DAIMON Toolbox Fact Sheets:

Methods to Study the Impact of Dumped Munitions on Marine Biota

Assessment category 2: Hazardous substances

Toolbox component: Biota chemistry

Fact Sheet 2.11: Analysis of explosives and metabolites via HPLC-QQQ-MS

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What is it?

Explosives compounds are as such rarely detected in marine biota. This results from the rapid degradation of the xenobiotic compounds through the detoxification mechanisms of the organism. Hence, metabolites of explosives are more likely to be found in biological samples than their precursors.

What does it tell you?

A sensitive detection method has been established that allows target compound analysis of selected substances as well as screening of samples for unknown nitro-aromatic compounds. The method is based on the coupling of a High Performance Liquid Chromatograph (HPLC) to a Triple Quadrupole Mass Spectrometer (QQQ-MS).

Type of Indicator (tick box)

☐ non-specific stress indicator
☐ specific for groups of contaminants incl. CWA or explosives
☐ CWA-specific indicator
☒ specific for substances related to explosives (e.g. TNT)

How to analyse samples on HPLC-QQQ-MS?

***Samples*:** Analytes are dissolved in Acetonitrile. 5 µL of the respective solution are usually injected on the HPLC for separation and subsequent analysis on the QQQ-MS.

***Equipment*:** Analytical brown glass vials with inserts are used as injection vials to prevent potential photocatalytic degradation of analytes. The HPLC is equipped with an Acclaim E2 explosives column (Thermo Fisher), which is maintained at 25 °C. Eluents used consist of A: H_20 (10mM NH_4Ac + 2.7mL Acetic Acid, pH 4) and B: MeOH (10mM NH_4Ac + 2.7mL Acetic Acid). Detection of compounds is performed on an ABSciex Triple Quadrupole Mass Spectrometer (QQQ-MS). Components are ionized via negative mode (neg) atmospheric

pressure chemical ionisation (APCI).

Method: The HPLC gradient separation is conducted as follows: 5 minutes isocratic at 45 % B, increase to 60 % B at 5 minutes, followed by a gradient from 60 to 95 % B from 5 to 40 minutes with a subsequent reconditioning of the column at 45 % B for 5 minutes.

The detection of target analytes is conducted in Multiple Reaction Monitoring (MRM) mode with concurrent recording of a full scan Enhanced Mass Spectrum (EMS). Ionization energies are optimized using commercially available standard substances. Conditional screening for nitroaromatics is performed via Neutral Loss (NL) scanning for loss of individual nitro groups and consecutive acquisition of Enhanced Product Ion (EPI) spectra of the nitro-group containing compound. For an example of the transitions analyzed in MRM mode, see Koske et al., 2019. Analysis of explosive compounds from marine biota samples is described in Koske et al. (in prep).

How to analyse and assess the data?

Quantification: The amount of the analyte present in the sample is quantified via the internal standard 1,4-DNB, added to the sample before extraction. Response factors for individual substances are determined through an external calibration. The standard mix used for calibration contains the following substances: HMX; RDX; DNBA; 1,4-DNB; TNT; 2,4-DNT; 2,5-DNT; 2-ADNT; 4-ADNT; TNAzoxyT and is analysed in concentration of: 5; 1; 0.1; 0.5; 0.01; 0.05; 0.001; 0 ng/µL and is routinely measured in duplicate.

Data: Data generated in the DAIMON project are available in the AMUCAD database provided by EGEOS.

References

Koske, D., Goldenstein, N.I., Rosenberger, T., Machulik, U., Hanel, R., Kammann, U., 2019. Dumped munition: New insights into the metabolization of 2,4,6-trinitrotoluene in Baltic flatfish. *in prep.*

Koske, D., Straumer, K., Goldenstein, N.I., Kammann, U., Lang, T., First evidence of munition compounds in fish caught near munition dumpsite, *in prep.*