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

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Abstract

Presented herein is an automation compatible, high throughput method for the determination of drug concentrations in serum or plasma samples. The MultiScreen® Deep Well Solvinert filter plate permits in-plate mixing and incubation of acetonitrile and serum to completely precipitate serum proteins. Filtration through the 0.45 µm PTFE membrane yields a protein-free solution with complete sample recovery for seamless integration with LC-MS/MS detection. Recoveries of three drugs (warfarin, testosterone and propranolol) from bovine serum are shown to be reproducible by LC-MS/MS over a broad concentration range with the deep well filter plate using Tecan Genesis®, Hamilton MICROLAB® STAR and Beckman Multimek™ automation systems. Correlation of automated methods to manual and centrifugal methods is presented and illustrates that sample preparation for LC-MS/MS analysis using a deep well filter plate is a fast and effective alternative to centrifugation.

Introduction

Determining the concentration of drug in plasma or serum at various time points after administration is necessary to calculate the pharmacokinetics (PK) of a drug. Quantitative analysis of drug concentrations in plasma or serum samples using LC-MS/MS requires sample preparation using methods such as protein precipitation, solid phase extraction or liquid/liquid extraction. Protein precipitation using a water-miscible organic solvent such as acetonitrile is preferred in a high throughput setting due to the minimal requirements for method development. Centrifugation has been the traditional method to separate precipitated proteins; however, separation by filtration has distinct benefits not offered by centrifugation, including automation compatibility and greater recovery of the protein free solution. Filtration using a 96-well plate can improve data quality by completely separating precipitated proteins from the analyte. This is important because even the slightest particulate contamination during sample preparation can have a detrimental impact on the LC-MS/MS analysis.

Experimental Methods

- Warfarin, Propranolol and Testosterone drug solutions were prepared by serial dilutions in serum to 10, 5, 1, 0.5 and 0.1 µM in a deep well block.
- Manual protein precipitation method: A Biohit Proline™ multichannel pipettor was used to add 1000 µL of acetonitrile to each well of a MultiScreen Deep Well Solvinert filter plate (#MDRPNP410). Using the pipettor's double aspiration program, 250 µL of the spiked serum solution was aspirated from the deep well block first, followed by 250 µL of acetonitrile from the filter plate to initiate protein precipitation in the pipette tip. The mixture was added back to the filter plate, which was then shaken vigorously for 2 minutes. Vacuum filtration was accomplished at >18″ Hg.
- Automated protein precipitation method: The previously described protein precipitation method was carried out separately on Tecan Genesis, Hamilton MICROLAB STAR and Beckman Multimek workstations. The Tecan and Hamilton robots were capable of running the entire protocol, while the Beckman robot required shaking and vacuum filtration offline.
- Centrifugation: Manual protein precipitation was carried out in a deep well block, shaken vigorously and spun in a Jouan CR 3.12 centrifuge at 2000 x g for 2 minutes.

LC-MS/MS Methods

•All samples were diluted with an equal volume of water after filtration prior to LC-MS/MS analysis. Final analyte concentrations injected to LC-MS/MS were 1000, 500, 100, 50 and 10 nM. The five concentrations of each drug were analyzed in 6 replicate wells per plate.

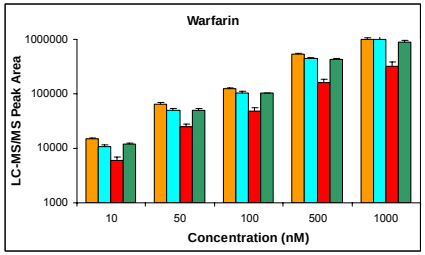
•LC-MS/MS analyses were performed using a Sciex API-2000 mass spectrometer coupled with an Agilent 1100 HPLC and well plate autosampler. A Phenomenex Synergi Hydro-RP (4 mm, 50x2 mm) C-18 column was used with a guard cartridge. For ESI-MS, Solvent A was 0.1% formic acid in water, solvent B was 100 %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

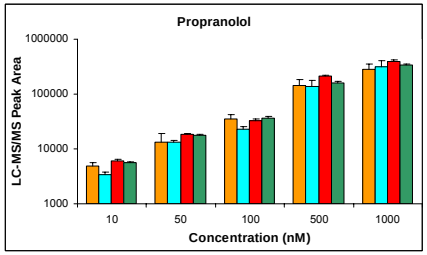
Warfarin: Injection volume: 15 µL/sample, flow rate = 300 µL/min, HPLC solvent of 80 % A to 10 % A in 4 min, then to 80 % A in 1 min. A TurbolonSpray (ESI) source was used in the positive mode monitored at m/z = 309/163.

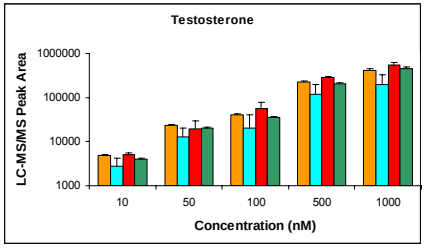
Propranolol: Injection volume: 5 µL/sample, flow rate = 300 µL/min, HPLC solvent of 100 % A for 2 min, gradient to 40 % A in 3 min, then returns to 100 % A in 1 min. A TurbolonSpray (ESI) source was used in the positive mode monitored at m/z = 260/116.

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 monitored at m/z = 289/97.

Comparison of Drug Recoveries from Filtrates Following Automated Protein Precipitation, Filtration and LC-MS/MS Analysis







Three drugs were spiked into bovine serum followed by protein precipitation and filtration through MultiScreen Deep Well Solvinert filter plates. The assay was carried out manually and on three robotic workstations. Drug concentrations in the filtrates were analyzed by LC-MS/MS and are compared. The linearity of results for both automated and manual methods (R^2 values ≈ 1) demonstrates that drug samples in serum can be reliably prepared by precipitation and filtration in a MultiScreen Deep Well Solvinert filter plate. The high degree of linearity indicates that filtrates generated following this procedure allow accurate LC-MS/MS analysis across a wide concentration range.

= Tecan = Hamilton
= Beckman = Manual

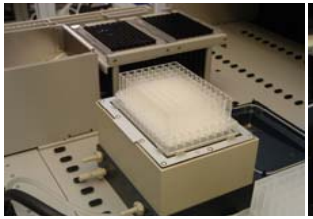
Drug	Linearity (R^2)	
	Automated	Manual
Warfarin	T = 0.997 H = 0.997 B = 0.996	0.999
Propranolol	T =0.999 H =0.998 B =0.998	0.999
Testosterone	T =0.998 H =0.990 B =1.000	1.000

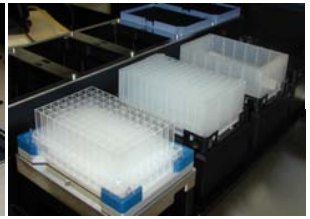
T = Tecan H = Hamilton B = Beckman

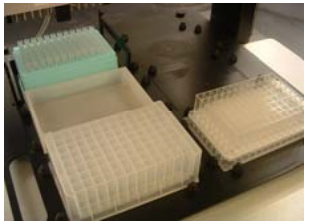
MultiScreen Deep Well Solvinert Filter Plate

- 96-well automation compatible filter plate
- 0.45 µm PTFE membrane with a 5 µm polypropylene pre-filter for fast flow and removal of particulates
- Compatibility with and ability to incubate organic solvents
- Clear plate construction for sample viewing
- Elevated spout design prevents contact with robot decks and plate shakers
- High drug recovery for reliable, accurate results
- Non-interfering levels of extractable compounds

Sample Preparation Using a MultiScreen Deep Well Solvinert Filter Plate is Compatible with Multiple Automation Platforms







Sample preparation for high throughput total drug analysis using a MultiScreen Deep Well Solvinert filter plate can be achieved using multiple automation platforms including the Tecan Genesis (top left), Hamilton MICROLAB STAR (above) and Beckman Multimek (left) workstations.

Conclusions

Sample preparation for high throughput Total Drug Analysis using filtration through a MultiScreen Deep Well Solvinert filter plate has several noteworthy features:

- ➢Filtrates generated are free of particulates and are ready for LC-MS/MS analysis
- ➢The filter plate is fully compatible with multiple automation platforms
- ➢Samples generated are highly linear across a broad concentration range
- ➢Manual and automated methods are consistent
- ➢Filtration yields equivalent results to centrifugation in less time (Data not shown*)
- ➢Low non-specific binding leads to high drug recovery (Data not shown*)
- ➢The filter plate is compatible with most solvent systems and has no interfering extractable compounds (Data not shown*)
- ➢For literature and additional information, please see: http://www.millipore.com/solvinert

* Please see Poster PS2004ENUS

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