

High Performance Liquid Chromatography

Introduction

This protocol describes the preparation of drug stock solutions for preparation of standards for quantitation of drug using reversed-phase HPLC or UPLC with UV/Vis detection.

Definitions

HPLC: High performance liquid chromatography

UPLC: Ultraperformance liquid chromatography

UV/Vis: Ultraviolet/Visible wavelength

Reagents and Materials

- Amber glass vials with caps (8 mL and 4 mL)
Manufacturer/Source: Fisher Scientific
- Disposable antistatic microspatulas
Manufacturer/Source: VWR Scientific
- Drug powder:
Manufacturer: In house (prodrugs) or BOC sciences (parent drug)
 - Drug _____, Lot/Batch # _____, Date Mfc _____
_____, Amount Weighed _____, Vial label _____
 - Drug _____, Lot/Batch # _____, Date Mfc _____
_____, Amount Weighed _____, Vial label _____
 - Drug _____, Lot/Batch # _____, Date Mfc _____
_____, Amount Weighed _____, Vial label _____
 - Drug _____, Lot/Batch # _____, Date Mfc _____
_____, Amount Weighed _____, Vial label _____
- HPLC grade methanol (A452-4, 4L)
Manufacturer: Fisher Scientific
- 50 mL screw-cap conical tube (polypropylene; Falcon # 352098)
Source: Fisher Scientific
- Parafilm
Manufacturer:
- Pipetmen (LTS or Classic)
P1000
P200
Manufacturer: Rainin

- Pipet tips
P1000
P200
Manufacturer: Rainin

Instrumentation

Mettler Analytical scale

Sonicator bath

Manufacturer: VWR B1500A-DTH,

Alternative Manufacturer: Branson 3510

Vortex mixer

Manufacturer: Scientific Industries Vortex Genie2

Protocol

For 1.0 mg/mL ultimate stock

1. Weigh out 2-7 mg drug powder with balance on 4th floor using antistatic microspatula into 8 mL amber glass vial
2. Carefully add enough HPLC-grade Methanol to vial to create a 1.0 mg/mL ultimate stock;
 - a. **Note:** saturate pipette tip thoroughly by pipetting up and down twice before adding to vial to avoid dripping
3. Cap the vial and vortex 10 seconds at high speed to mix and ensure all drug powder is dissolved in the methanol
4. Place in sonicating bath for 1 minute to help dissolve any remaining drug then vortex 10 seconds at high speed to mix
5. Label vial with: ***Drug name; 1 mg/ml in methanol; date; your initials***

For 200 ug/mL working stock

6. Carefully add 800 uL HPLC grade Methanol to a 4 mL glass amber vial;
 - a. **Note:** saturate pipette tip thoroughly by pipetting up and down twice before adding to vial to avoid dripping
7. Carefully add 200 uL of 1.0 mg/mL drug stock to the methanol in the 4 mL vial;
 - a. **Note:** saturate pipette tip thoroughly by pipetting up and down twice before adding to vial to avoid dripping
8. Pipette the solution up and down several times to rinse tip after adding to methanol;
9. Cap and vortex 10 seconds at high speed to mix
10. Label vial with: ***Drug name; 200 µg/ml in methanol; date; your initials***
11. Tightly wrap caps of both vials with parafilm to ensure they are tightly sealed; store each at -80°C