

Apple Dimer Purification

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Introduction

This is taken from taken from Li, C.; Trombley, J.D.; Schmidt, M.A.; Hagerman, A.E. Preparation of an acid butanol standard from fresh apples. *Journal of Chemical Ecology* 2010, 36, 453-460.

Procedure

1. Wash and dry Granny Smith apples (from the grocery store or market)
2. Cut the apple(s) into small cubes (200g can be used at once, however one apple typically weighs 170-190g cut, so cutting one apple at a time reduces browning)
3. Place the cut apple into a 1L round bottom flask and add four volumes of MeOH (the mass of the apples multiplied by four is the amount in mLs needed)
4. Reflux for 1.5hrs (setting "2" on our heating mantle will be enough to boil the MeOH without boiling over). You need an ordinary cold water condenser for the reflux step.
5. Remove the flask from the mantle, and filter the MeOH extract through a large number 1 Whatman filter paper in a conical funnel
6. Reflux with fresh MeOH two more times (the MeOH extract can be stored in the cold room until ready to evaporate under reduced pressure)
7. Reduce the volume of the extract to 1/10 the original volume by rotary evaporation (easiest to do this by starting with 300mL of extract in a 1L round bottom and continually add extract to the flask until the desired volume is reached)
8. While rotovaping wash the XAD-7 resin with water to remove any organic solvents
9. Add the concentrated extract and the XAD-7 resin to a 1L beaker and swirl for 45 mins
10. Remove the liquid by allowing the resin to settle, and pouring off as much liquid as possible without pouring off any resin (discard liquid from this step)
11. Add 200mL of H₂O and swirl for 45 mins, again remove as much liquid as possible by pouring off & discarding the liquid.
12. Repeat one more time, discarding the liquid
13. Add 200mL of EtOH to the resin and swirl for 45mins, remove the EtOH by vacuum filtration through a fritted funnel with filter paper on the frit. Keep the EtOH.
14. Rotovap the EtOH to dryness, then redissolve the solid material in the flask with 50mL of H₂O

15. Partition the water against an equal volume of ethyl acetate in a separatory funnel to select for the small phenolics, repeat two more times. At each extraction, keep the ethyl acetate (top layer) and re-extract the water (bottom layer).
16. Add a small volume of water to the ethyl acetate, and rotovap until the volume is less than that of the added water
17. Repeat steps 2-15 for as many apples as needed
18. Freeze dry the sample(s)
19. Dissolve approximately 0.1 to 0.2g of the crude phenolic material in 2mL of MeOH
20. Run on a Toyopearl column using MeOH as the mobile phase, and collecting approximately 5mL fraction (normally 80 fraction is more then enough)
21. Record the UV absorbance at 280nm for the fractions collected
22. Run HPLC of the highest absorbing fraction for each peak seen at 280nm to assess peaks.
23. Combine the fractions of the correct 280nm peak for each Toyopearl run
24. Add 50mL H₂O and rotovap to a volume less then 50mL
25. Freeze dry the sample
26. Run HPLC-MS and compare to published spectra to confirm identity of purified material