***Analytical Observation concerning…***

☑ ***a compound*** ☐ ***a matrix*** ☑ ***a method*** ☐ ***other***

# Analysis of Dodine by the QuEChERS Method - Interactions in the injector and role of matrix

Reported by: EURL-SRM

Version 1 (last update: 23.02.2015)

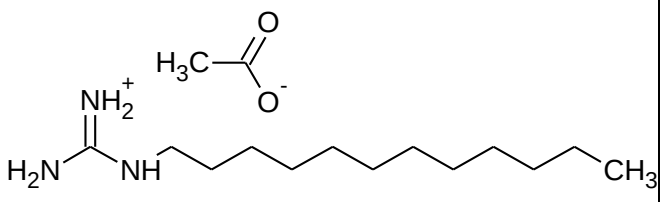
## Brief description of problem/observation/solution:

In LC-MS/MS analysis dodine often shows overestimated recovery rates when quantified using calibration solution in pure solvent. These effects can vary considerably from instrument type to instrument type and typically temporarily improve when cleaning the injector block. These effects are attributed to the tendency of dodine to interact with surfaces especially in absence of any competitive components and could be effectively eliminated when calibration standards prepared from QuEChERS extracts of blank commodities were used or when the solvent based calibration standard was acidified (e.g. with acetic or formic acid).

## Compound details

**Dodine free base** (CAS: 112-65-2), IUPAC: 1-dodecylguanidine
**Dodine** (CAS: 2439-10-3), IUPAC: 1-dodecylguanidine monoacetate

| Parameter | Value | Notes |
|---|---|---|
| **Molecular Mass** | Free base<br>Monoacetate | 227.4 g/mol<br>287.4 g/mol |
| **Pka** | 9 [1] | |
| **LogPow** | 1.65 [1] | |
| **Water solubility** | 630 mg/L | |
| **Stability** | Stable | |
| **Residue definition EU** | Dodine | |
| **Authorized /Approved** | Reg. 540/2011/EU<br>AT, BE, BG, CY, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IT, LU, LV, NL, PL, PT, RO, SI, SK, UK | |




Dodine is a local systemic foliar fungicide with protective and some curative action used for control of scab on apples, pears, and pecans; leaf spot diseases of cherries, olives, blackcurrants, celery, and other crops; and foliar diseases of strawberries. Also used on other fruit, vegetable, nut, and ornamental crops, and on shade trees [1]. The Acceptable Daily Intake (ADI, EFSA 2010) is 0.1 mg/kg bw/day and Acute Reference Dose (ARfD, EFSA 2010) is 0.1 mg/kg bw.

**Observations and experiments conducted:**

**Experiment 1:**
*Aim*: localize the problem
*Description*: Standard solution containing dodine at equal concentrations were prepared in pure solvent (solvent-based standards) and in blank extracts (matrix-based standards) and injected to the LC-MS/MS composed of a *Waters* UPLC with a loop-based injector and an *API4000 ABSciex* MS/MS component.
*Observation*: The peak area obtained from the pure solvent-based standard was much smaller compared the one obtained when injecting matrix-based standard. See Figure 1:

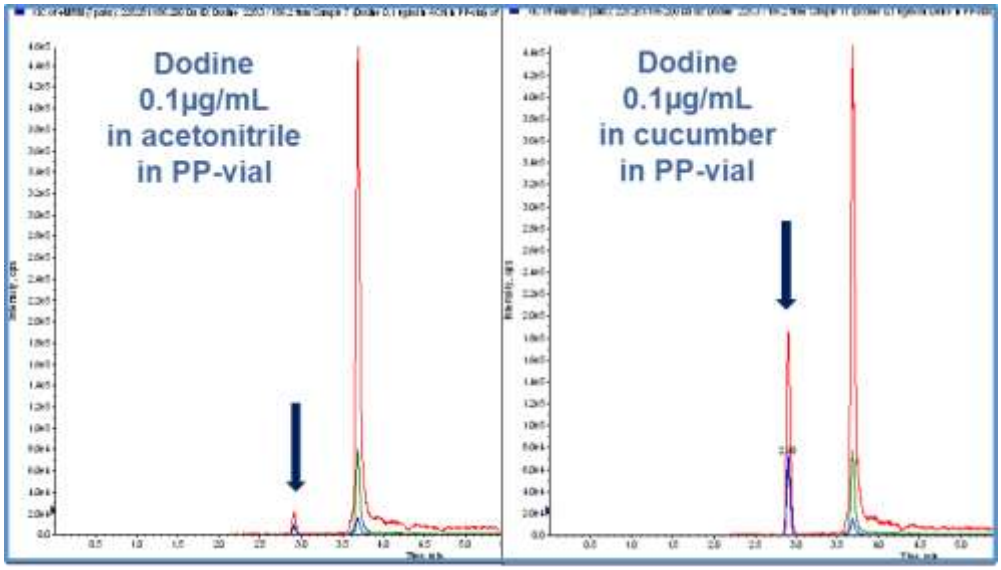



*Figure 1: Dodine response in acetonitrile and in cucumber extract (Waters UPLC, API 4000 ABSciex)*

As possible reasons were considered:

- Signal enhancement in the source of the MS
- Analyte losses due to adsorption to the (glass) vials
- Analyte losses due to adsorption during injection which may be reduced in presence of matrix components due to competition.

**Experiment 2:**
*Aim*: Check whether the above effects can be reduced by acidification
*Description*: the above solvent- and matrix-based standard solutions were acidified and re-injected as follows. a) Addition of 10 µL diluted formic acid (5% in ACN), short shaking and re-injection; b) Addition of 10 µL formic acid (conc.) to the same vial; short shaking and re-injection.
*Observation*: The addition of diluted formic acid considerably increased the peak area of dodine in the solvent-based standard, whereas the area in the matrix-based standard remained the same. The subsequent addition of 10 µL of formic acid (conc.) led to further in-

crease of the peak area that reached the level of the matrix-based calibration. The addition of acid to the matrix-based solution improved the signal intensities only very slightly.
*Conclusion*: a) Signal enhancement in the ion-source due to the presence of matrix is unlikely to be the source for the observed effect, as formic acid separates from the analyte during the chromatographic run; b) both, presence of matrix and acidification obviously reduce interactions with surfaces and minimizes losses; c) both interaction in the vial and interaction in the injector cannot be ruled out.

**Experiment 3:**
*Aim*: Investigate whether there is interaction with the walls of the glass vial.
*Description*: Equally concentrated solvent- and matrix-based dodine solutions were prepared both in PP-vials and in normal glass vials, kept for 3 hours and injected. After injection the vials were acidified with 10 µL formic acid (conc.) and re-injected. A similar set of standard solutions was kept one week in advance and injected in parallel.
*Observation*: Signals for dodine from the fresh solvent-based solutions were comparable for both glass- and PP-vials and both considerably lower than the respective areas obtained when injecting the matrix-based standards, which were themselves also comparable in intensity. The addition of 10 µL formic acid (conc.) resulted again in equal signals for the dodine solutions with and without matrix. When injecting the 1-week stored solutions in pure solvent the dodine signal obtain from the extract stored in the PP-vial was slightly higher (ca. 20 %) than the signal obtained when stored in the glass vial.
*Conclusion*: The adsorption to the walls of glass-vials can be considered as having a rather minor impact on the above described effect and only in absence of matrix.

**Experiment 4:**
*Aim*: To find out whether the above described effects are instrument-specific.
*Description*: A set of standards prepared with and without matrix / acidification and containing equal concentrations of dodine were injected to the instrument in which experiments 1-3 were conducted but following intensive injector cleanup. In addition they were also injected to other LC-MS/MS systems available in our laboratory. The chromatographic parameters (injection volume, LC-column, gradient) were kept the same.
*Observation:* Using the system on which experiments 1-3 were conducted (*Waters ACQUITY UPLC with a loop-based injector* + ABSciex, API4000) but following intensive injector cleanup (elution with + injection of a series of acidic and neutral solvents that were directly routed to waste), effects could be reduced significantly but gradually increased again as more and more samples were injected. Using a system composed of a *Waters ACQUITY UPLC I-Class* with an FTN (flow-through needle) mechanism and an *ABSciex API3200 MS/MS* the effects were much lower than with the loop-based injector, but were still significant. Using a system composed of an Agilent 1200 HPLC with a loop injector and an *ABSciex API4000* MS/MS the differences between matrix- and solvent-based solutions were minimal.
*Conclusion:* Adsorption effects vary from injector type to injector type and also depend on the cleanness of the surfaces that come into contact with the sample.

**Overall Conclusions:**

**In absence of matrix components dodine shows a variable tendency of adsorption within the injector unit depending on the type of the LC-injector and its condition. The effect can be avoided using matrix-based solutions or by adding 10 µL of formic acid to 1 mL of solvent-based solutions. To make sure that dodine residues are not overestimated one of the two options has to be implemented in the lab routine, see Table 1.**

*Table 1: Results for samples analyzed from the German market 2014 using solvent- or matrix-based calibration solutions*

| Real samples | Calculated dodine level (mg/kg) when using for calibration a … | | Overestimations when calibrating with standard in pure solvent |
|---|---|---|---|
| | Matrix-based Standard (cucumber) | Solvent-based Standard | |
| **Pears** | 0.064 | 0.090 | 141% |
| **Pears** | 0.22 | 0.58 | 264% |
| **Sour cherries** | 0.047 | 0.22 | 468% |
| **Cherries** | 0.084 | 1.5 | 1786% |
| **Apples** | 0.015 | 0.047 | 313% |
| **Apricots** | 0.28 | 0.77 | 275% |
| **Apricots** | 0.012 | 0.034 | 283% |
| **Gooseberries** | 0.021 | 0.058 | 276% |
| **Endive** | 0.014 | 0.022 | 157% |
| **Spinach** | 0.003 | 0.009 | 300% |

[1] The Pesticide Manual 14th Edition, C D S Tomlin, BCPC 2006

**History**

| Action | When | Version |
|---|---|---|
| Conduction of experiments | June 2013 – February 2014 | |
| Method placed on-line | February 2015 | V1 |