Rapid Sample Preparation Method for High Throughput Total Drug Analysis by LC-MS/MS

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Abstract

An automation compatible, high throughput sample preparation method for total drug analysis from serum or plasma samples in 96-well MultiScreen® Deep Well Solvinert filter plates is demonstrated. Following in-plate protein precipitation, incubation and filtration, the filtrates were analyzed by LC-MS/MS to assess correlation and reproducibility for three different compounds (warfarin, propranolol and testosterone) in adult bovine serum. This filtration-based method provides comparable results to centrifugation with advantages of ease, speed, reproducibility, and consistent removal of precipitated protein. Furthermore, the impact of any extractable species on LC-MS/MS and LC-UV analyses is presented in which analytes extracted from plasma have been shown to be unaffected at concentrations as low as 1 nM.

Introduction

Requiring minimal method development, protein precipitation from serum or plasma with an organic solvent is a preferred sample preparation technique for total drug analysis in a high throughput setting. Samples are often centrifuged to remove precipitated species and the supernatant is extracted for analysis. The method requires careful sample handling to avoid interference from the precipitated protein with analysis. This process can be labor-intensive and may or may not be automation compatible.

Alternatively, protein precipitation can be performed in a solvent-resistant 96-well filter plate (MultiScreen Deep Well Solvinert plate). After protein precipitation and incubation in the device, vacuum filtration separates the filtrates from the precipitated proteins, providing clear filtrates from all 96 wells. This process is readily integrated into automated analytical techniques.

Experimental

General procedure for protein precipitation and filtration: To a MultiScreen Deep Well Solvinert plate (catalog # MDRP NP4 05), 800 µl of acetonitrile was added. Adult bovine serum (200 µl) spiked with drugs was pulled into the pipette tip followed by 200 µl of acetonitrile from the 800 µl contained in the appropriate well(s) of the Deep Well plate to initiate precipitation. The 1:1 ACN:serum solution was added to the 600 µL of acetonitrile remaining in the well(s) to afford a final 4:1 ACN:serum mixture with a total volume of 1 mL. The plate was shaken for 2 minutes, incubated at 4 °C for 1 hr[1], then filtered at 20 "Hg or higher.

Three drugs, testosterone, propranolol, and warfarin, were tested at initial concentrations in the serum of 0.1 µM, 0.5 µM, 1 µM, 5 µM, and 10 µM.

Centrifugation: All protein precipitation, incubation and centrifugation steps were performed in a Greiner polypropylene 96 well Masterblock® plate in a Jouan CR 312 Centrifuge at 2000 x g for 5 minutes.

Standards: Analyte of interest was spiked into a 4:1 ACN:serum mixture that had been filtered through the MultiScreen Deep Well Solvinert plate to the appropriate concentration.

All samples were diluted with an equal volume of water after filtration and prior to LC-MS/MS analysis. Thus, final analyte concentration injected to LC-MS/MS is one-tenth of the initial drug concentration in serum. Each concentration was studies in 6 replicate wells with 3 injections per well.

LC-MS/MS Method

LC-MS/MS analyses were performed using a Sciex® API-2000 mass spectrometer coupled with an Agilent 1100 HPLC and well plate autosampler. A Phenomenex Synergi Hydro-RP (4 µm, 50x2 mm) C-18 column was used with a guard cartridge. For ESI-MS, Solvent A was 0.1 % formic acid in water, solvent B was 100 % methanol. For APCI, solvent A was water, solvent B was 100 % methanol.

Warfarin: Injection volume: 15 µL/sample, flow rate = 300 µL/min, HPLC solvent of 80 % A to 10 % A in 4 min, then to 80 % A in 1 min. A TurboIonSpray (ESI) source was used in the positive mode with MS/MS monitored at m/z 309/163.

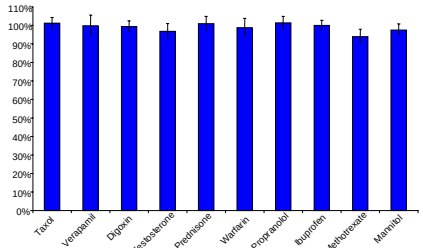
Propranolol: Injection volume: 5 µL/sample, flow rate = 300 µL/min, HPLC solvent of 100 % A for 2 min, gradient to 40 % A in 3 min, then returns to 100 % A in 1 min. A TurboIon-Spray (ESI) source was used in the positive mode with MS/MS monitored at m/z 260/116.

Testosterone: Injection volume: 30 µL, flow rate = 500 µL/min, 50 % A to 0 % over 2.5 min, 0 % A to 50 % over 1.5 min, then remained at 50 % A for 2 min. A Heated Nebulizer (APCI) source was used in the positive mode with MS/MS monitored at m/z 289/97.

MultiScreen Deep Well Solvinert Filter Plate

- Hydrophilic and hydrophobic 0.45 µm PTFE membrane
- With and without 5 µm polypropylene prefilter
- 1.9 mL total volume capacity per well
- Filtration via vacuum or centrifugation
- Automation compatible
- Precipitation/incubation/ filtration all in one plate

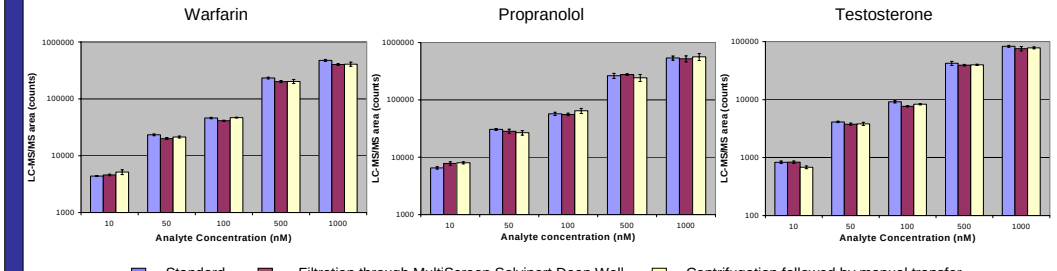
Drug recovery



A panel of ten radio labeled compounds at 1 µM in 4:1 ACN:H2O were analyzed for drug recovery in a Multiscreen Solvinert Deep Well Filter plate. Compound recovery is shown to be > 94% for all compounds examined.
For further details, please see: http://www.millipore.com/catalogue.nsf/docs/c8871

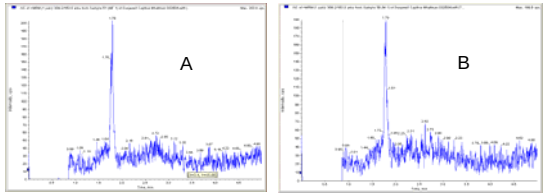
[1] Polson, C.; Sarkar, P.; Incledon, B.; Raguvaran, V.; Grant, R. Optimization of protein precipitation based upon effectiveness of protein removal and ionization effect in liquid chromatography–tandem mass spectrometry, *J. Chrom. B*, **2003**. *785*, 263-275.

Comparison of MultiScreen Deep Well Solvinert Filtration and Centrifugation/Manual Transfer

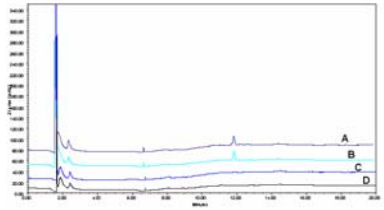


Drug recovery at different concentrations by filtration through MultiScreen Deep Well Solvinert filter plates as compared to centrifugation followed by manual transfer is illustrated. Coefficient of variation: *warfarin:* standard 1.9~4.1 %; filtration 2.6~4.0 %; centrifugation 1.7~9.6 %; *propranolol:* standard 3.5~10 %; filtration 3.8~12 %; centrifugation 4.3~14 %; *testosterone:* standard 2.9~8.3 %; filtration 2.7~8.6 %; centrifugation 2.0~6.2 %.

Study of impact from extractable species on LC-MS/MS and HPLC-UV

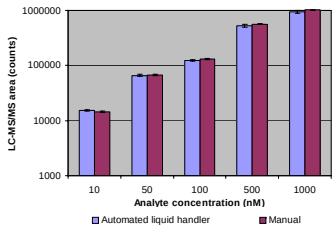


Raw LC-MS/MS chromatograms of warfarin is shown above: Ultrafiltrate of ACN and serum (4:1, v/v) was obtained. Portion of this was spiked with 1 nM warfarin, diluted with equal volume of water and analyzed with LC-MS/MS (A). Another portion was spiked with 1 nM warfarin, filtered through MultiScreen Deep Well Solvinert, diluted and analyzed (B). ***No ion suppression or other interference from extractable species is detected in the analysis.***



HPLC-UV Chromatograms (214 nm) for warfarin is shown above: Chromatogram A: control sample for warfarin at 5 µM in 80 % acetonitrile; B: Warfarin at 5 µM in 80 % ACN after 1 hr incubation and filtration through the device; C: 80 % ACN after 1 hr incubation and filtration through the device (extractables); D: 80 % ACN only. ***No interference from extractable is detected in the analysis.***

Automation Compatibility (warfarin)



For further details, see http://www.millipore.com/publications.nsf/docs/pc1052en00

Conclusions

A method for total drug analysis from plasma with analysis by LC-MS/MS has been developed using the MultiScreen Deep Well Solvinert filter plate that is:

- Solvent resistant
- Precipitation/incubation/filtration all in one plate
- Particulate free filtrate
- Comparable to existing centrifugal methods
- automation compatible
- Low binding, high recovery
- No interfering extractable to LC-MS/MS or HPLC-UV