

## Protocol

# Soxhlet extraction for isolating poly-halogenated terpenes from marine algae

By James Tannahill

Extraction using a Soxhlet extractor efficiently isolates compounds from algae and plant material. Soxhlet extraction is a semi-continuous method whereby the sample is automatically percolated with a desired solvent. Solvent is heated to evaporation. Solvent vapor enters the condenser, cools, and the new liquid percolates down to sample chamber. The chamber fills with solvent after several minutes. When sufficient solvent occupies chamber, the siphon triggers solvent to flow through sample and to the flask. This cycle repeats several times per hour. For each cycle, non-volatile compounds are dissolved in to the solvent. After ~ 50-70 cycles, a desired product is concentrated in the flask. Soxhlet extraction allows for solvent to be recycled through the sample.

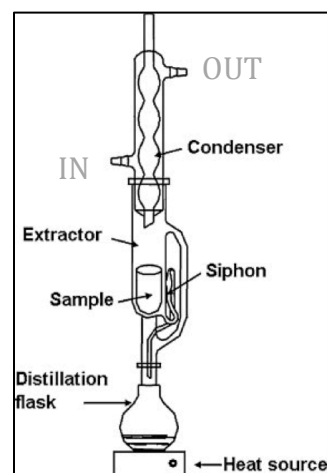
## MATERIALS

Reagents	Solvent	Dipole	Dielectric	Class	BP (°C)
	Hexane	0.00	1.9	NP	68.0
	Chloroform	1.04	4.8	NP	61.2
	Dichloromethane	1.60	9.1	PA	39.6
	Ethyl Acetate	1.78	6.0	PA	77.1
	Methanol	1.70	33	PP	64.7

NP=nonpolar;PA=polar aprotic;PP=polar protic (data from MOC)

## Equipment

Soxhlet extractor  
 Condenser (Allihn or Liebig)  
 Roundbottom flask;boiling flask ( $\leq 500$  mL)  
 Heat mantel;water bath  
 Rotovap  
 Cellulose thimble  
 Fume hood  
 Ice bath



## METHOD

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### Sample Preparation

1. Remove dried sample from 4° C and place at RT. Pulverize to a small size to increase surface area.
2. Load sample into thimble and weigh powder + thimble for each replicate.
3. Place sample + thimble into sample chamber; connect water and start heating.
4. Decant ~ 150 mL of desired solvent(s) to flask.

### Extraction

5. Reflux for ~ 8 to 12 hours at appropriate temperature (~ 60 °C).
6. Weigh cellulose thimble and compute % extraction for each replicate.

$$\% \text{ Extraction} = (m_1 - m_2 / m_1) \times 100$$

$m_1$  = thimble + sample before;  $m_2$  = thimble + sample after

7. Collect product and combine replicates of identical solvent runs.  
*Product may be recrystallized by setting flask in ice bath*

### Product

8. Save enough of each product for fractionation\*, bioassay, and LC-MS/MS.  
*\*Fractionation will require the greatest quantity of sample, followed by bioassay and MS/MS steps*

## REFERENCES

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Harwood, L.M., & Moody, C.J. (2012). Experimental organic chemistry: Principles and Practice. 122–5.

Master Organic Chemistry (MOC). (2016). All About Solvents: Polar Protic? Polar Aprotic? Nonpolar? Accessed on May 1, 2016 from [masterorganicchemistry.com/2012/04/27/polar-protic-polar-aprotic-nonpolar-all-about-solvents/](http://masterorganicchemistry.com/2012/04/27/polar-protic-polar-aprotic-nonpolar-all-about-solvents/).