Protocol for Preparation of Formulation for Drug Quantitation by HPLC-UV/Vis

Introduction

This protocol describes the processing of nanoformulation samples for quantitation of drug using reversed-phase HPLC with UV/Vis detection. This is a standard method of drug quantitation and has been used in our numerous publications for formulation quantitation. The mobile phase used for separation and the wavelength used for detection are compound specific.

Definitions

HPLC: High performance liquid chromatography
UV/Vis: Ultraviolet/Visible wavelength
PTFE: Polytetrafluoroethylene

Reagents and Materials

- Drug formulation prepared in the laboratory:
  Manufacturer: In house (Record formulation name and manufacture date)

- HPLC grade methanol (A452-4, 4L)
  Manufacturer: Fisher Scientific

- 50 mL screw-cap conical tube (polypropylene; Falcon # 352098)
  Source: Fisher Scientific

- 1.7 ml microcentrifuge tubes, snap cap (catalogue # 05-408-129)
  Manufacturer: Fisher Scientific

- Waters maximum recovery glass vials (12x32, 9mm, screwneck, Lectrobond cap, preslite, PTFE silicone septa, part # 186000327c)
  Manufacturer: Waters Inc.

- HPLC grade water (18 ohm)
  Source: Millipore water filtration system in laboratory
  Alternative source: Fisher Scientific (W5-4, 4L)

- Pipetmen (LTS or Classic)
  P1000
  P200
  Manufacturer: Rainin

- Pipet tips
  P1000
  P200
  Manufacturer: Rainin

- Ice pack (8-12 oz)
  Source: ULINE Cold Pack S-7361, 8 oz)

Instrumentation

Refrigerated microcentrifuge
Manufacturer: Eppendorf 5430R or 5417R

Sonicator bath
Manufacturer: VWR B1500A-DTH,
Alternative Manufacturer: Branson 3510

Vortex mixer
Manufacturer: Scientific Industries Vortex Genie2

HPLC system (Waters Alliance HPLC System with TUV detector)
Manufacturer: Waters Inc.

Protocol

1. Pour 10 mL HPLC grade methanol from stock bottle into a 50 mL conical tube
2. Dilute formulations 100-fold in HPLC grade methanol by adding 10 µL sample to 990 µL fresh HPLC grade methanol in 1.5 ml microcentrifuge tube
3. Sonicate samples for 8 minutes in water bath sonicator; place small ice pack into water bath to avoid high temperatures from sonication
4. Vortex each sample for 10 seconds at highest speed to ensure it is well mixed
5. Centrifuge samples at 20,000 rcf for 10 minutes at 4°C
6. In clean microcentrifuge tube, dilute sample supernatant **either**:
    a. 10-fold (100 µL sample into 900 µL fresh HPLC grade methanol) **or**
    b. 100-fold (10 µL sample into 990 µL fresh HPLC grade methanol)
    Note: dilution is based on target drug concentration range (middle of standard range of 0.048 µg/mL –50 µg/mL) and expected drug concentration in formulation based upon drug and polymer amounts added to the formulation mixture before manufacture
7. Vortex 10 seconds on highest speed to mix thoroughly
8. Pipet 80 µL of sample into glass maximum recovery HPLC vial
9. Carefully cap each vial
10. Flick vial with finger to make sure there is no air bubble at the bottom of the vial
11. Place vial into HPLC autosampler tray (Waters Alliance HPLC-TUV System) for analysis (see protocol for HPLC-UV/Vis quantitation)