



Evaluation of SPE Formats for Improved Extraction and Quantification of Diquat and Paraquat in Drinking Water

Evan Walters

Diquat & Paraquat

Background & Timeline of EPA Method 549

- » Diquat and Paraquat are both quaternary amine herbicides
- » Both are commercially available in the US, used primarily in the agricultural industries
- » Various degrees of toxicity to humans and animals
- » US Monitors Drinking Water following EPA Method 549.2

EPA 549.1
(Rev 1.0)
1992



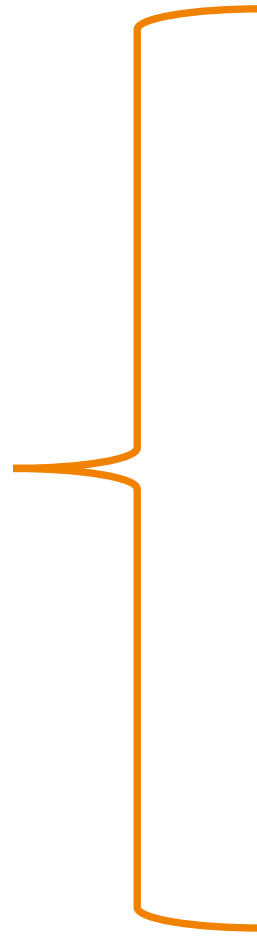
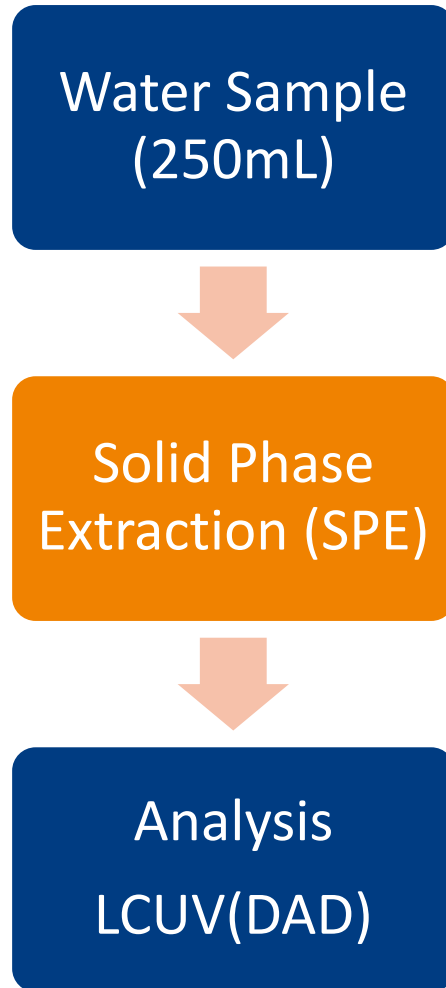
EPA 549.2
(Rev 1.0)
1997



Today – Labs Still
Running 549.2

Method Summary

EPA 549.2



Step	Solvent
Conditioning (8 Steps)	Deionized water
	Methanol
	Deionized water
	Conditioning Solution A
	Deionized Water
	Methanol
	Deionized Water
	Conditioning Solution B
Sample Load	Load Sample
Wash	Methanol
Dry	Vacuum Dry
Elute	Eluting Solution

Solid Phase Extraction Media

EPA 549.2

Section (Format)	Description
6.6.1 (SPE Cartridge)	Liquid solid extraction cartridges, C8, 500 mg or equivalent.
6.6.3 (SPE Disk)	Liquid solid extraction disks (C-8 Empore, 47 mm, or equivalent).

2019 Empore™ C8 Discontinuation

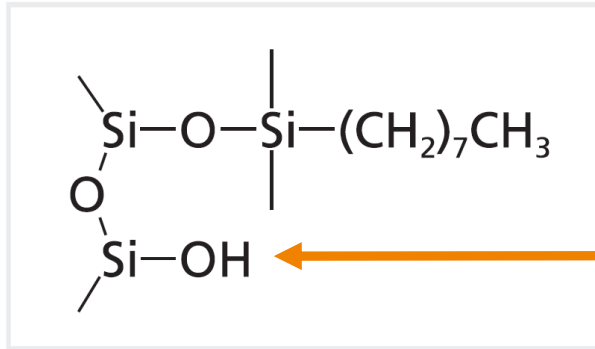
- PTFE Disk “Impregnated” with C8
- Laboratories Started reaching out looking for SPE alternatives
- **This is where our story starts!!**

LSE = Liquid Solid Extraction = SPE

Disk Sorbent Evaluation

C8 vs. C8EC

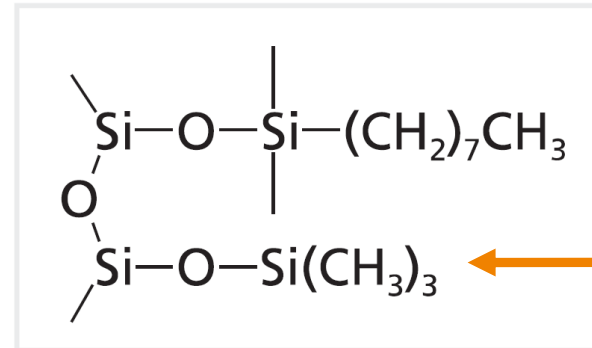
ISOLUTE® C8



Chemical structure of C8 silane covalently bonded to the surface of a silica particle

Average particle size	50 μm
Pore diameter	60 Å
Sorbent Type	Non-Polar

ISOLUTE® C8(EC)

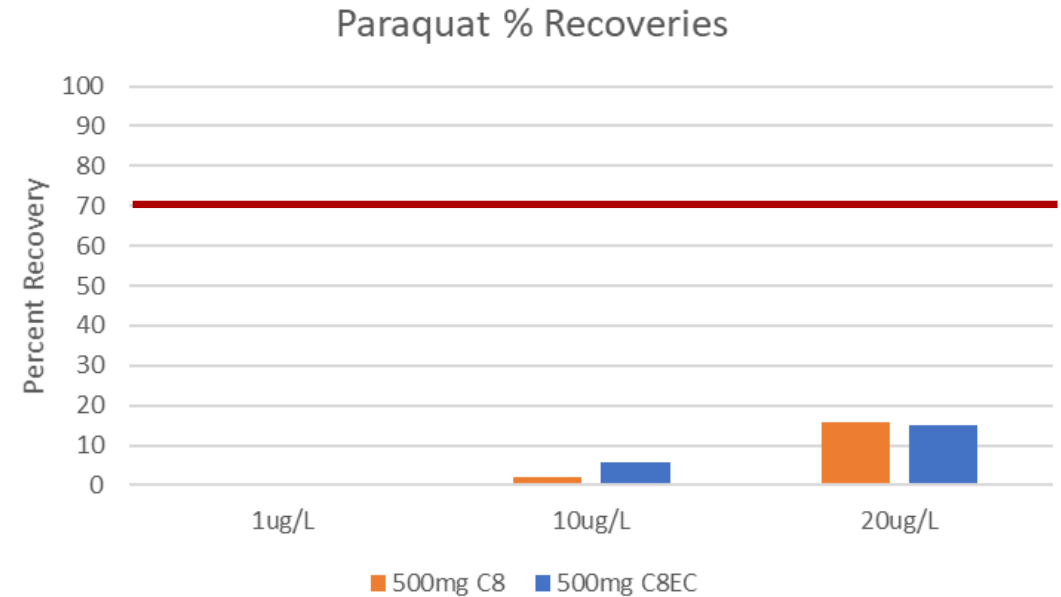
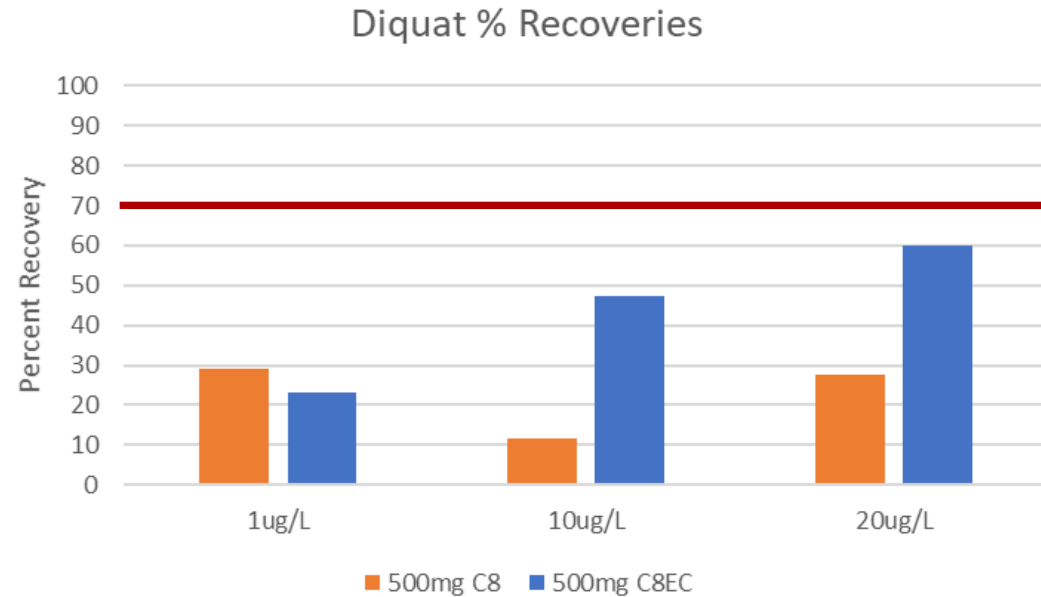


Chemical structure of C8 silane and trimethyl silyl group covalently bonded to the surface of a silica particle

Average particle size	50 μm
Pore diameter	60 Å
Sorbent Type	Non-Polar

Sorbent Evaluation

C8 vs. C8EC



3 Spike Levels Evaluated (Low, Med, High)

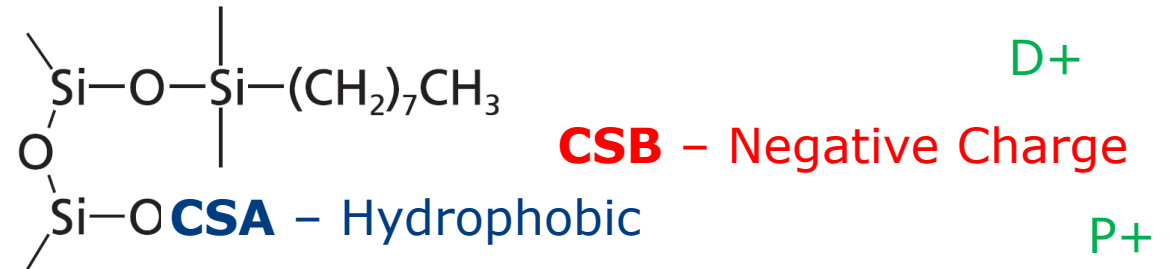
- Diquat Recoveries were higher than Paraquat
- Higher Spikes Levels Performed Better
- EC performed better than non-EC
- Acceptance Criteria (70-130%) was not reached at any spike level

Why EC over non-EC?

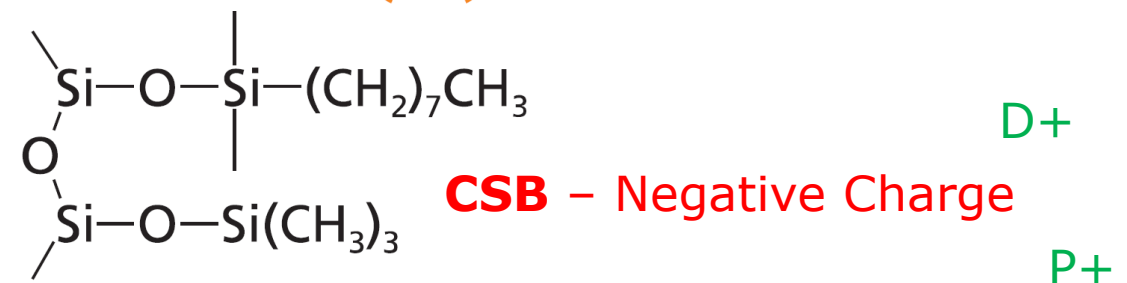
Conditioning Steps

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ISOLUTE® C8



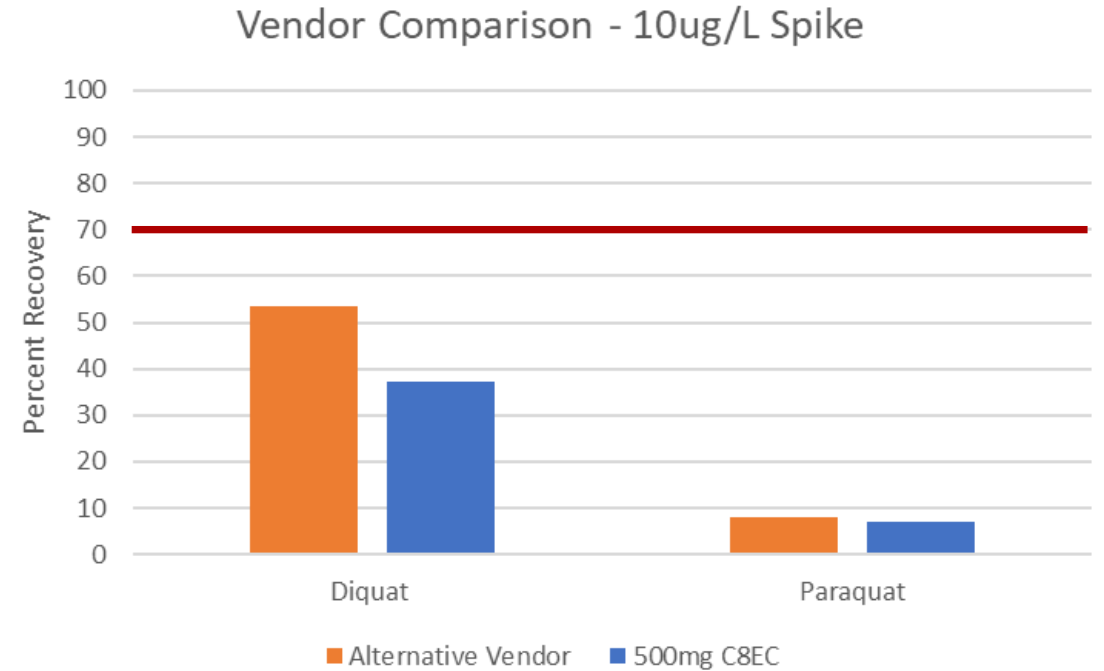
ISOLUTE® C8(EC)



Vendor Disk Comparison

Recovery Comparison

1. Only a select few vendors outside of the Empore brand had 549.2 (C8) Disks available for purchase
2. Evaluated an alternative vendor against our C8EC disk and compared recoveries at 10ug/L Spike level
3. Alternative vendor option outperformed our disk
4. Both options failed to meet acceptance criteria
5. Noticed that alternative vendor SPE disk was thin compared to ours
6. Were the analytes passing through the disks or bound too tightly?



Disk Packing

Dispersion vs. Dry Packing

Dispersion Packing

- Has a benefit of uniformly distributing the sorbent
- Result is thick SPE disk (~3mm)

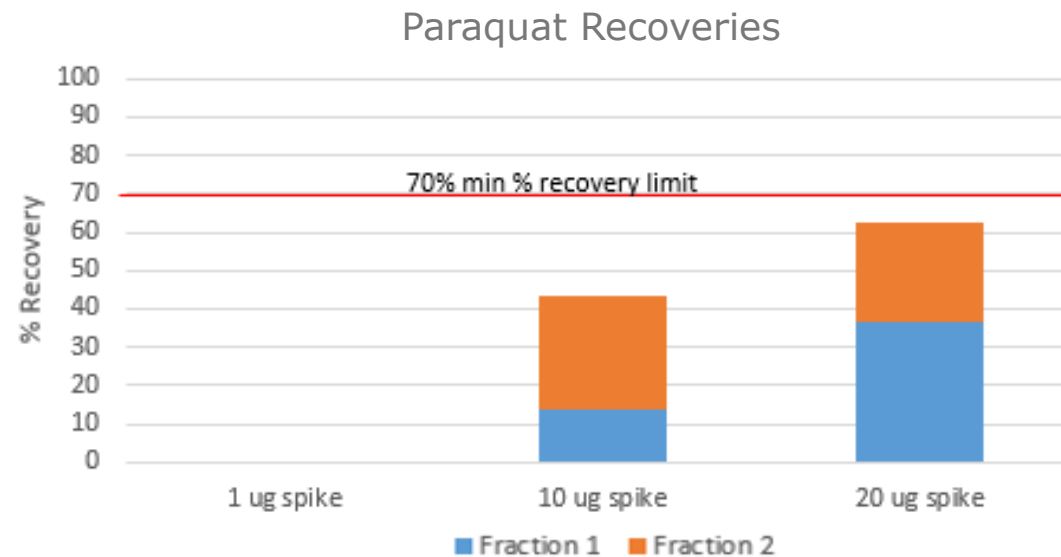
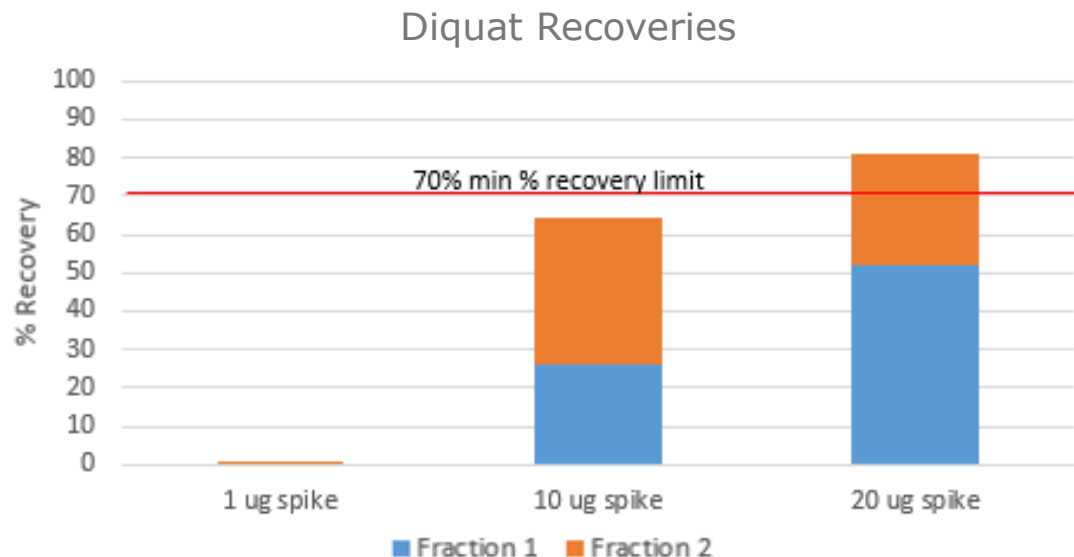
Dry Packing

- Risk of uneven distribution of the disk media
- Allowed for a thin disk (~1.5mm)

All tests at this point had been performed on disks that were **dispersion packed**

Packing Evaluation

Dispersion (Thick) 500mg C8EC

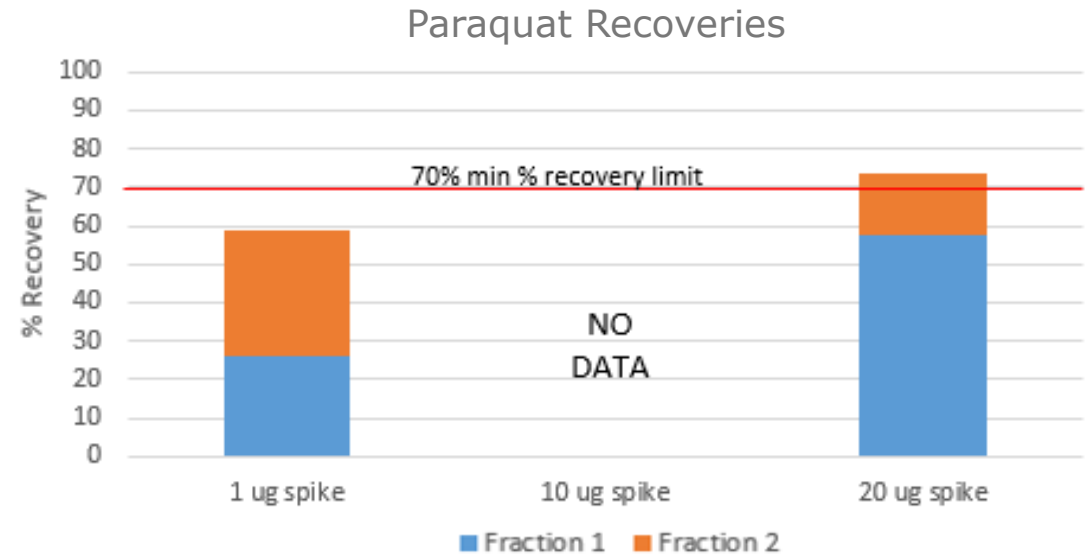
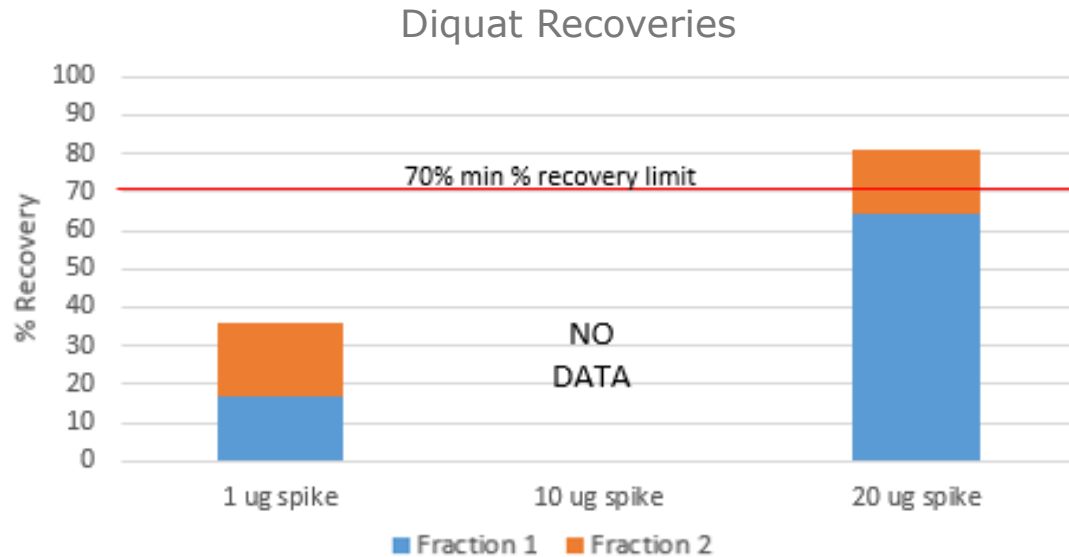


3 Spike Levels Evaluated (Low, Mid, High)

- 2x Elutions were collected in separate fractions
- Higher Spikes Levels Performed Better, limited recovery at low level
- Both fractions for Mid & High spikes had detectable amounts of Diquat & Paraquat, suggesting the compounds were binding too strongly to the SPE disks
- Acceptance Criteria (70-130%) was not reached without using more elution volume (i.e. addition of both fractions)

Packing Evaluation

Dry (Thin) 500mg C8EC



2 Spike Levels Evaluated (Low & High)

- 2x Elutions were collected in separate fractions
- High Spikes Level Performed Better
- Dry Packed (Thin) SPE Disk performed better than the Dispersion Packed (Thick) SPE
- Acceptance Criteria (70-130%) was not reached without using more elution volume (i.e. addition of both fractions)

Disk Material

PTFE vs. Glass Fiber

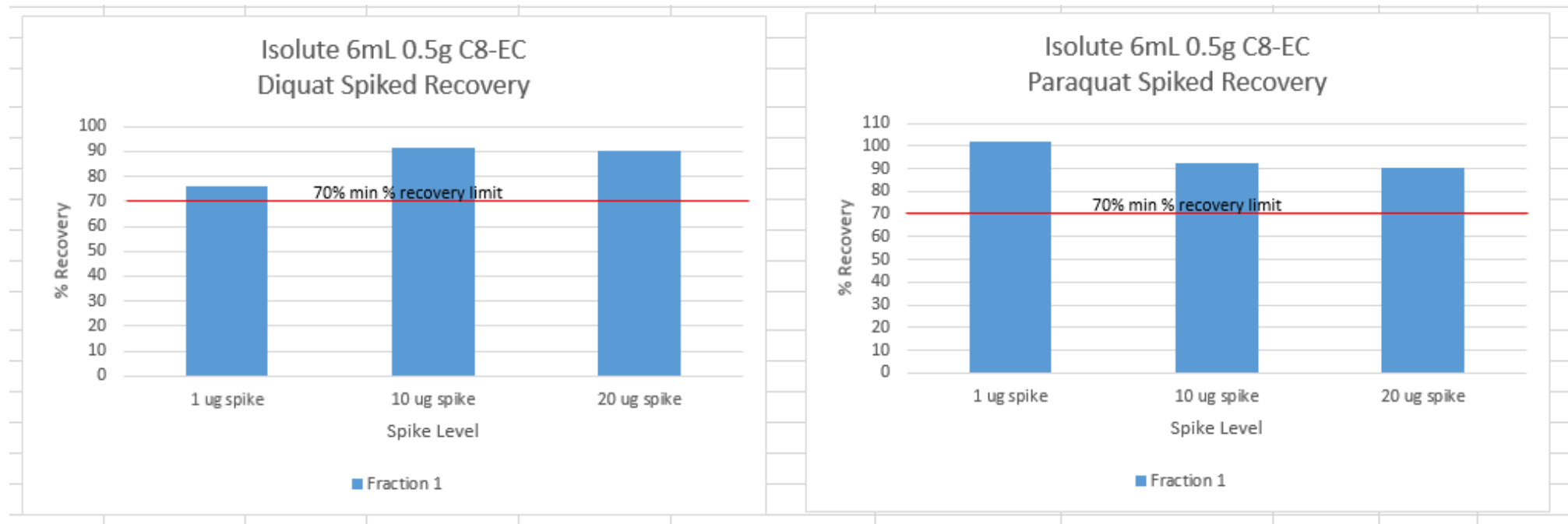
1. PTFE Material to make C8 “impregnated” disks similar to Empore brand, were difficult to source
 - PTFE = Polytetrafluoroethylene
2. Alternative disk options currently available are composed of glass fiber
 - Found Diquat & Paraquat bind to glass fiber which lowers recoveries and reproducibility
3. Evaluation of cartridge format containing ISOLUTE C8EC was next option.

Cartridge Evaluation

500mg C8EC – 6mL Cartridge



- Polypropylene Tube
- Polyethylene Frits
- 500mg C8EC



Automation Optimization

Two Lab Study - Biotage 5000



Initial Demonstration of Capability (IDC)

Two Lab Study



- » LAB A – 4xLFBs at 100 µg/L
- » LAB B – 4xLFBs at 10ug/L & 4xLFBs at 1ug/L
- » Accuracy Criteria \pm 30% of the true value (70-130%R)
- » Acceptance criteria (precision): RSD less than 30%

Laboratory	Lab A (100ug/L)		Lab B (10ug/L)		Lab B (1ug/L)	
	Paraquat %R	Diquat %R	Paraquat %R	Diquat %R	Paraquat %R	Diquat %R
LFB 1	95.43	88.80	98.9	97.4	81.0	92.0
LFB 2	93.60	90.46	100.6	99.1	84.0	88.0
LFB 3	93.95	93.16	100.1	98.1	88.0	96.0
LFB 4	96.79	91.68	93.6	92.2	88.0	97.0
Average %R	94.94	91.68	98.3	96.7	85.3	93.3
RSD (%)	1.54	2.03	3.3	3.2	4.0	4.4

Method Detection Limit

Two Lab Study



- » The EPA 549.2 method, Revision 1.0, June 1997, cites MDLs:
 - » Diquat = 0.72ug/L
 - » Paraquat = 0.68ug/L
- » LAB A – 5xLFBs at 0.8µg/L
- » LAB B – 8xLFBs at 0.5µg/L

Laboratory	Analyte	Target Conc. (µg/L)	MDL 1 (µg/L)	MDL 2 (µg/L)	MDL 3 (µg/L)	MDL 4 (µg/L)	MDL 5 (µg/L)	MDL 6 (µg/L)	MDL 7 (µg/L)	MDL 8 (µg/L)	Std. Dev.	Calculated MDL (µg/L)
Lab A (n=5)	Paraquat	0.80	0.75	0.74	0.73	0.90	0.87	NA	NA	NA	0.079	0.298
	Diquat	0.80	0.62	0.60	0.66	0.73	0.74	NA	NA	NA	0.063	0.237
Lab B (n=8)	Paraquat	0.50	0.51	0.45	0.40	0.42	0.45	0.39	0.46	0.46	0.038	0.113
	Diquat	0.50	0.53	0.57	0.52	0.52	0.54	0.47	0.54	0.56	0.031	0.092

Summary

549.2 SPE Evaluation

- » Endcapped C8 Sorbent Outperformed Non-Endcapped C8
 - » Reduces risk of Secondary interaction of Silanol groups within C8 Silica media
- » Glass Fiber C8 SPE Disks can cause low recoveries and reproducibility
 - » PTFE C8 Disks can be difficult to source
- » C8EC SPE Cartridges Outperformed Glass Fiber Disks Evaluated
 - » Cartridges are made from PP and frits are PE
- » ISOLUTE C8EC Cartridges Performed Excellent at High Sample Load Rates
 - » EPA Method 549.2 Recommends load rate of 3-6mL/min (40-80min)
 - » IDC & MDL Studies using ISOLUTE C8EC: load rate of 25-40mL/min (6-10min)
- » The Biotage 5000 and ISOLUTE C8EC Workflow
 - » Complete Automation of EPA 549.2 Extraction
 - » IDC & MDLs exceeded Acceptance Criteria
 - » Ability to perform 3x549.2 Extractions in less than 20minutes

Thank You!

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EPA 549.2
Application Note

