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Extraction of Drugs in Plasma by Automated Liquid-Liquid Extraction For Downstream Analysis

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Introduction

Liquid-liquid extraction (LLE) is labor intensive and prone to pipetting errors. Here an automated LLE technique is demonstrated for screening extraction solvents for drugs in plasma. Automation involves a programmable offline autosampler or workbench that mixes and vortexes various combination of solvents in glass vials for LC-MS analysis. The best extraction condition would provide minimal matrix interference and maximum sample recovery.

An automated liquid-liquid extraction of drugs from plasma was performed using Agilent 7696A Sample Prep WorkBench. The extracted drug sample was analyzed using a Agilent 1290 Infinity LC System coupled to an Agilent 6490 Triple Quadrupole LC-MS. The removable vial racks used in LLE on WorkBench are placed directly in the autosampler for LC-MS/MS analysis.

The optimized automated method was used to perform reproducibility studies to demonstrate the feasibility for analyzing drug in large batches of plasma with minimal manual intervention. Another automated method was also developed in order to perform calibration curves by serial dilutions.

Figure 1: Agilent 7696A Sample Prep WorkBench used in automating LLE.

Experimental

Sample Prep WorkBench is used, (see Figure 1). LLE solvents optimization was performed for carbamazepine in plasma and analyzed using UHPLC coupled to triple quadrupole mass spectrometer. In the first step, drugs in plasma are loaded in the autosampler vials. To thirty three different LCMS glass vials containing equal amount of drugs, three different pH (3.0, 7.0 and 10.0) aqueous buffers and eleven different combinations of hexane and ethyl acetate are pipetted using the WorkBench. After mixing, the top hexane/ethyl acetate layer is dried in offline rotator evaporator. The dried vials are reconstituted in appropriate buffer using the WorkBench. The holder containing sample vials from the WorkBench is placed directly into the autosampler of the 1290 Infinity LC system. Optimal extraction condition was used to extract fifty different plasma samples. Another automated method was also developed to perform calibration curves by serial dilutions.

Table 1. Instrumentation

Parameters	Details
Automated platform	Agilent 7696A Sample Prep WorkBench
LC System	Agilent 1290 Infinity LC System
LC-MS/MS System	Agilent 6490 Triple Quadrupole System

Table 2. LC Parameters

Solvent A	0.1% formic acid in water
Solvent B	0.1 % formic acid in acetonitrile
Column	Agilent Zorbax Eclipse Plus 2.1 X 50 mm, 1.8 µm
Column Temp	40°C
Injection volume	15 µL
Needle wash	15 sec, 70% acetonitrile-30%water
Gradient	%B Time (min) 25 0.5 56 2.5 95 2.7 95 3.5 46 3.7 46 5.4 25 5.5 Post time 0.5

Experimental

Table 2. MS Parameters

Ionization Mode	Positive with Agilent JetStream Technology
Gas Temp	320°C
Gas Flow	15 L/min
Nebulizer	40 psi
Sheath Gas Temp	400°C
Sheath Gas Flow	10 L/min
Capillary Voltage	2500 V
Nozzle Voltage	0 V
Time Filtering	0.03 min
Fragmentor	250
High Pressure RF	120 V
Low Pressure RF	80 V

Table 3. MRM Parameters

	Carbamazepine	Carbamazepine, 10,11epoxide (ISTD)
Precursor Ion	237.1	252.9
MS1 Resolution	Wide	Wide
Product Ion	193.9	180
MS2 Resolution	Unit	Unit
Dwell	200	200
Collision Energy	15	30

Table 4. Sample Prep WorkBench Setup

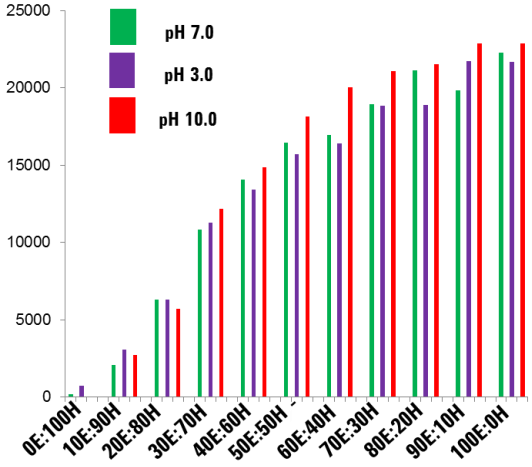
Parameter	500 µL front tower (Dispense pump)	500 µL front tower (Dispense setting)	100 µL back tower (Dispense pump)	100 µL back tower (Dispense Setting)
Number of washes	1	-	2	-
wash/pump volume (µL)	50	-	20	-
draw speed (µL/min)	1500	1500	300	300
Dispense speed (µL/min)	4000	4000	6000	6000
Needle depth offset (mm)	-1	-1	0	0

Results and Discussion

Optimizing the extraction conditions for LLE

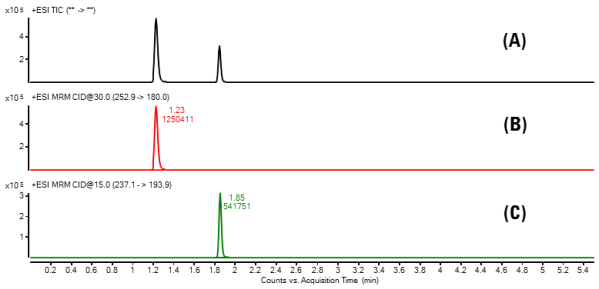
The Sample Prep WorkBench was programmed to add three different aqueous buffers: (50 mM ammonium acetate in water with 1% ammonium hydroxide, approximately pH 10; 50 mM ammonium acetate in water, approximately pH 7; and 50 mM ammonium acetate in water with 1% formic acid, approximately pH 3) and 11 different extraction solvent combinations of hexane and ethyl acetate (100/0, 90/10, 80/20 . . . 20/80, 10/90, 0/100, v/v) [Ref1]. These 33 different combinations were added to plasma sequentially and vortexed using WorkBench. Although, centrifugation and rotatory evaporation was performed using external instrument, the pipetting of top layer and dilutions were performed by the WorkBench.

Figure 2: Area of carbamazepine peaks from various combination of buffer and organic automated additions.



Results show that pH 10.0 and 90% ethyl acetate-10% hexane gives the maximum peak area. Extraction was performed from aqueous carbamazepine samples.

Figure 3: (A) total ion chromatogram, (B)MRM for ISTD, (C) MRM for carbamazepine performed under optimized condition.

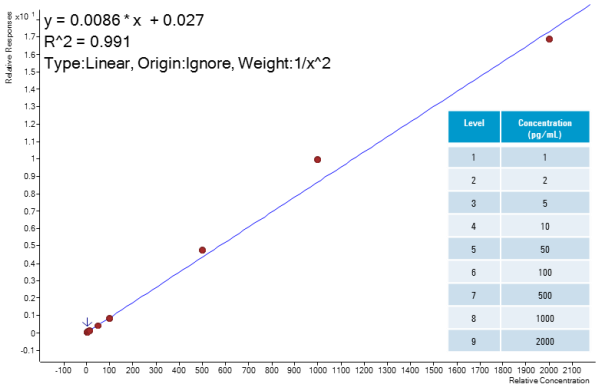


Results and Discussion

Calibration curve by serial dilutions using the Sample Prep WorkBench

The Sample Prep WorkBench was used to prepare 9 concentration levels by serial dilutions. A constant volume of standard from each aqueous dilution was spiked into constant volume of plasma. Post spiking and mixing, sample in each level was subjected to LLE using the optimized extraction protocol..

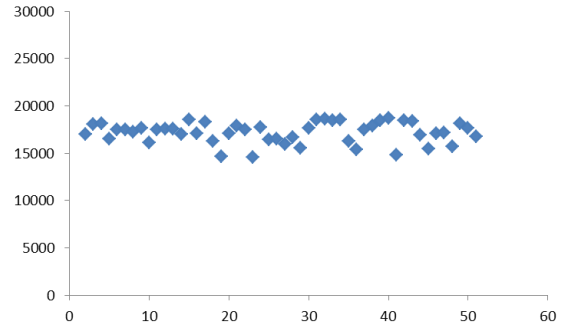
Figure 4. Carbamazepine shows a linear response with R^2 >0.99 and L1 of 1 pg/mL.



Analysis of plasma samples extracted using LLE by automated Sample Prep WorkBench.

A 50 plasma samples were extracted using LLE and their response recorded to determine the reproducibility of the automation method.

Figure 5. Reproducibility of 50 different plasma samples extracted by LLE.

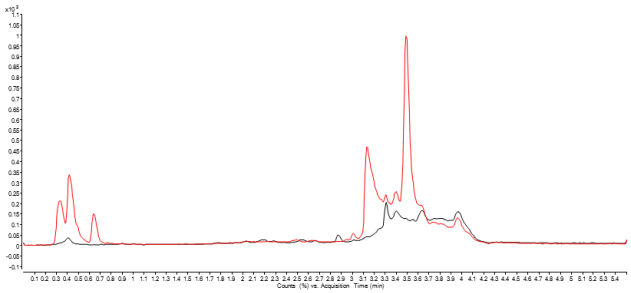


Extraction of 50 different aqueous carbamazepine samples display consistent peak area having RSD of 6%

Advantages of working with glass vials compared to polypropylene vials for the LLE on the WorkBench.

The Sample Prep WorkBench uses 2 mL HPLC glass vials while LLE is usually carried out in polypropylene tubes. Due to the use of organic solvents it is possible that leachables enter the sample from plastic tubes. LLE experiments were performed on blank plasma samples by both SamplePrep WorkBench that uses glass vials and by hand using polypropylene tubes. The extracted samples were monitored for leaching compounds

Figure 6. Total ion chromatogram showing red upper trace of LLE performed by hand using polypropylene tubes while lower blank trace is from the WorkBench using glass vials. Significantly less leachables are observed by glass vials.



Conclusions

Automated liquid-liquid extraction readily optimizes LLE conditions and yields less variable results for large numbers of analyses.

- Automated LLE and serial dilutions demonstrated using the Agilent 7696A Sample Prep WorkBench for bioanalytical workflow.
- The high sensitivity of 6490 Triple Quadrupole Mass Spectrometer is able to achieve sensitivity < 1 pg/mL
- Reproducibility studies shows good LLE extracted from 50 samples
- LLE using glass vials is advantageous over plastic vials.
- Removable vials racks in the WorkBench helps to easily transport and place vials in 1290 autosampler.

References

Guowen Liu, et.al., "Strategy of Accelerated Method Development for High-Throughput Bioanalytical Assays Using Ultra High-Performance Liquid Chromatography Coupled with Mass Spectrometry," Anal. Chem. 2009, 81, 9225-9232.