reported foreign substances:

<table>
<thead>
<tr>
<th>Lab code</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Xylene mixture of isomers, containing ethyl benzene</td>
<td>Dimethyl disulfide</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>N-Methyl-2-pyrrolidone</td>
<td>5,7- dimethyl-1,3-diaza-adamantan-6-one</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1-Methyl-2-pyrrolidone</td>
<td>Propylene glycol</td>
<td>In sample 1 we also found p-xylene</td>
</tr>
<tr>
<td>4</td>
<td>p-Xylene</td>
<td>Butylated hydroxytoluene</td>
<td>Butylated hydroxytoluene has also been identified in sample 1</td>
</tr>
<tr>
<td>5</td>
<td>Xylene (isomeric mixture)</td>
<td>4-Hydroxy-4-methyl-2-pentanone</td>
<td>In sample 1 ethylbenzene (a chain isomer of xylenes) was also detected</td>
</tr>
<tr>
<td>6</td>
<td>1-Methyl-2-pyrrolidone</td>
<td>N,N-Dimethylformamide</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1-Butanol</td>
<td>Propylene glycol</td>
<td>Sample 1 - xylenes and the accompanying substance ethylbenzene were also identified. Also 1-methyl-2-pyrrolidone was identified in a very low concentration</td>
</tr>
<tr>
<td>8</td>
<td>Xylene (ortho and para)</td>
<td>Propylene glycol</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>p-Xylene</td>
<td>Propylene glycol</td>
<td></td>
</tr>
</tbody>
</table>

Sample 1 - analysis by the organiser prior to the PT:
- N-methyl-2-pyrrolidone
- xylenes, ethylbenzene
- 1-butanol

All reported substances are present but...

Sample contained isobutanol

1-butanol can be considered an impurity of this co-formulant and thus should be excluded
N-methyl-2-pyrrolidone shows largest peak and was therefore selected as target compound.

This compound is of special interest from a toxicological and legal point of view:

- toxic to reproduction category 1B
- forbidden co-formulant according to Annex III of Reg 1107/2009

Interestingly, none of the laboratories reporting xylenes did at least mention the presence of N-methyl-2-pyrrolidone.

Assessment of reported results:

- N-methyl-2-pyrrolidone: correct identification
- Xylenes: wrong identification but acceptable result
- 1-butanol: wrong identification, unacceptable result
Results of the proficiency tests
PT 2023 – substance identification – sample 2

- Sample 2 - analysis by the organiser prior to the PT: propylene glycol
- Quantified at 48 g/l
- Higher content than co-formulant glycerol → not a contamination of glycerol
- Hardly to be missed in chromatogram

Results of the proficiency tests
PT 2023 – substance identification

- Overall evaluation:

<table>
<thead>
<tr>
<th>Lab code</th>
<th>Target foreign substance identified correctly</th>
<th>Correct identifications</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N-Methyl-2-pyrrolidone (sample 1)</td>
<td>Propylene glycol (sample 2)</td>
</tr>
<tr>
<td>1</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>2</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>3</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>4</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>5</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>6</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>7</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>8</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>9</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Overall</td>
<td>3 (+5) / 9</td>
<td>4 / 9</td>
</tr>
</tbody>
</table>
Results of the proficiency tests
PT 2023 – substance quantification

❖ Selected foreign substance for quantification: N-methyl-2-pyrrolidone

❖ Statistical data and z-score graph:
  ❖ Number of results: 9
  ❖ Mean: 1.02 g/kg
  ❖ Median: 1.00 g/kg
  ❖ Standard deviation: 0.19 g/kg
  ❖ RSD: 18.5%
  ❖ Assigned value (median): 1.00 g/kg
  ❖ Target standard deviation: 0.075 g/kg

❖ Legal limit (EU): 0.1% (=1 g/kg) !!

Results of the proficiency tests
Method parameters

❖ For both identification and quantification of the foreign substance(s) the participants were asked to provide various details concerning their employed methods

❖ These method parameters included for the identification part:
  extraction solvent, concentration of measured solution, GC column type, mobile phase, injection mode, GC run time, MS scan range

❖ For the quantification part the details requested encompassed the same method parameters as for the identification part and additionally: type of detector, MS mode, internal standard, type of quantification

❖ The aim was to see which conditions were favoured by the majority of participants and whether any conclusions in terms of especially useful/problematic conditions could be drawn
Results of the proficiency tests
Method parameters – concentration of sample solution

❖ Foreign substance identification:

❖ Foreign substance quantification:

➢ Wide range of sample concentrations was employed ranging from >100 g/l to <1 g/l
➢ Generally, lower sample concentrations were used for the screening (identification) part than for the quantification part
➢ Over the first three PTs no clear picture emerged regarding advantageous sample concentrations (evaluation of PT 2023 data still outstanding)
Results of the proficiency tests
Method parameters – GC run time

❖ Foreign substance identification:

❖ Foreign substance quantification:

➢ GC run time ranged from <15 min to >60 min
➢ There was a slight tendency to employ shorter run times for quantification than for identification
➢ Successful foreign substance identification was also possible with (very) short GC run times, with long run times being no guarantee for correct findings
➢ The results for the substance quantification parts showed that the run time is not a decisive factor for achieving good z-scores
While there was no clear preference for either split or splitless injection in the methods used for identification of the foreign substance(s), split injection was predominantly used for foreign substance quantification.

Over all three proficiency tests wrong results for foreign substance identification were never observed with splitless injection, only with split injection (and other techniques).

For substance quantification no clear picture emerged.
Results of the proficiency tests

Method parameters – others

❖ GC column type:
  ❖ 5% phenyl / 95% methylpolysiloxane stationary phase (DB5, etc.) was used most often
  ❖ Various other column types used by single participants
  ❖ All column types can be used successfully

❖ Detection used for foreign substance identification:
  ❖ MS in SIM mode used most frequently
  ❖ FID also employed successfully

❖ Overall, different analytical approaches and choices of method details can yield good results – laboratory experience seems to be the most important factor

Lessons learned

Each PT came with its own surprises

❖ As an organiser of PTs targeting foreign substances it is practically impossible to cover all possible method variations that will be employed by participants

❖ Despite thorough analysis by the organiser some participants will report an unexpected, yet not untrue, result

❖ Evaluation of the qualitative analysis (substance identification) thus needs to take such findings into account in a flexible way

❖ Possible challenges envisioned by the organiser may be tackled successfully by most or all participants, while an “easy” sample can turn out to yield unsatisfactory performance of many laboratories
Lessons learned

Aspects to consider for GC-MS screening methods

❖ The choice of extraction solvent can in some instances be highly critical
  ❖ A careful selection a suitable, i.e. mid-polarity, solvent available in high purity is essential
  ❖ The use of two different solvents may be advantageous
❖ A preference should be given to splitless injection over split injection; otherwise methods with different choices for other variables (e.g. GC column, temperature program) can all work well
❖ Data evaluation is the most important step, experience needs to be built
❖ Expect foreign substances at low concentrations at the legal limit and way above it
❖ A good knowledge of ppp formulations and typical impurities of co-formulants is of great value

Conclusions

Good performance but still room for improvement

❖ PTs were set up to reflect “real life” situation (market sample, available knowledge)
❖ Overall participating laboratories have a high competence in correctly identifying and quantifying foreign substances in ppps using GC-MS screening methods
❖ However, in the PT round of 2023 the rate of false identifications was very high with only a third of participants achieving a satisfactory result
❖ Especially astonishing was that only four out of nine laboratories correctly identified propylene glycol at a content of almost 5%
❖ Quantification of foreign substances generally yielded comparable results, especially considering that an “ad hoc” method had to be used
Future proficiency tests according to the established concept (foreign substance identification and quantification, analysis of relevant impurities and co-formulants) will be conducted if demand is there.

Official laboratories who did not take part yet or not in the last proficiency test are warmly invited to do so (again).

Any feedback on improvement in terms of concept, evaluation etc. is welcome.

The first laboratories have started to utilise LC-high resolution mass spectrometry for screening ppvs, so this may be an interesting technique to target in a future PT and encompass non-volatile ppp constituents.

THANK YOU!

All colleagues helping in handling PT samples

Austrian authority allowing the use of market control samples for PTs

All participants
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