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 µL 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: : Using this equation calculate the amount of powder required

$$Mass\ required = (Mass\ of\ the\ tablet) / (250\ \square\square g) \times 200\ \square\square g$$

*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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