MILLIPORE

Protocol note

Date: **April 2004**
Lit. No.: **PC1052EN00**
Title: **MultiScreen® Deep Well Solvinert Filter Plate: Automated Protein Precipitation Method**

Overview

To determine the total concentration of a drug in biological samples, current analytical methods require extraction of the compound from plasma, serum or other biological medium prior to analysis. Total Drug Analysis is typically accomplished with the addition of the biological fluid to a water miscible organic solvent such as acetonitrile or methanol. The solvent precipitates interfering species such as proteins and salts, as well as solubilizes the compound. Precipitated species are removed via centrifugation or filtration to provide the compound in a protein-free solution suitable for analysis by HPLC-UV or LC-MS/MS.

The MultiScreen Deep Well Solvinert filter plate has been developed and optimized for use in a wide range of aqueous and organic sample applications with a volume of ≤ 2 mL including total drug analysis and sample preparation prior to HPLC. The plate has been designed, tested and proven to retain a wide range of organic solutions over extended incubations without leakage. The device is compatible with both vacuum and centrifugal modes of filtration and is designed to fit with standard 96-deep well receiver blocks for use in filtrate collection. It enables discrete and complete filtrate transfer without cross talk. It has excellent flow properties, exhibits low non-specific binding (NSB), and has little or no UV or Mass Spectrometry (MS) detectable or interfering extractable components. The device meets published standards 2004 ANSI-SBS 1,2 and 4 for dimensional guidelines and tolerances for automation compatibility.

The total drug analysis assay in the MultiScreen Deep Well Solvinert filter plate with supporting methodology produces 96 samples for the determination of (total) drug in a biological sample, typically serum, following protein precipitation and filtration. The device is designed for in-plate mixing and incubation of total sample volumes ranging from 0.35 to 1.5 mL. The automated filtration-based assay as described below is robust, reliable and precise, capable of total sample volumes to 1.25 mL.

Materials and Methods

Reagents

- Acetonitrile
- Serum

Materials

- MultiScreen Deep Well Solvinert filter plates with the hydrophobic PTFE membrane with a prefilter (cat #MDRP NP4 10)
- Deep Well collection plate
  - Greiner Deep Well Receiver Plate #780285 is recommended
- Robotic liquid handling system
- 1000 mL disposable tips
- Pipettor or Multichannel pipettor such as
  - Biohit™ Proline 1200 µL multichannel pipettor #710800
  - 1200 µL pipette tips #780043 (Biohit—Helsinki, Finland; Fisher catalog # 21-165-20 and 21-165-53) are recommended if using the in-tip mixing procedure.

Equipment

- Multiscreen Vacuum Manifold #MAVM 096 0R with the stainless steel grid removed.
- Multiscreen Deep Well Ring with Gaskets #MAVM 096 0T
- Plate shaker
  - Lab-Line titer plate shaker #4625 (Lab Line—Dubuque, IA)

General Method

A general protocol for automated sample preparation for total drug analysis from plasma or serum samples using a deep well plate is as follows:

1. Add 1.0 mL of acetonitrile to all wells of the Deep Well Solvinert filter plate. (Note 1)
2. Draw 250 µL of serum or plasma into the tips.
3. With the serum or plasma in the tips, draw 250 µL of ACN into the tips from the corresponding wells of the MultiScreen Deep Well filter plate. (See Note 2)
4. Immediately dispense the mixture into the wells containing the remaining 750 µL of ACN.
5. Mix for 2 minutes on a plate shaker. (**Note 3**)
6. Filter at maximum vacuum (> 18" Hg), leaving the vacuum on for approximately an additional 30 seconds upon complete filtration.

Note 1: User controlled variables include solvent selection and ratio of solvent to plasma/serum. An excess of an organic solvent is ideal for protein precipitation and compound extraction, optimized conditions include a 4:1 ACN:Serum ratio with a maximum volume of 1.25 mL. Use of other solvents such as methanol will require optimization for reliable performance.
Note 2: The "in-tip" procedure requires both plasma and ACN in the pipette tips, in which mixing and precipitation initiates immediately, resulting in a cloudy solution in the tip. Transfer of the precipitating solution into the corresponding wells should be immediate.
Note 3: RPM settings for shakers can vary from instrument to instrument. It is recommended to shake as rapidly as possible without spilling.

- All 96 wells of the device must be filled to an equal volume to ensure complete filtration, if only a portion of wells are used all empty wells must be sealed with a mat cap

(www.marchbio.com AB-0566), sealing tape (Corning #6569) or equivalent to ensure good vacuum.

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