Enrichment of supernatants/rotary evaporator

1. fill up samples with MQ-water to 30 ml
2. adjust pH with hydrogen chloride (HCl) to a pH under 4 (to protonate the samples)
3. fill up samples with MQ-water to 50 ml
4. use a separatory funnel for a 1:1 dilution in ethyl acetate
   -> 50 ml sample in 50 ml ethyl acetate
   Attention: close the stopcock before filling in solutions!
5. close separatory funnel with the stopper, invert and shake it
   Attention: carefully open the stopcock from time to time to release gas! Don't train the bottom tap on people!
6. the organic phase (upper phase) and the aqueous phase (lower phase) are visible
7. drain out the aqueous phase into a measuring cylinder
   -> note volume
8. drain out the organic phase into a round bottom flask
9. close stopcock of the separatory funnel and fill in the aqueous phase again
10. add the same volume of ethyl acetate
11. act as in step 5
12. pour the aqueous phase away and drain out the organic phase into the flask of step 8
13. fold the filter and put it into a cone
14. fill up the filter with sodium sulfate up to the half
    Attention: Sodium sulfate should only be filled into the filter, not aside from the filter!
15. Pour the organic phase onto the sodium sulfate and let it run through
16. Discard the filter
17. Repeat the last 4 steps
18. Dehydrated samples were loaded onto the rotary evaporator
    -> attach the round bottom flask with a clamp to the evaporator
    Attention: ensure that the clamp does not touch the edge of the water bath!
19. Choose following settings: temperature: 40°C, pressure (P): 200 mbar, pressure difference (ΔP) 30 mbar
20. Use the lever arm to bath the round bottom flask in the water
    Attention: the water level should be above the sample's liquid level
21. Close the tap of the vacuum pump (on the left side), start the rotor
22. Wait for the temperature reaching 40°C, then lower the pressure by pressing the "STOP" button, adjusting the pressure with the arrow key and pressing the "START" button
    Attention: lower the pressure in 20 mbar steps until 150 mbar, then use 10 mbar steps
23. Evaporate the sample for about 40 min
24. Check if the sample's liquid is evaporated: Take the flask out of the water bath, by pushing the lever arm upwards. Dry the flask paper cloth. If the inner of the flask is still wet, evaporate further.
25. If the flask is dried turn off the rotary evaporator: First turn off the rotor and then the vacuum pump. Open the tap of the vacuum pump (on the left side). Remove the clamp.
26. Prepare speed vacuum concentrator (Speed VaC) tubes by weighing every tube with its lid on the special accuracy weighing machine

-> labes tubes with this weight!

Attention: transparent tubes belong to green lids and brown tubes belong to black lids!

27. resuspendate the sample in 2 x 100µl methanol and transfer it into a Speed VaC-tube
    -> label tube on the white area!
28. resuspendate the sediment in 150 µl DMSO
29. transfer sample in MS-tubes with glass (-> conic form)
30. store the MS-tubes in the MS-fridge at 4°C