Blow flies as remote sampling devices: Detection of insensitive munitions and their degradation products in the environment using LC-MS

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Overview

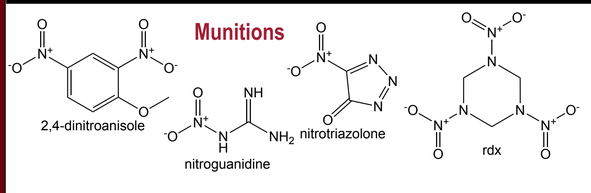
- Insensitive munitions (IM) are alternatives to traditional explosives that are less shock and temperature sensitive, resulting in less unintentional detonations[1].
- Due to incomplete consumption in a blast, IMs are likely to deposit in the surrounding environment.
- IMs undergo abiotic, biotic, and UV-mediated transformation resulting in transformation products sometimes more toxic than the agent itself.
- This work aims at using blow flies as environmental sampling devices for detecting insensitive munitions and their transformation products in the environment.
- An untargeted LC-MS method was developed to detect these polar chemistries in a single method.
- Controlled feeding experiments were performed to determine that the agents could be detected in the blow fly sample matrix.

Methods

- LC-MS assay developed for a group of insensitive munitions and their degradation products.
- Trifunctional amide, XBridge BEH Amide column (2.1x100 mm) HPLC column.
- Hydrophilic interaction liquid chromatography (HILIC) mode of separation selected due to the polar nature of the analytes.
- Isocratic elution in 10 minutes using 95/3/2 acetonitrile/methanol/water with 10 mM ammonium acetate as the mobile phase.
- Q-Exactive Hybrid Quadripole-Orbitrap mass spectrometer operated in Full MS using positive/negative polarity switching.
- Calibration curves made using calibrators ranging from 15 to 1215 ppb.
- Quality control samples (135 ppb) used for feeding experiments.
- Flies extracted using methanol sonication for 30 minutes.
- Following sonication, samples were centrifuged for 10 minutes.
- 75 µL of sample pipetted into autosampler vial and diluted with 225 µL of ACN.
- Injection volume was 2 µL and flow rate was 0.3 mL/min.

Figure 1. Dionex Ultimate 3000 HPLC connected to a Q-Exactive Orbitrap Quadrupole Mass Spectrometer

LC-MS Assay Development



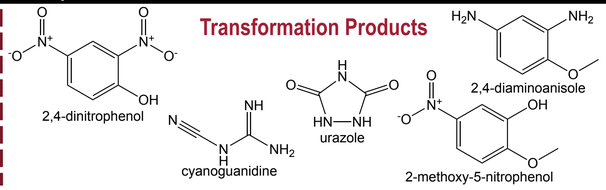


Table 1. Select IM and IM transformation products detected in LC-MS/MS method

Analyte	Analyte Type	m/z	Polarity	Retention Time (min)	Internal Standard	Avg. r^2	Avg. LOD (ng/sample)
royal demolition eXplosive (RDX)	Munition	281.0487	-	1.52	2,4-DNP-d_3	0.9934	4.4
2-methoxy-5-nitrophenol (2,5-MNP)	Transformation Product	168.0303	-	1.65	2,4-DNP-d_3	0.9909	4.5
2,4-diaminoanisole (2,4-DAAN)	Transformation Product	139.0866	+	1.72	Tol-d_3	0.9042	16.7
2,4-dinitrophenol (2,4-DNP)	Transformation Product	183.0048	-	1.79	2,4-DNP-d_3	0.9966	2.7
nitroguanidine (NQ)	Munition	103.0264	-	3.60	CQ[^{13}C]	0.9800	6.7
cyanoguanidine (CQ)	Transformation Product	83.0364	-	3.95	CQ[^{13}C]	0.9954	3.2
urazole (UZ)	Transformation Product	97.9994	-	6.17	2,4-DNP-d_3	0.9843	14.4
toluidine-d_3 (Tol-d_3)	Internal Standard	111.0997	+	1.50			
2,4-dinitrophenol-d_3 (2,4-DNP-d_3)	Internal Standard	186.0236	-	1.79			
cyanoguanidine-^{13}C (CQ[^{13}C])	Internal Standard	85.0431	-	3.95			

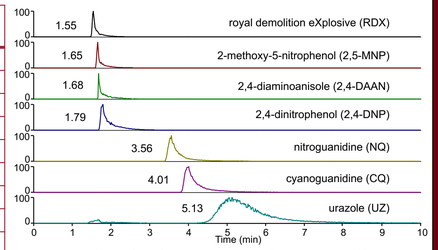


Figure 2. Extracted ion chromatograms (EICs) of select IMs and IM transformation products

Fly Feeding Experiments

Feeding Experimental Conditions

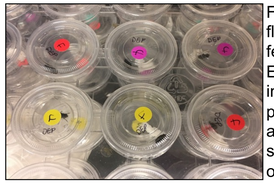


Figure 3. Adult blow flies undergoing feeding experiment. Each fly is maintained in an individual 1 oz portion cup for 4h with a small kimwipe soaked in either water or IM.

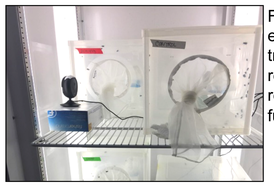


Figure 4. Flies exposed to each treatment were released into their respective cages for further monitoring.

Feeding Results

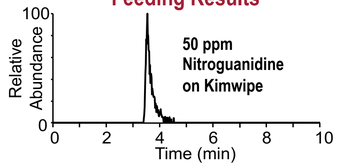


Figure 5. Extracted ion chromatogram of IM detected in fly after fed 50 ppm solution

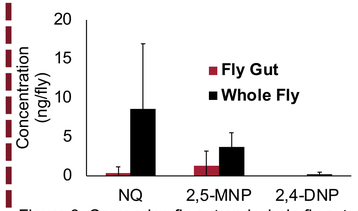


Figure 6. Comparing fly gut and whole fly extraction

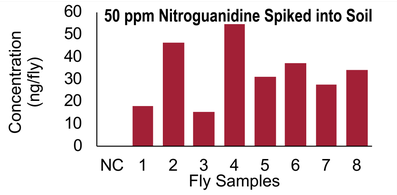


Figure 7. Concentrations detected in flies after exposed to IM contaminated soil

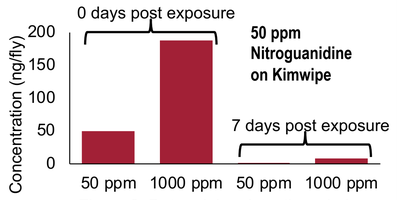


Figure 8. Determining detection window

DNAN and NTO Retention

- Original analyte group used to develop HILIC method did not contain 2 major munitions components: DNAN and NTO.
- On the HILIC method, DNAN co-eluted with 2,4-DNP. During ionization, DNAN demethylates to form an ion at m/z 183.0048 (same as 2,4-DNP).
- NTO had a RT of 17 minutes and took 7 minutes to elute.
- A C18 method was developed on a Hypersil Gold (100 mm x 2.1 mm x 3 µm) column to solve these problems.
- 15 minute gradient (A: 98/2 water/methanol with 5 mM ammonium acetate; B: 98/2 methanol/water with 5 mM ammonium acetate).

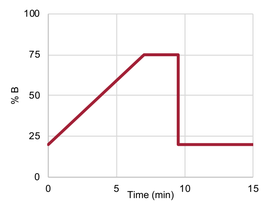


Figure 9. C18 method gradient

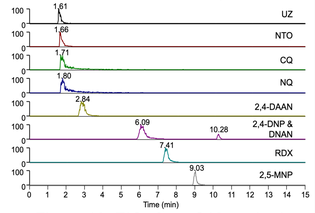


Figure 10. EICs from C18 method

Conclusions & Future Work

- Developed a HILIC HPLC-MS method to retain IM compounds and their transformation products with LODs at ng levels.
- Showed proof-of-concept studies detecting IMs and IM transformation products in fly matrix.
- C18 method developed for DNAN separation and to improve NTO peak shape.
- Use DNAN, NTO, and NQ for future feeding experiments such as longevity experiments at a variety of temperature and humidity conditions[2].
- Assess the detectability in environmental matrices such as different soil types.
- Identify IM transformation products due to fly metabolism of IM components.

References

1. Russell, A.L., et al., Analysis of munitions constituents in IMX formulations by HPLC and HPLC–MS. Talanta, 2014. 128: p. 524-530.
2. Dowling, S.N., et al., *Insects as Chemical Sensors: Detection of Chemical Warfare Agent Simulants and Hydrolysis Products in the Blow Fly Using LC-MS/MS*. Environmental Science & Technology, 2022. **56**(6): p. 3535-3543.

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