Achieving High Recovery & Reproducibility in High Throughput Sample Preparation Using Silica/Polymer Composite 96 Deep-Well SPE Plates

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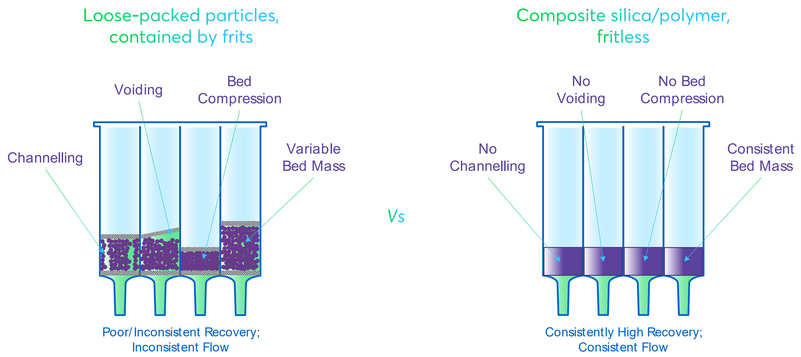
1. Background

- Loose-packed SPE plates can suffer from channelling, voiding, compression and mass variation of sorbent between wells.
- Composite SPE technology eliminates these issues, achieving more uniform flow, enabling higher analyte recovery and improved precision.
- Reduced batch failure rate and solvent consumption are achievable via composite SPE.
- Composite SPE does not require frits to contain material within plate wells, lowering hold-up volume, further increasing analyte recovery over traditional loose-packed SPE media at low elution volumes.
- Enhanced assay sensitivity achieved with greater composite SPE recovery.

2. Superiority of Composite SPE Media vs Loose-packed

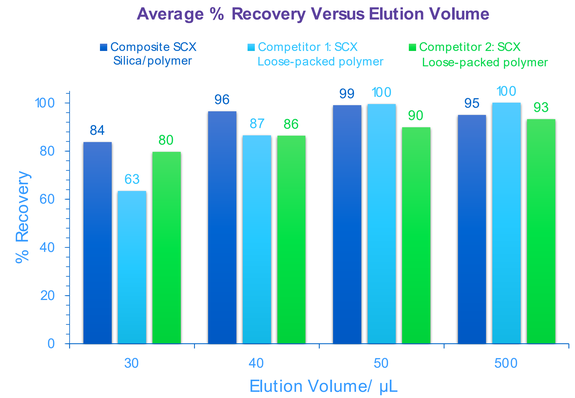
- Composite SPE sorbent beds have more uniform mass, geometry and fixed positioning.
- Samples flow and interact with the sorbent more consistently well-to-well and plate-to-plate.
- Avoiding channelling facilitates higher analyte recovery and improved assay robustness.



- Removing frits reduces well hold-up volume, further increasing recovery and precision at lower elution volumes.
- Removal of hydrophobic frits reduces effort required to load high-aqueous content samples onto sorbent.

3. Low Elution Volume SPE Recovery Data

- Superior recovery at low elution volumes for remacemide via composite silica/polymer than loose-packed polymer equivalent SPE plates.
- Higher recovery provides greater assay sensitivity.



- Maximum recovery achieved with a 40 µL elution volume via composite SPE, whereas 50 µL or higher required for loose-packed plates.
- Lower elution volumes reduce solvent waste and increase throughput via faster sample dry-down.

Sample Preparation

SPE Plates: A) Composite SCX 96-well SPE plate, 2 mg
B) Competitor 1: Loose packed SCX 96-well SPE plate, 2 mg
C) Competitor 2: Loose packed SCX 96-well SPE plate, 2 mg

Condition: 200 µL 0.1% formic acid in MeOH v/v

Equilibration: 200 µL 0.1% formic acid (aq) v/v

Sample Load: 50 µL of 200 µg/mL remacemide sample

Elution: 30-500 µL 5% NH_3 in MeOH v/v

Post Elution: Evaporate & reconstitute in 500 µL 0.1% formic acid (aq) v/v

Analysis: Samples analysed by LC-UV

Chromatography

Column: Avantor® ACE® HTP-MS

Particle Size: 2 µm

Dimensions: 10 x 2.1 mm

Mobile Phase: A) 0.1% formic acid (aq) v/v
B) 0.1% formic acid in MeCN v/v

Gradient:

Time (mins)	% B
0.0	0
0.5	0
1.5	100
2.0	100
2.1	0
4.0	0

Flow Rate: 0.4 mL/min

Injection: 2 µL

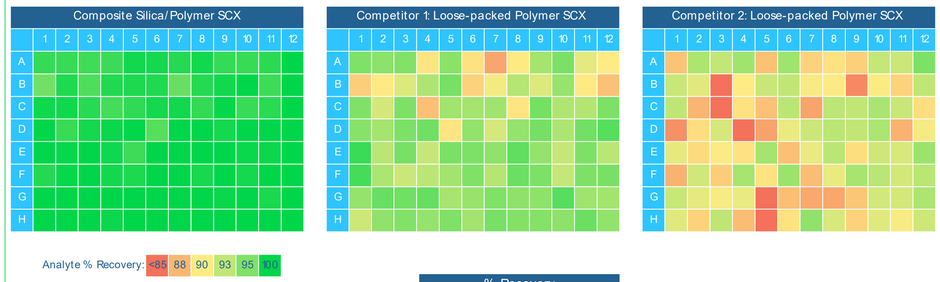
Column Temp: 40 °C

Detection: UV; 192 nm

4. Improved Data Precision with Composite SPE

- 96 wells from all three SPE plates were evaluated via the same SPE method as in Section 3, using a 50 µL microelution volume.
- Results demonstrate the superior precision of silica/polymer composite SPE over loose-packed SPE microelution plates.
- Greater analyte recovery also obtained via the composite SPE plate than the loose-packed SPE plates.

Precision Data: Full Plate SPE % Recovery



	% Recovery			
	Min	Max	Mean	% RSD
Composite SCX	96	104	100	1.53
Competitor 1 SCX	87	98	94	2.39
Competitor 2 SCX	80	95	90	3.18

- Higher throughput is achievable through reduced likelihood of batches failing precision criteria.
- Greater repeatability attained via composite SPE enhances confidence in analytical results.

5. Summary and Conclusions

- Composite SPE media avoids channelling, voiding, compression and sorbent mass variability issues inherent with loose-packed SPE plates.
- Greater uniformity of flow and interaction with the sorbent result in superior recovery and precision at low elution volumes.
- Reduced batch failure rate and higher throughput are achievable via composite SPE.
- Optimal recovery at lower elution volumes facilitates reduced solvent consumption and time spent drying down samples; further increasing throughput.
- Excellent analyte recovery delivers higher assay sensitivity.