Standard Operating Procedure

Lignin methoxy cleavage via the Zeisel method with liquid-liquid extraction of iodomethane

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A. PURPOSE

This is a protocol to cleave methoxy (-OCH_3) groups such as found on lignin molecules for targeted analyses on the methyl group (e.g., quantification or isotopic composition). The Zeisel method uses hydroiodic acid to cleave ether bonds and generates iodomethane (CH_3I), a volatile liquid at room temperature (boiling point 42°C). Here, we describe the experimental procedure to isolate iodomethane from lignin polymers or monomers, or lignin bearing substrates (e.g., wood, peat, lignite and coal) and to dissolve the evolved CH_3I in isooctane, ready for analysis via gas chromatography (GC).

B. NECESSARY MATERIALS

Reagents:

1. Hydroiodic acid (HI), 55% (H1020, Spectrum Chemicals, 25 mL size)
2. Potassium hydroxide (KOH), 5M
3. Isooctane, GC grade *(not lower grade, as pentane, hexane contamination in lower grades would interfere with the iodomethane analyte)*

Consumables:

4. Clear 2 mL GC crimp top vials (24383 Restek)
5. Al crimp caps, PTFE/rubber TF2 septum gas tight septa (21175 Restek)
6. Amber 2 mL GC screw top vials
7. Polyprolylene screw thread caps, PTFE-silicone-PTFE septa

Apparatus:

8. Fume hood – *all acid and solvent work to be performed in fume hood.*
9. Hot block, with GC vial holder
10. Microbalance
11. Centrifuge (optional), with GC vial holder
12. Micropipette, variable volume pipette and pipette tips, (100-200 µL range)
13. Cap crimper (23396 Restek 11mm size)/decapper (23397 Restek 11mm size)
14. 250 µL syringe dedicated to potassium hydroxide addition (cone-tipped)
15. 250 µL syringe dedicated to isooctane addition (flat-tipped)
16. 250 µL syringe for sample extraction (flat-tipped, gas tight)

C. PROCEDURE

Zeisel reaction and neutralization *(after Zeisel, 1885; Li et al., 2012; Feakins, et al., 2013)*

1. Use a microbalance to weigh the sample, then place in a clear crimp 2 mL vial.

2. Heat a hot block with GC vial holder to 120°C (takes ~30 mins to reach 120°C).

3. In a fume hood, use a micropipette (polyethylene tips) to add 100-150 μL of 55 % HI into each vial (HI in excess). Cap vials immediately with crimper tool.

4. React for 30 min at 120°C, cover with Al foil to block off light, lower fume hood sash.

5. Remove vials from the hot block and place in a vial rack and leave to reach ambient temperature (30 min), while covered with Al foil to block off light.

6. Use a syringe (dedicated to KOH) to add 150 μL of 5M $KOH_{(aq)}$ through the septum. Shake and let the vial cool for 30 sec (neutralization is exothermic). If solution is still red/pink, add 20-50 μL KOH, one drop at a time, until the color disappears.

Liquid-Liquid Extraction (following Baker, 1996)

7. Add 250 μL isooctane with a syringe (dedicated to isooctane) through the already pierced septum, then shake vigorously for 30 sec.

8. Let the aqueous (lower) and organic (top) layers settle, or centrifuge to aid separation.

9. Extract the top (isooctane) layer with a syringe (dedicated to samples) through the pierced septum, taking care to record the volume extracted, and transfer to an amber screw thread vial. Cap the vial (PTFE/silicone/PTFE septa).

10. Repeat steps #7-10 two more times adding a total of 750 μL isooctane. Record final volumes extracted, which denotes recovery. Cap the vial.

    Tip to simplify accounting while also obtaining consistent extraction fractions: if extraction volume 1 is 150 μL, extraction 2 is 220 μL, extraction 3 is 250μL, for a total of 620 μL and 95% fractional extraction.

11. Rinse the sample syringe 3 times from each of 3 vials of isooctane (9 rinses total) before proceeding to the next sample.

12. Start liquid-liquid extraction from step #7 for the next sample.

13. Capped GC vials containing iodomethane in isooctane are ready for GC analysis.

D. REFERENCES

Baker, S.M., 1996. Rapid methoxy analysis of lignins using gas chromatography. Holzforschung 50, 573–574.

Feakins, S.J., Rincon, M., Pinedo, P., 2013. Analytical challenges in the quantitative determination of $^{2}H/^{1}H$ ratios of methyl iodide. Rapid Communications in Mass Spectrometry 27, 430–436.

Li, H., Chai, X.S., Zhan, H., Liu, M., Fu, S., 2012. A Novel Method for Determination of Ethoxyl content in ethyl cellulose by headspace gas chromatography. Analytical Letters 45, 1028–1035.

Zeisel, S., 1885. Über ein Verfahren zum quantitativen Nachweise von Methoxyl. Monatshefte für Chemie 6, 989–997.