



Determination of a Basic Drug Extraction from Plasma using Caliper's Zephyr SPE Workstation and Biotage's Evolute CX 96 Well SPE Plate

Introduction

Sample cleanup prior to analysis is critical for achieving high quality analytical results. As increased sample loads and decreased headcounts have driven up the sample loads, many laboratories are turning to the 96 well format for sample clean-up and injection. Here we will evaluate a basic drug extraction from plasma using Biotage's Evolute CX; a resin based mixed-mode strong cation exchange SPE clean-up plate used on Caliper's Zephyr SPE Workstation.

Materials and Methods

Instrumentation

The sample extractions were performed on a Zephyr SPE Workstation from Caliper Life Sciences. Post extraction evaporation was performed using Caliper's TurboVap 96 Workstation for evaporation from 96 well plates. Analysis was performed on a Waters 2795 liquid handling system interfaced to a Quattro Ultima Pt triple quadrupole mass spectrometer using electrospray ionization.

Consumables

Tips – Caliper Life Sciences BioRobotics 96 Pipet Tips, Part Number 109081 200 µL 96 Racked Tips

SPE Plate – Biotage Evolute CX 601-0025-P01, 25 mg, 96-well Plate

Reservoirs – Nalgene Robotic Reservoirs, Flat Bottom, 300mL Part Number 1200-1300

Sample Plate and Collection Plate – Costar Assay Block, 2mL 96 Well Standard, Non-Sterile, Polypropylene Part Number 3961

Reagents

Methanol HPLC Grade

0.05M NH₄OAc pH 6.0

95/5 (v/v) methanol/ NH₄OH

Blank human plasma was obtained through the Welsh Blood Service (Pontyclun, UK).

PREPARING THE SAMPLE PLATE

Sample Pre-treatment: Plasma sample diluted with 50 mM ammonium acetate buffer at pH 6 (1:3, v/v)

Column Conditioning: Methanol (1 mL)

Column Equilibration: 50 mM ammonium acetate buffer at pH 6 (1 mL)

Sample Loading: Pre-treated plasma sample (400 µL)

Interference Elution 1: 50 mM ammonium acetate buffer at pH 6 (1 mL)

Interference Elution 2: Methanol (1 mL)

Analyte Elution: 5% (v/v) NH₄OH in methanol (1 mL)

AUTOMATED METHOD

SPE method Run: Caliper Life Sciences Zephyr SPE Method

Method Name: Biotage Evolute CX

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*** Method Steps: ***

Condition with 1000 µL C3 - Methanol and vacuum to waste for 30 secs

Pre-Air gap 10 µL, Post Air Gap 10 µL, Asp Speed 40 µL/sec, Disp Speed 50 µL/Sec, Retract Speed 0 percent

Condition with 1000 µL C4 - 0.05M NH4Ac pH 6.0 and vacuum to waste for 30 secs

Pre-Air gap 20 µL, Post Air Gap 5 µL, Asp Speed 40 µL/sec, Disp Speed 50 µL/Sec, Retract Speed 0 percent

Load - 500 µL from Sample Plate to SPE Plate and vacuum to waste for 60 secs

Pre-Air gap 10 µL, Post Air Gap 10 µL, Asp Speed 40 µL/sec, Disp Speed 50 µL/Sec, Retract Speed 0 percent

Wash with 1000 µL C4 - 0.05M NH4Ac pH 6.0 and vacuum for 45 secs

Pre-Air gap 10 µL, Post Air Gap 10 µL, Asp Speed 40 µL/sec, Disp Speed 50 µL/Sec, Retract Speed 0 percent

Wash with 1000 µL C3 - Methanol and vacuum for 45 secs

Pre-Air gap 10 µL, Post Air Gap 10 µL, Asp Speed 40 µL/sec, Disp Speed 50 µL/Sec, Retract Speed 0 percent

Elute with 500 µL of B3 - 95/5 Methanol/NH4OH and vacuum for 45 secs

Pre-Air gap 10 µL, Post Air Gap 10 µL, Asp Speed 40 µL/sec, Disp Speed 50 µL/Sec, Retract Speed 0 percent

*** Method Setup Parameters: ***

+ **Selected Method Options:** + Clogged Well detection activated with 2 retry cycles using 26mm Threshold Value.

+ **Checked Steps to Perform:** + Load Sample onto SPE Plate.

+ **Volume Definitions:** + Sample Block Initial Volume (B2): 1000 µL

SPE Plate Maximum Well Volume (A2): 1800 µL

Tips for Condition & Load (A3) Maximum Volume: 200 µL

Tips for Wash & Elute (A4) Maximum Volume: 200 µL

+ **Reagent Definitions:** +

Deck Location Reagent Name Initial Volume

B3 95/5 Methanol/NH4OH 300mL

B4 Reagent2 300mL

C2 Reagent3 300mL

C3 Methanol 300mL

C4 0.05M NH4Ac pH 6.0 300mL

Results

Blank human plasma (n=7) was spiked at a concentration of 50 ng/mL and extracted using the SPE procedure above. The extracts were evaporated to dryness and reconstituted in 1 mL of 80:20 (v/v) H₂O/MeOH for subsequent LC-MS/MS analysis. Comparison of recoveries using both manual and automated processing using the Zephyr SPE Workstation is shown in Figure 1. Relative RSD's for both methods were below 10% for all analytes in this study.

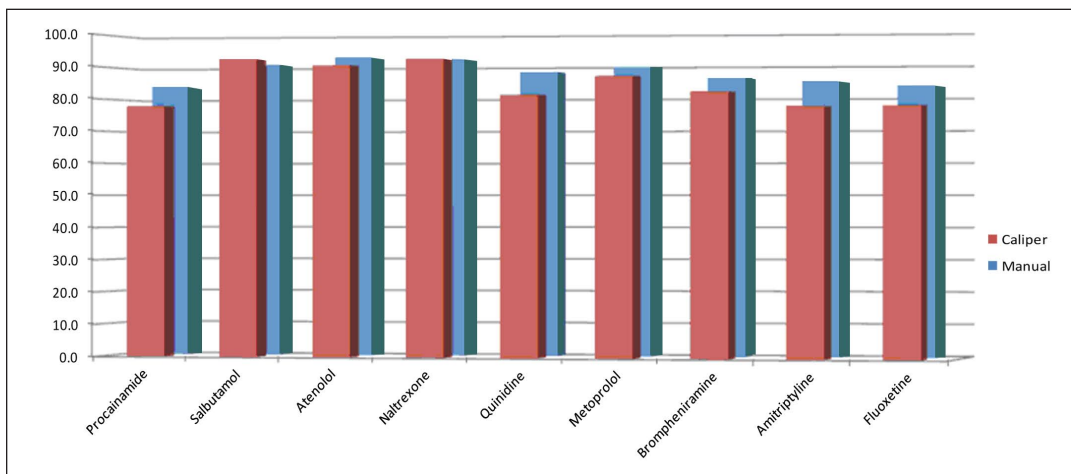


Figure 1. Recovery of basic analytes comparing manual and automated processing using the Zephyr SPE Workstation.

Conclusion

Good analyte recoveries and corresponding RSD's were obtained when repeating manual SPE extractions on the Zephyr SPE Workstation. The automated procedure has the advantages of being "Walk Away Automation", allowing the user to work on other tasks. The automated system is also not subject to operator-to-operator variability in how techniques are performed.



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SC-AP-724 Feb 09