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Method Article

Validation of an optimised protocol for quantification of microplastics in heterogenous samples: A case study using green turtle chyme

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ABSTRACT

Quantifying the extent of microplastic (<5 mm) contamination in the marine environment is an emerging field of study. Reliable extraction of microplastics from the gastro-intestinal content of marine organisms is crucial to evaluate microplastic contamination in marine fauna. Extraction protocols and variations thereof have been reported, however, these have mostly focussed on relatively homogenous samples (i.e. water, sediment, etc.). Here, we present a microplastic extraction protocol for examining green turtle (*Chelonia mydas*) chyme (i.e. ingested material and digestive tract fluid), which is a heterogeneous composite of various organic dietary items (e.g. seagrass, jellyfish) and incidentally-ingested inorganic materials (sediment). Established extraction methods were modified and combined. This protocol consists of acid digestion of organic matter, emulsification of residual fat, density separation from sediment, and chemical identification by Fourier transform-infrared spectroscopy. This protocol enables the extraction of the most common microplastic contaminants>100 μm: polyethylene, high-density polyethylene, (aminoethyl) polystyrene, polypropylene, and polyvinyl chloride, with 100% efficiency. This validated protocol will enable researchers worldwide to quantify microplastic contamination in turtles in a reliable and comparable way.

Abbreviations: SLS, sodium lauryl sulphate; PE, polyethylene; HDPE, high density polyethylene; PVC, polyvinyl chloride; PP, polypropylene; PET, poly(ethylene terephthalate); AM-PS, (aminoethyl) polystyrene; RO, reverse osmosis; ATR-FTIR, attenuated total reflectance Fourier transform-infrared spectroscopy.

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- Optimization of microplastic extraction from multifarious tissues by applying established methods in a sequential manner.
- Effective for heterogenous samples comprising organic and inorganic material.

ARTICLE INFO
Method name: Sequential protocol for extracting microplastics from heterogenous sample matrices
Keywords: Plastic ingestion, Marine debris, Plastic contamination, Extraction technique, Chemical digestion, Density separation, Fourier transform-infrared spectroscopy
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Specifications Table

Subject area	*Environmental Science*
More specific subject area	*Marine pollution*
Method name	*Sequential protocol for extracting microplastics from heterogenous sample matrices*
Name and reference of original method	*Nitric acid digestion:* Claessens, M., Van Cauwenberghe, L., Vandegehuchte, M.B., Janssen, C. R., 2013. New techniques for the detection of microplastics in sediments and field collected organisms. Mar. Pollut. Bull. 70, 227–233. doi:https://doi.org/10.1016/j.marpolbul.2013.03.009 *Density separation:* Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine environment: A review of the methods used for identification and quantification. Environ. Sci. Technol. 46, 3060–3075. doi:https://doi.org/10.1021/es2031505 *FTIR spectroscopy:* Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine environment: A review of the methods used for identification and quantification. Environ. Sci. Technol. 46, 3060–3075. doi:https://doi.org/10.1021/es2031505 Kroon, F., Motti, C., Talbot, S., Sobral, P., Puotinen, M., 2018. A workflow for improving estimates of microplastic contamination in marine waters: A case study from North-Western Australia. Environmental Pollution, 238, 26–38. Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at sea: Where is all the plastic? Science 304, 838. doi:https://doi.org/10.1126/science.1094559 Jung, M.R., Horgen, F.D., Orski, S. V, C, V.R., Beers, K.L., Balazs, G.H., Jones, T.T., Work, T.M., Brignac, K.C., Royer, S., Hyrenbach, K.D., Jensen, B.A., Lynch, J.M., 2018. Validation of ATR FT-IR to identify polymers of plastic marine debris, including those ingested by marine organisms. Mar. Pollut. Bull. 127, 704–716. doi:https://doi.org/10.1016/j.marpolbul.2017.12.061
Resource availability	Hardware: Extraction: • Aluminium trays and foil • Block heater (80 °C, model AIM500, SEAL Analytical) • Branson® 2200 Ultrasonic Clearer • Covered glass dishes (shallow, flat bottomed, 5 cm diameter) • Freezer (−20 °C) • Fridge (4 °C) • Glass beakers (200 mL) • Glass Pasteur pipette • Glass stirring rod • Glass test tubes (50 mL) • Glass Vacuum-filter apparatus (glass frit Buchner funnel with metal clamp and vacuum Erlenmeyer flask; Millipore) • Hot plate (50 °C, Stuart CB160) • Lint free tissue • Maggylamp (×2 magnification) • Metal bucket • Metal needles • Metal tweezers • Metal teaspoon • Millipore HA cellulose nitrate/acetate 0.45 μm pore membrane filters