

# **HPLC** Analysis Cheat Sheet

by MJC3 via cheatography.com/212269/cs/46152/

#### Overview

- 1. Weigh tablet Record to 4 significant figures (e.g., 1.000 g).
- 2. Grind tablet to fine powder.
- 3.**Transfer** powder to 10 mL volumetric flask
- 4.Add ~5 mL mobile phase, do not shake.
- 5. Sonicate for 5 mins.
- 6.**Top up** to 10 mL mark with mobile phase, then mix.
- 7. Filter into a beaker using syringe + filter disc.
- 8.Pipette 50 µL into 20 mL flask.
- 9.Fill to 20 mL with mobile phase, mix.
- 10.**Transfer** ~1 mL to HPLC vial for analysis.

### Transfer TP to volumetric and add MP

- 1.Transfer your tablet powder into the **10 mL volumetric flask**.
- 2.Use a small amount of mobile phase (1-2 mL) to wash any powder left on the weighing boat into the flask.
- 3.Add approximately **5mL of the mobile phase** to the volumetric flask.
- 4.Do not stir or shake the flask at this step!
- 5. Sonicate the flask for 5 minutes
- 6.While your sample is in the sonicator you can start to prepare your next sample (if required).
- 7.The mobile phase is irritant so please wear gloves and goggles. Wash off immediately if it comes into contact with skin.

# Full Method

- 1. Put on gloves and goggles
- 2. Weigh the tablet record to 4 significant figures.
- 3.Calculate the required amount of powdered tablet that contains 200 mg paracetamol
- 4.Crush the tablet (try to keep powder towards centre of the bowl)
- 5. Tare the balance with the weighing boat. Transfer as accurately as possible the calculated amount of powder to the weighing boat.
- 6.Add the powder to the 10 mL flask, wash any residual power into the flask with mobile phase
- 7.FILL THE FLASK HALFWAY with approximately 5 mL of mobile phase and do not shake the content!
- 8. Take your sample to the sonicator.
- 9.Once sonicated, fill your flask to the 10 mL line and mix it.
- 10.Filter your sample and put 50  $\mu$ L in the 20 mL flask using a mechanical pipettor.
- 11.Fill the 20 mL flask to the line with mobile phase, mix and transfer to HPLC vial.

#### Dilution in 20ml flask

TRANSFER: Collect 50 µL of filtrate and

transfer into the 20 mL volumetric flask.

- 1. Put a tip on the pipettor
- 2. Push the pipette plunger down to the first stop position and hold.
- 3.Insert the tip into the filtered solution, draw plunger back to the top, once the tip is filled take it out of the solution.
- 4.Push the pipette plunger down all the way to the 2nd stop position to release all the solution into the 20 mL flask.

FILL: Fill the flask up to 20 mL line

with the mobile phase

MIX: Mix thoroughly

TRANSFER: Transfer approximately 1

mL of the solution to the HPLC vial using the

pipettor.

**Note:** as you are filling the flask make sure you are looking at the line at the **eye level** to avoid parallax



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# Weigh and Grind tablet

**WEIGHING:** tare the balance with the weighing boat on it, then add 1 tablet and record this value to **4 significant figures**, e.g., 1 g = 1.000 g or 0.12 g = 0.1200 g

CALCULATION: :Mass required=Using this equation(Mass of thecalculate thetablet )/(250  $\square$ amount of powder $\square g$ ) x 200  $\square$ required $\square g$ 

\*Mass of the tablet is the actual value weighed and recorded which indicates the actual amount in the weighing boat.

**GRINDING:** when crushing the tablet try not to spread the powder onto the walls as this will make collection harder for the next stage

# Fill up to the 10 mL line and mix.

Fill up to 10 mL line with mobile phase Now you can mix it by shaking.

Note: Note: as you are filling the flask make sure you are looking at the line at the eye level to avoid parallax.

Filter the solution

- 1.Transfer the content of the 10 mL flask into the larger beaker.
- 2.Draw up some of the solution into the syringe without the filter attached.
- 3. Screw the filter disc on the syringe
- 4. Filter the solution into the small beaker.

Note: the syringe is quite stiff with the filter disk on, so don't be afraid to push hard.



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