Monitoring DEET in Water: Potential Interference from Solvents During LC-MS/MS Analysis

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Overview of DEET History & Environmental Occurrence

- DEET (N,N-Diethyltoluamide) is an insect repellent ingredient developed by the US Army in the mid-1940s.
- Incorporated in about 140 products, DEET is now widely used by the US population.
- Contained in products applied on the skin, DEET is washed off during showering and therefore becomes a common wastewater contaminant.
- Due to incomplete removal during wastewater treatment, DEET may reach both surface water and ground water. However, evaluation according to standardized testing guidelines indicates that DEET is readily biodegradable.
- One of the first studies on wastewater contaminants in US streams by Kolpin et al. (2002) reported the occurrence of DEET in 74% of 54 samples with a maximum concentration of 1.1 µg/L.
- Several subsequent studies confirmed the wide distribution of DEET at generally much lower concentrations in the environment.

Issues raised by DEET Monitoring in water

- DEET is commonly detected in laboratory blanks.
- Reported DEET concentrations are unexpectedly high.
- There is a lack of geographical and seasonal pattern in DEET concentrations in surface waters.
- The amount of DEET extrapolated from concentrations reported in water appears to exceed the annual production of this insect repellent ingredient.

Hypothesis

- A mimic of DEET or an interfering compound occurs in the water sample or is introduced during the analysis itself.

Since DEET is often detected in laboratory blanks, the present study focuses on identifying a potential bias in the LC-MS/MS analysis. The solvents used for the mobile phase are liquids resulting from purification processes and are therefore a major potential source of interference.

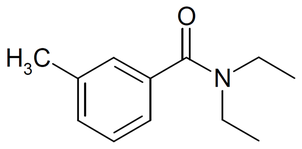

Figure 1: *Chemical structure of DEET*

Approach

Step 1: Test the accumulation of interfering compound on the column

- Two mobile phases, water/acetonitrile (95:5, %v:v) then water/methanol (95:5, %v:v), were considered in this study and two brands of each solvent were tested.
- Before each analysis of DEET, the mobile phase was allowed to flow through the column for 0, 5, 10, 20, 30 or 60 minutes. Because of the low percentage of organic solvent (5%), any interfering compound occurring in the mobile phase would not likely be eluted and therefore would accumulate on the column head.
- To avoid bias, analysis of DEET after the different column equilibration times was performed on a 0 µL injection so that the amount detected reflects only the amount accumulated from the mobile phase.
- The experiment was repeated on 3 different column types.

Step 2: Concentrate the solvents and the interfering compound

- Each solvent tested was concentrated 20:1, evaporating 10 mL down to 0.5 mL.
- Non-concentrated solvent was analyzed right before the concentrated one in order to compare the intensity of the DEET signal.
- Four transitions were monitored for improved specificity.

Materials

Solvent and standards

- HPLC grade Water procured from Fisher and Honeywell
- HPLC grade Methanol procured from Fisher and Honeywell
- HPLC grade Acetonitrile procured from EMD and Honeywell
- DEET (96.7% purity) procured from Sigma-Aldrich

Analytical columns

- Phenomenex Synergi MAX-RP 80 A; 4µ; 250 x 4.60 mm (C12 HPLC column)
- Agilent Eclipse Plus C18; 1.8µ; 2.1 x 50 mm (C18 UHPLC column)
- Agilent Zorbax Bonus RP; 1.8µ; 2.1 x 50 mm (C18 UHPLC column)

Mass Spectrometer

- Agilent 6460 Mass spectrometer with Agilent 1290 liquid chromatography apparatus

Results

Detection of DEET in Blanks (0 µL injection)

- A signal corresponding to DEET was detected with each mobile phase and each column even with 0 µL injection (Figure 2).
- The ratio of all 4 transitions was consistent with DEET (Figure 3).
- Carryover would be unlikely to explain the results since they were obtained avoiding any contact between injection needle and vials as well as employing a 10 sec needle wash before each analysis.
- The DEET standard was injected after all the 0 µL injections on brand new columns so a residual of DEET on the column from previous runs can be excluded.

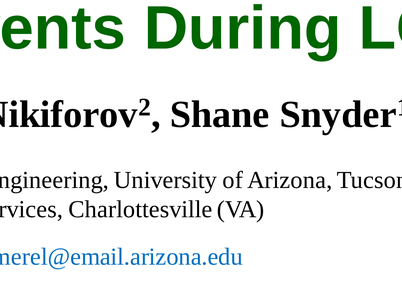

Figure 2: *Detection of DEET with 0 µL injection*

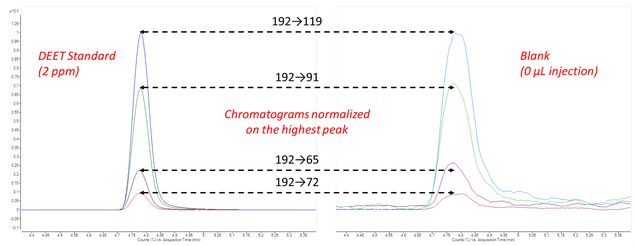

Figure 3: *Comparison of the transitions between the signal observed with a 0 µL injection and the signal of DEET*

Impact of column equilibration time on DEET signal

- Area of signal associated with DEET transitions increased with the column equilibration time with both mobile phase (water/methanol and water/acetonitrile) and both types of column (HPLC and UHPLC), as presented in Figure 4.
- Area of interfering signal depended on the type of organic solvent, e.g. methanol was "cleaner" than acetonitrile.
- Area of interfering signal also appeared to depend on solvent brand, e.g. solvents from Honeywell were generally "cleaner".
- Area of interfering signal seemed to be higher with a HPLC column than with a UHPLC column (longer post-time required for HPLC columns may be a partial explanation).

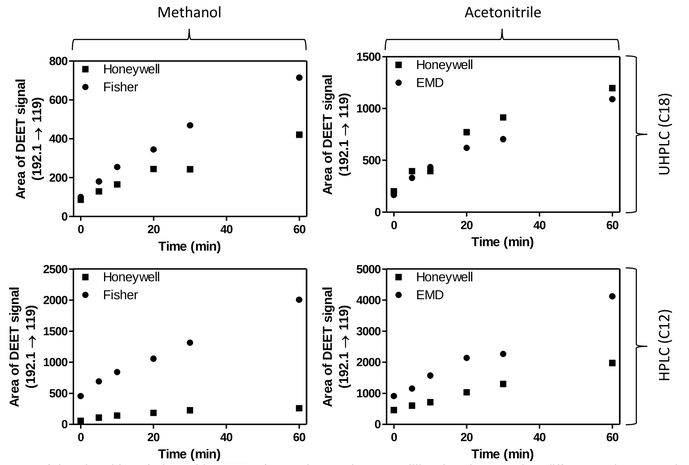

Figure 4: *Area of the signal interfering with DEET after various column equilibration times, using different solvents and columns*

DEET signal in concentrated and non-concentrated solvents

- Intensity of signal interfering with DEET was consistently higher in concentrated solvent (Figure 5), even if the ratio was not 20:1 as expected.
- According to these results, both organic solvents and water seemed to contain compounds interfering with DEET quantitation.

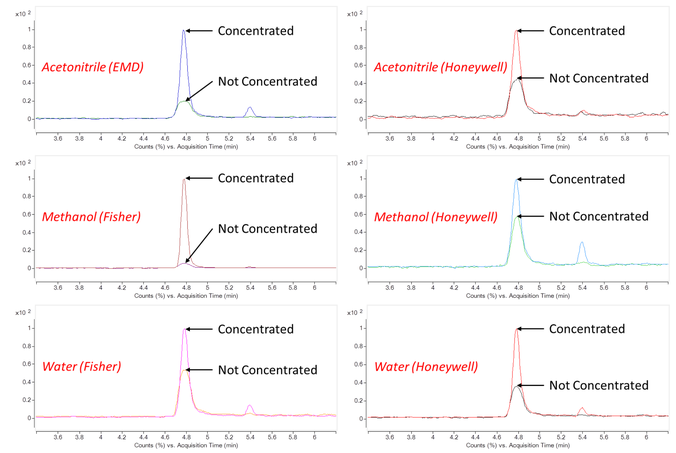

Figure 5: *Comparison of the DEET signal in concentrated versus non-concentrated solvents*

Conclusions

- An interference with LC-MS/MS analysis of DEET seemed to be introduced through the mobile phase.
- Interference depended on the type and brand of commercial analytical solvent used.
- Further analysis on LC-QTOF should allow the identification of the interfering compounds.

Acknowledgement

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Reference

Kolpin et al., ES&T 2002, 36, 1202-1211.