Quantitation of Drug by UV/Visible Wavelength Detection

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Introduction

This protocol describes the processing of cell extract 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 cell extract quantitation. The mobile phase used for separation and the wavelength used for detection are compound specific.

Definitions

UPLC: Ultraperformance liquid chromatography
UV/Vis: Ultraviolet/Visible wavelength

Reagents and Materials

- Frozen cell extracts from experiment:
  Source: In house experiment (Record cell experiment and date)

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

- 70% Ethanol
  Manufacturer: prepared in-house from 200 proof ethanol using HPLC-grade water

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

- 96-Well round bottom plate (Fisherbrand; cat # 12565500)
  Manufacturer: Fisher Scientific

- 96-well plate cover (Polypropylene Mat Caps; cat # 186002483)
  Manufacturer: Waters Inc.

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

- Pipet tips
  P1000
  P200
  Manufacturer: Rainin

- Microcentrifuge tube rack
  Manufacturer: Fisher Scientific or USA Scientific

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

- Kimwipes

- 250 ml glass beaker

Instrumentation

QSonica Ultrasonic Processor with microtip sonication probe (Model CL-188)
Manufacturer: Cole Parmer

Refrigerated microcentrifuge
Manufacturer: Eppendorf 5430R or 5417R

Vortex mixer
Manufacturer: Scientific Industries Vortex Genie2

UPLC-TUV system (Waters Aquity H-class UPLC with TUV detector)
Manufacturer: Waters Inc.

Protocol

1. If samples have been stored, remove samples from -80°C freezer and let warm to Room temperature on bench in microcentrifuge rack
2. Pour 10-20 mL fresh HPLC grade methanol from the bottle into a 50 mL conical tube
3. Add 200 µL HPLC grade methanol to each sample tube
4. Sonicate each sample for 2 seconds each using the sonication probe set at 20% amplitude
5. Between each sample:
    a. Rinse probe with 70% ethanol using a squirt bottle into a waste beaker
    b. Clean by immersion in 100% HPLC-grade methanol and sonication for 2-5 seconds
    c. Wipe dry with Kim Wipe
6. After sonication, vortex each sample for 10 seconds at high speed to ensure each is well mixed
7. Centrifuge samples at 20,000 rcf for 10 minutes at 4°C
8. Prepare standard curve for UPLC (see UPLC/HPLC stds protocol)
9. Pipet 75 µL of each standard and sample supernatant into a separate well of a 96-well plate; record what standard or sample is in each well
10. Carefully cover plate with 96-well plate cover; make sure to avoid any cross contamination between samples
11. Place plate in UPLC-TUV Sample Manager FTN for analysis