Automated liquid-liquid extraction (LLE) of drugs from plasma

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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.

Table 1. Instruments used.

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

Table 2. LC/MS experimental details.

Parameters	Details
Solvent A	0.1% formic acid in water
Solvent B	0.1% formic acid in acetonitrile
Column	Agilent Zorbax Eclipse Plus 2.1X50 mm, 1.8 µm
Column temperature	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
Ionization	Positive with Agilent Jet Stream 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 – 2500 V Nozzle Voltage – 0 V Time filtering – 0.03 min Fragmentor – 250 High Pressure RF voltage – 120 Low Pressure RF voltage - 80

Table 3. MRM details

	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
Cell acceleration voltage	0	0
Delta EMV	200	200

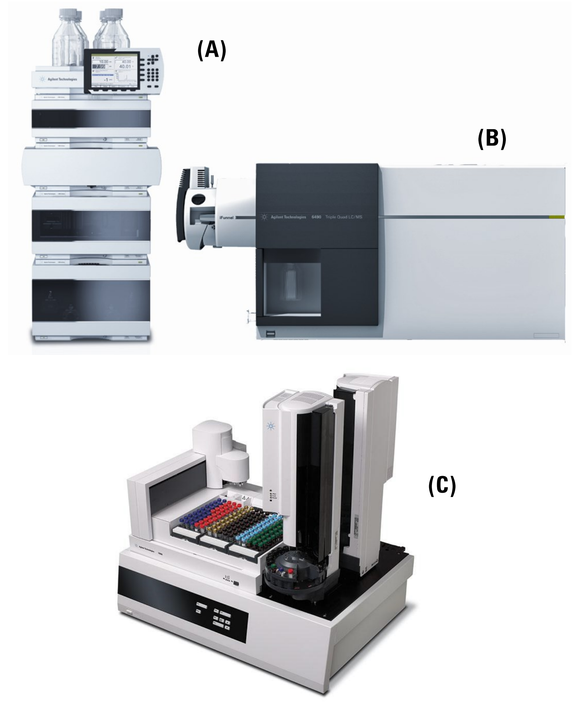
Table 4. Sample Prep WorkBench Setup

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

Experimental

Instrumentation

Agilent 1290 Infinity LC System (A); Agilent 6490 Triple Quadrupole LC/MS System (B) and Agilent 7696A Sample Prep WorkBench (C)

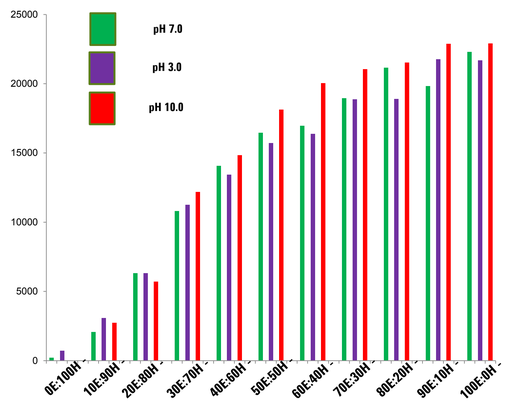


Results and Discussion

Optimizing the extraction conditions for the LLE:

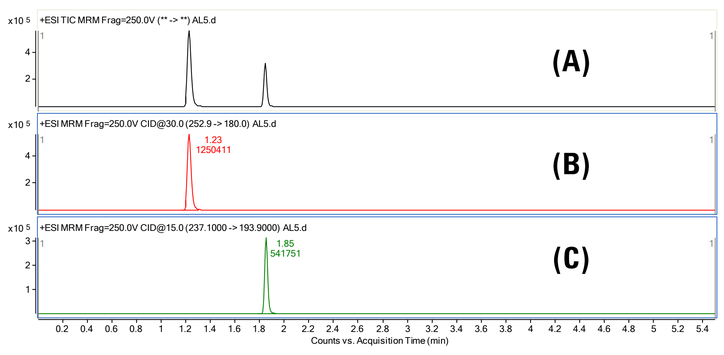
The Sample Prep WorkBench was programmed to add three different aqueous buffers: (50 mM ammonium acetate in water with 1% ammonium hydroxide, approximately pH 10; 50 mM ammonium acetate in water, approximately pH 7; and 50 mM ammonium acetate in water with 1% formic acid, approximately pH 3) and 11 different 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 1: Area of carbamazepine peaks from automated addition of various combination of buffer and organic additions.



Results show that pH 10.0 and 90% ethyl acetate-10% hexane gives the maximum peak area

Figure 2: An overlaid chromatogram of (A) total ion chromatogram, (B) MRM of ISTD and (C) MRM of carbamazepine performed under optimized condition.

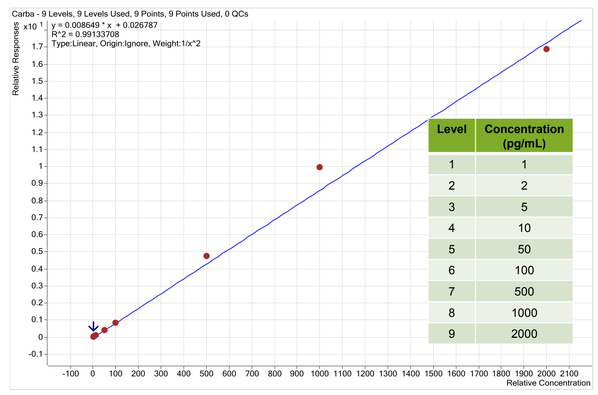


Results and Discussion

Calibration curve by serial dilutions using the Sample Prep WorkBench:

The Sample Prep WorkBench was used for serial dilutions to obtain 9 concentration levels. A constant volume of standard from each dilution was spiked into constant volume of plasma. Post spiking and mixing, sample in each level was subjected to LLE.

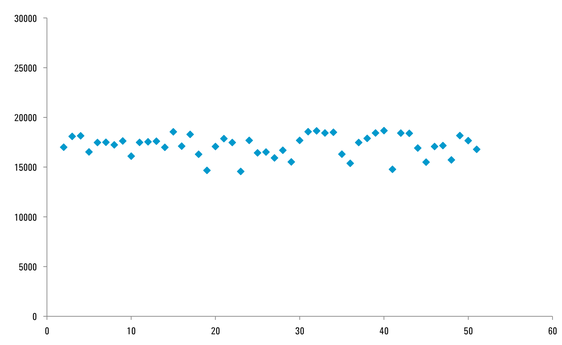
Figure 3. Carbamazepine shows a linear response of R^2 >0.99 and L1 of 1 pg/mL.



Analysis of large number of drugs in plasma 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 WorkBench

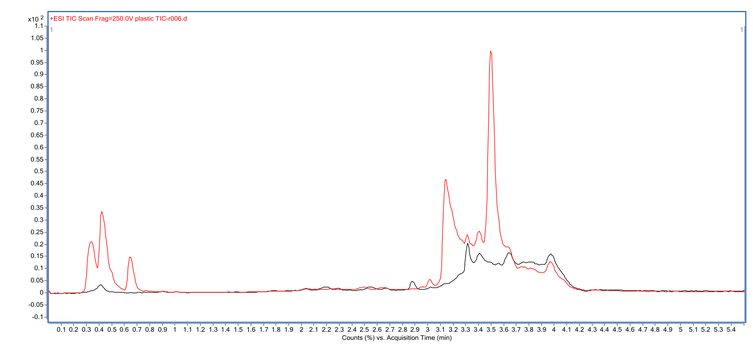
Figure 4. Reproducibility of 50 different plasma samples extracted by LLE is shown by plotting the area. A constant area is achieved having an RSD of 6.4.



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

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.