

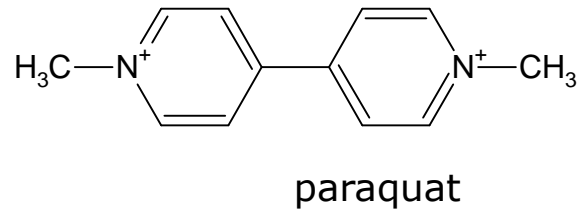
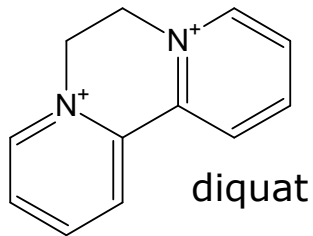
Improved SPE for UPLC/MS Determination of Diquat and Paraquat in Environmental Samples

Michael S.Young, Jeremy C. Shia,
Kim vanTran, Kevin M. Jenkins
and Masayo Yabo

Waters Corporation
34 Maple Street, Milford, MA 01757

- Paraquat and Diquat are quaternary ammonium compounds (quats) used worldwide as herbicides
- Quats are ionic at all pH and solvent conditions
 - Reversed-phase retention (LC or SPE) is accomplished with the aid of an ion-pairing reagent such as an alkyl sulfonic acid
- Oasis WCX provides excellent SPE performance
 - No ion-pairing reagents required
- LC-MS provides the analyst with a more sensitive and highly selective analytical method for quats
 - Traditional ion-pairing reagents (sulfonates) are incompatible with LC-MS
 - Volatile (MS compatible) ion-pairing reagents suppress MS response
 - Ion-pair reversed-phase not recommended
- LC on HILIC type column is compatible with Mass-Spec

Diquat and Paraquat



Quaternary Ammonium Compounds

- Can be retained for LC or SPE
 - Using reversed-phase with ion pairing (EPA 549.x)
 - Using weak cation-exchange (Oasis WCX)

Ion-Pairing SPE

In this procedure (EPA 549.2), a sulfonate ion-pairing reagent is added to the sample prior to SPE with a pre-treated C8 or C18 silica SPE cartridge. However, this procedure is cumbersome and not well suited for LC-MS.

Mixed-Mode Strong Cation-Exchange (Oasis® MCX)

Loading capacity for aqueous samples is very high; 300 mL may be loaded on 60 mg **Oasis® MCX cartridge**. However the concentrated salt solution required for elution produces an extract that is **not compatible with LC-MS**.

Mixed-Mode Weak Cation-Exchange (Oasis® WCX)

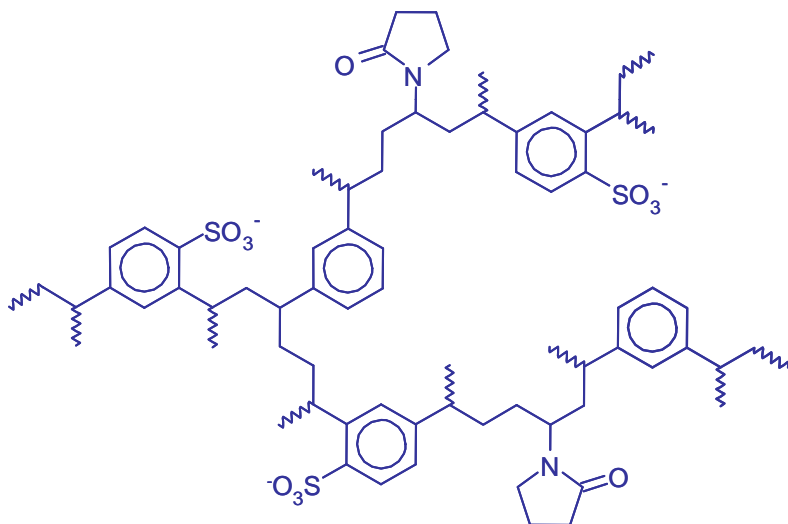
NEMC 2010 presentation

HPLC method required 30 mL sample.
Did not incorporate internal standards.
Did not include paraquat.

Mixed-Mode Cation Exchange SPE Sorbents

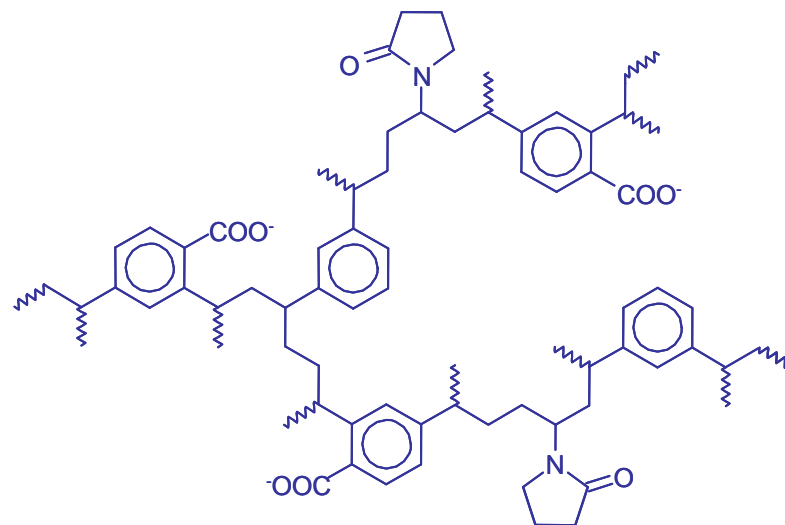
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Oasis® MCX strong ion-exchange



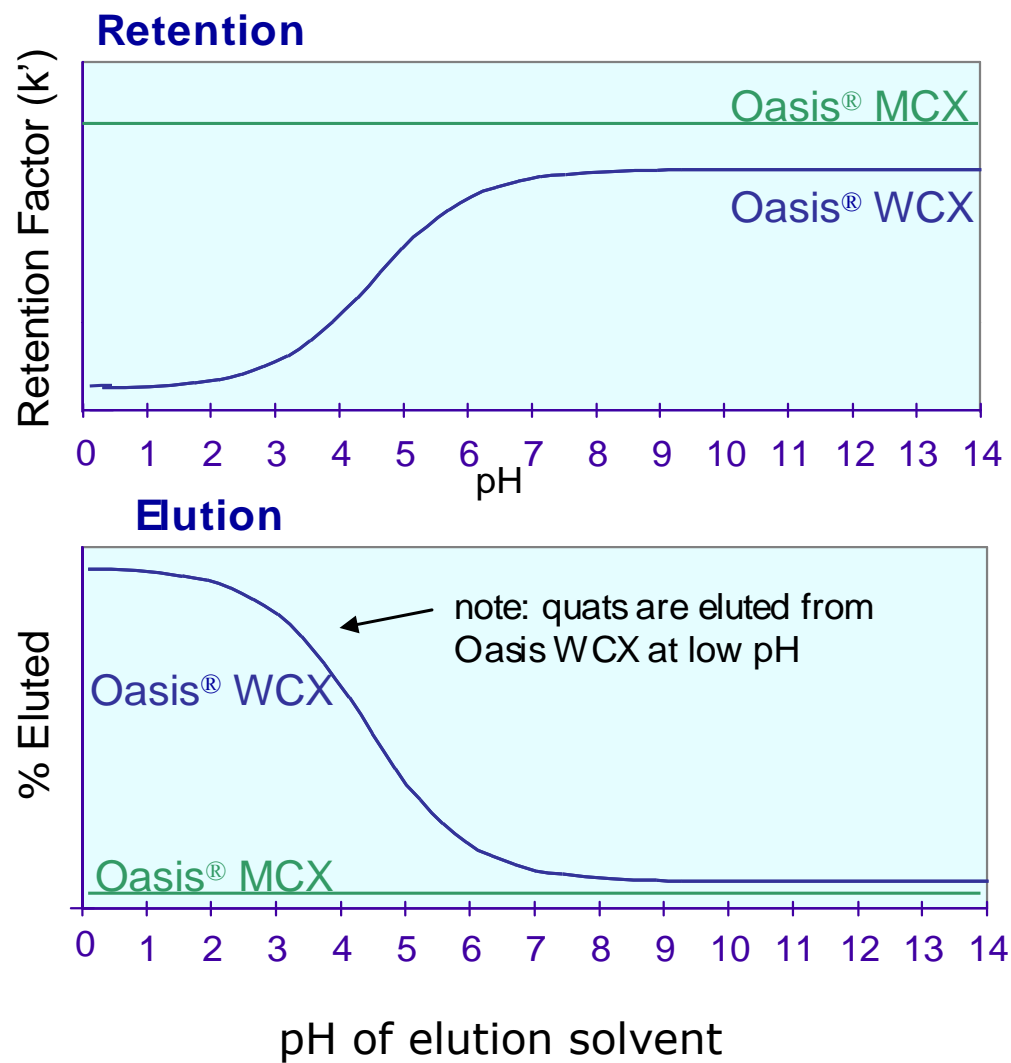
Sulfonate
Charged at all pH > 0

Oasis® WCX weak ion-exchange



Carboxylate
Charged at pH > 6.5

Retention and Elution of Quats on Mixed-Mode Sorbents



HPLC Method Performance 50-1000 ng/L in Groundwater (ppt)

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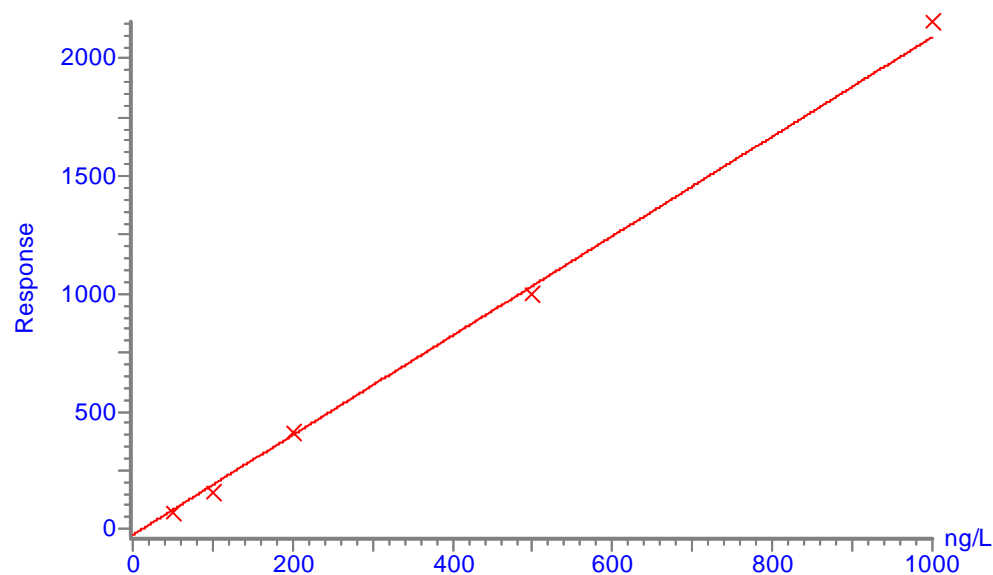
Compound name: diquat
Correlation coefficient: $r = 0.997513$, $r^2 = 0.995033$
Calibration curve: $2.11553 * x + -23.6641$
Response type: External Std, Area
Curve type: Linear, Origin: Include, Weighting: 1/x, Axis trans: None

External Standard

Results (n = 6):

Recovery:

@200 ng/L 75 % (9 % RSD)



Diquat $r^2 = 0.995$
Calibration in groundwater

Improvements Since NEMC 2010 Presentation

- Optimized UPLC Separation
- Incorporation of ISTDS for Paraquat/Diquat
- 10 mL sample size
- Detection limits below 40 ng/L (ppt)
- Simplified SPE method

New SPE Protocol

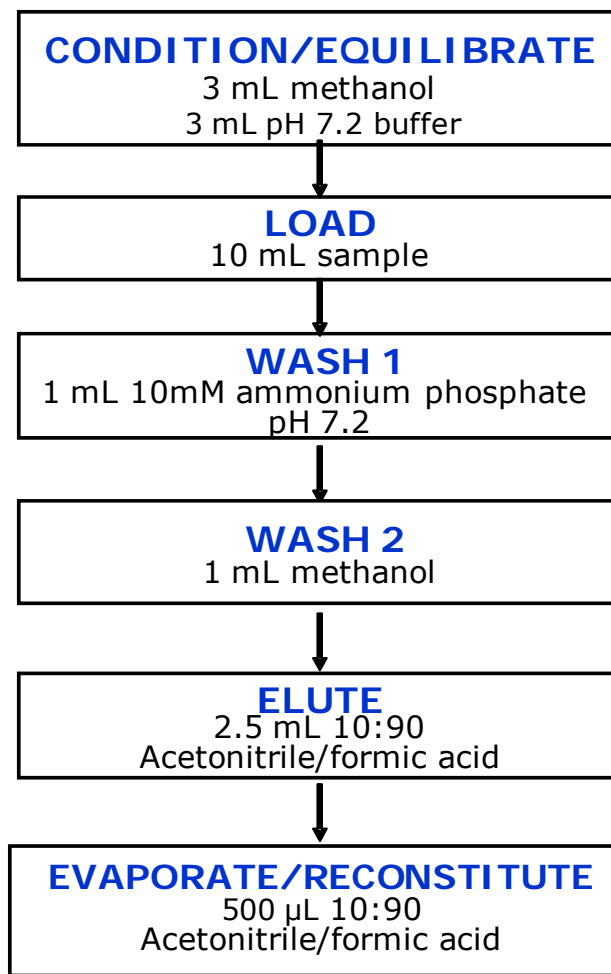
Diquat/Paraquat Analysis/10 mL Sample

OASIS® WCX, 3 cc 60mg, 30µm (pn 186002495)

SAMPLE PRETREATMENT

- To each 10 mL sample add 25 µL of 400 mM pH 7.2 phosphate buffer and 10 mg of sodium thiosulfate

- Spike with ISTDs



← For high ppt detection limits no evaporation required

UPLC Conditions

UPLC System:	ACQUITY UPLC H-Class system
Column:	ACQUITY BEH HILIC
Mobile phase:	40:60 A/B (Isocratic)
	A: 150 mM ammonium formate buffer (pH 3.7)
	B: Acetonitrile
Injection volume:	10 µL
Column Temperature:	30 °C
Weak Needle Wash:	50:50 methanol:water (600 µL)
Strong Needle Wash:	50:50 methanol:water (600 µL)
Seal Wash:	10:90 acetonitrile: water
Flow rate:	0.50 mL/min

LC/MS Conditions/MRM Transitions

Mass Spectrometer: Waters ACQUITY TQD
Ion Mode: Positive Electrospray
Source Temperature: 150° C
Desolvation Temperature: 350° C
Desolvation Gas Flow: 800 L/hr
Cone Gas Flow: 30 L/hr
Collision Gas Flow: 0.20 mL/min (argon)
Data Management: MassLynx v4.1

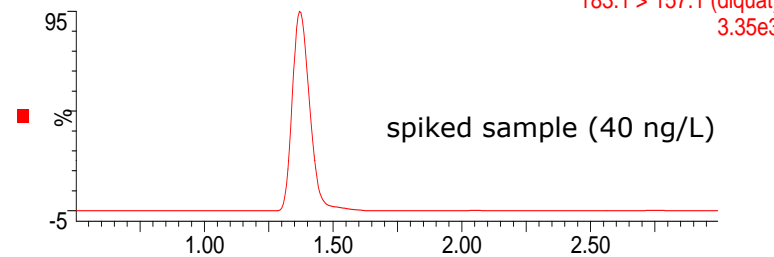
Compound	MRM	Cone (V)	CID (eV)
diquat	183.1 > 157.1	50	25
	183.1 > 130.1	50	30
d4-diquat	186.1 > 158.1	43	30
paraquat	185.1 > 170.1	38	22
	171.1 > 77.0	45	40
d8-Paraquat	183.2 > 178.1	35	25

Typical LC/MS(MS) chromatograms

40ppt spiked shrewsbury de-chlorinated8

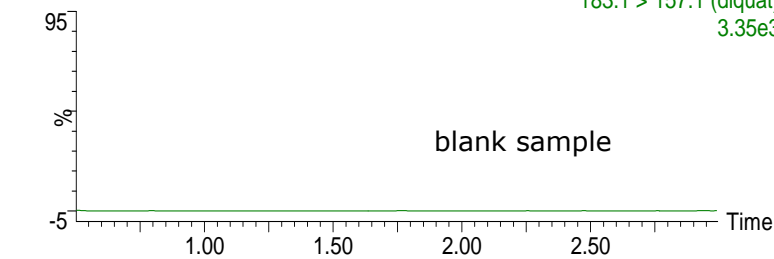
DiBEH15DEC11_39

1: MRM of 3 Channels ES+
183.1 > 157.1 (diquat)
3.35e3



DiBEH15DEC11_40

1: MRM of 3 Channels ES+
183.1 > 157.1 (diquat)
3.35e3

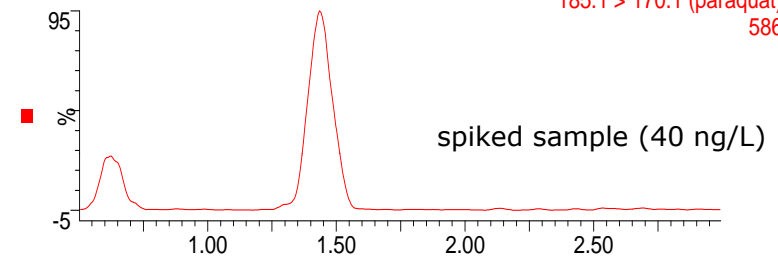


diquat

40ppt spiked shrewsbury de-chlorinated8

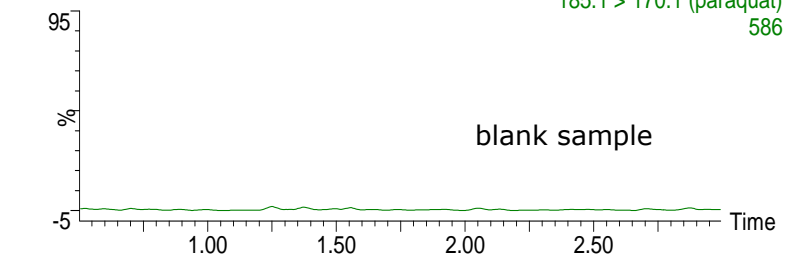
DiBEH15DEC11_39

2: MRM of 3 Channels ES+
185.1 > 170.1 (paraquat)
586



DiBEH15DEC11_40

2: MRM of 3 Channels ES+
185.1 > 170.1 (paraquat)
586



paraquat

HPLC-MS Separation

500 ppb Sample

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HPLC: Waters Alliance 2695

MS: Waters Quattro Micro™ mass spectrometer,
Waters 2487 dual wavelength UV detector.

Column: Atlantis HILIC, 2.1 x 150 mm, 3.5 μm.

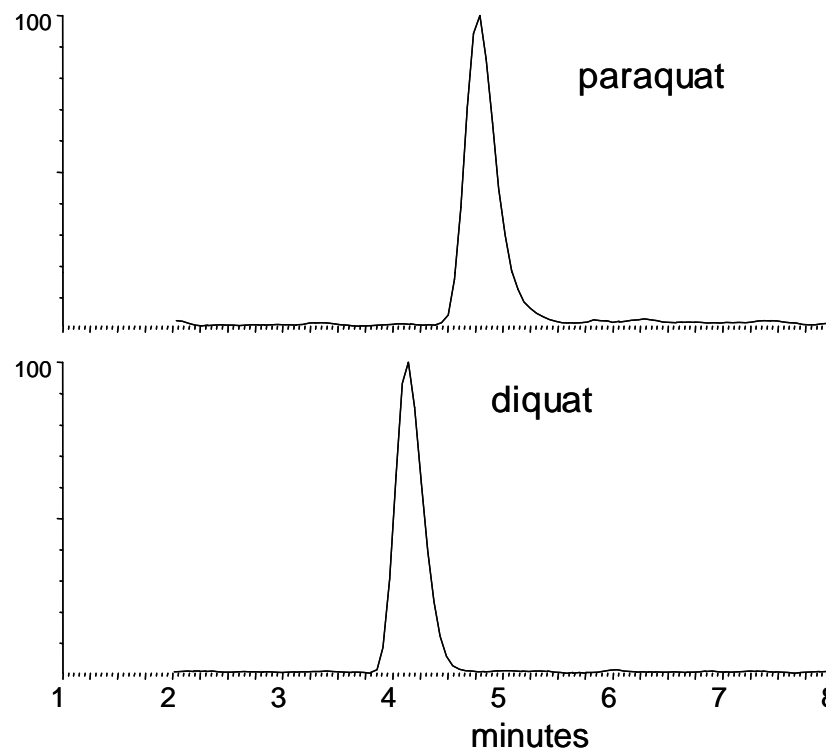
Mobile Phase: 40% acetonitrile, 60% aqueous
buffer pH 3.7 (250 mM ammonium formate).

Flowrate: 0.4 mL/min

Injection Volume: 20 μL

Column Temperature: 30 °C

- Advantage
 - Better separation of quats compared with UPLC on BEH HILIC
- Disadvantage
 - Twice the time for analysis



HPLC-UV Separation

500 ppb Sample

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Intrument: Waters Alliance 2695 with Waters 2487 dual wavelength UV detector

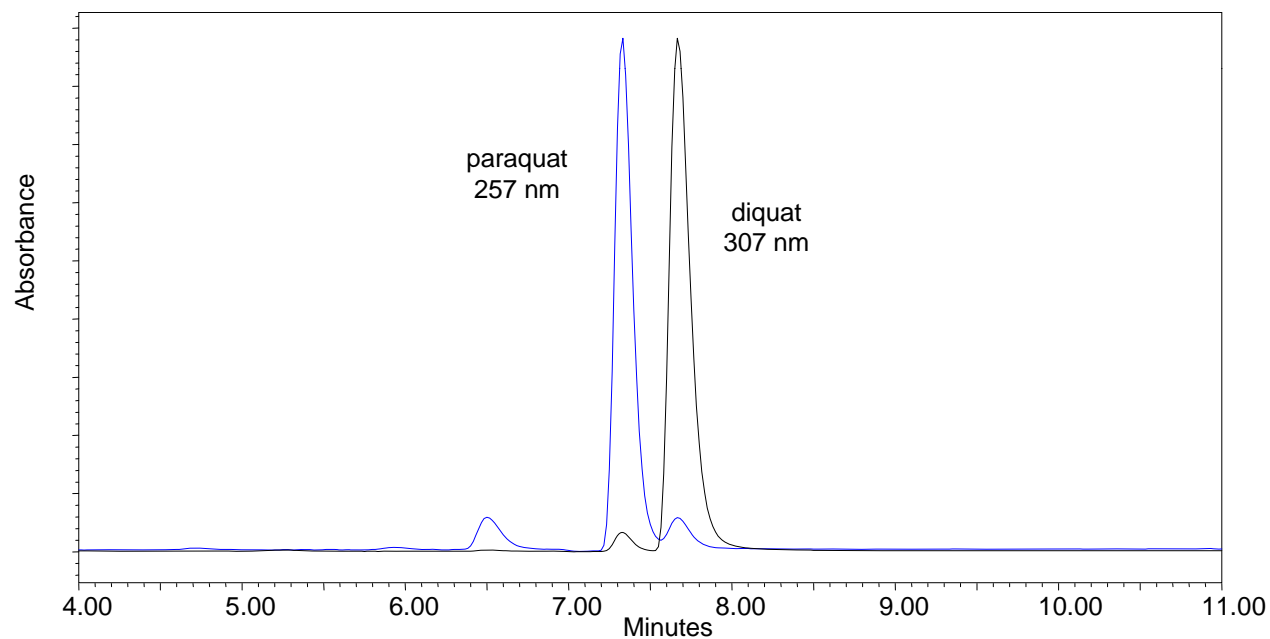
Column: Atlantis dC18, 2.1 x 150, 3.5 μ m

Mobile Phase: 25:75 acetonitrile/0.1 % heptafluorobutyric acid in water

Flowrate: 0.3 mL/min

Injection Volume: 20 μ L

Column Temperature: 30 $^{\circ}$ C



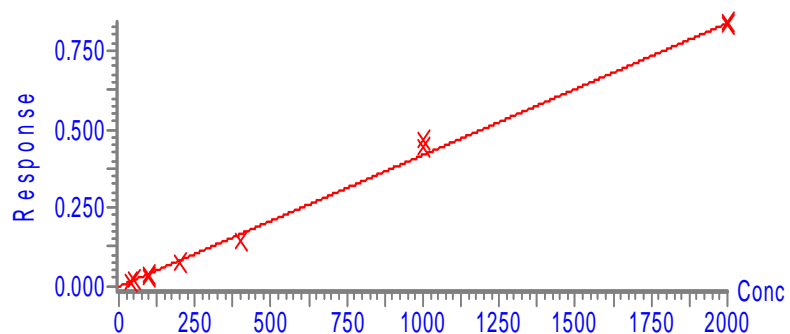
SPE Modifications

- Load 20 mL sample
- Evaporate/reconstitute in mobile phase

LC/MS(MS) Calibration Curves

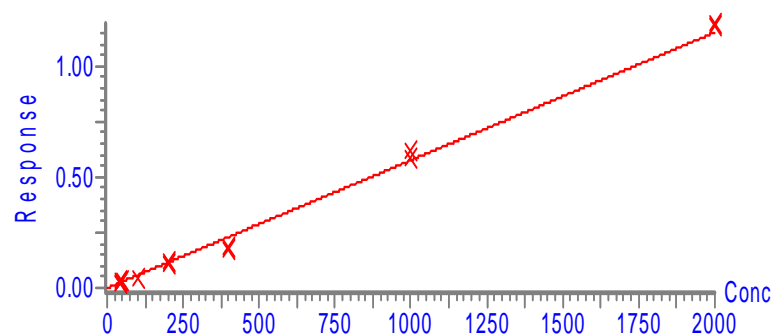
ISTD Calculation

Compound name: Diquat
Correlation coefficient: $r = 0.996129$, $r^2 = 0.992272$
Calibration curve: $0.000420002 * x + -0.00197468$
Response type: Internal Std (Ref 3), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: 1/x, Axis trans: None



diquat

Compound name: paraquat
Correlation coefficient: $r = 0.995100$, $r^2 = 0.990223$
Calibration curve: $0.000575997 * x + -0.000150743$
Response type: Internal Std (Ref 4), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



paraquat

Diquat/Paraquat Recovery Data

(n = 7, 40 ng/L spike level)

Sample Type	% recovery (% RSD)	
	Diquat	Paraquat
Groundwater	82 (8)	89 (16)
Tapwater (chlorinated)	84 (5)	88 (8)
River water	83 (4)	89 (19)

Internal Standard Method

■ Drawbacks

- Expensive
- ISTDS not very stable in water
 - Deuterium exchange requires standards preparation in D₂O

■ Benefits

- More reproducible MS response
- More consistent response from various waters

Tandem LC-MS (ESI+) provides outstanding sensitivity for paraquat and diquat when no ion-pairing reagents are employed

- detection limits < 50 ng/L
- analysis times under 4 minutes

Oasis WCX SPE protocol provides good recovery and sufficient enrichment

- 10 mL sample
- 10 minute SPE protocol
- 20 fold sample enrichment

Although UPLC analysis is fast, sensitive and reproducible, improvements can be made

- better paraquat/diquat separation would be desirable

DIQUAT/PARAQUAT SPE ANALYSIS SOP (3 cc Cartridges)

1. SCOPE AND APPLICATION

This is a UPLC/MS method for the determination of diquat (1,1'-ethylene-2,2'-bipyridilium dibromide salt) and paraquat (1,1'-dimethyl-4,4'-bipyridilium dichloride salt) in drinking water sources and finished drinking water. Based on laboratory work for this SOP, the LOQ for this analysis is well under 100 ng/L based on a 10 mL sample and a final volume after SPE of 0.5 mL.

2. SUMMARY OF METHOD

A 10 mL sample is adjusted to pH 7. Solid-phase extraction (SPE) is performed using a weak cation exchange/mixed mode cartridge (Oasis WCX). After the sample is loaded, the cartridge is washed with pH 7.2 buffer and methanol. The cartridge is then eluted with 2.5 mL of 10:90 formic acid/acetonitrile (ACN). The eluent is evaporated and reconstituted in 0.5 mL of the elution solvent. The resulting extract is analyzed by LC/MS using a Waters Acquity BEH HILIC column.

3. LC/MS APPARATUS

3.1. Isocratic or gradient pumping system capable of ultra high performance liquid chromatography (Waters Acquity UPLC or H-class with UPLC).

3.2. Manual injector or automatic injector, capable of delivering 10 µL.

3.3. Column heater capable of 30°C operation.

3.4. Analytical column – Acquity BEH HILIC 2.1mm x 100mm.

4. SOLID-PHASE EXTRACTION (SPE)

4.1 Oasis WCX cartridges (3cc, 60 mg, 30 µm)

4.2. Vacuum manifold or automated system capable of reproducible delivery of conditioning, sample, wash and elute solutions for SPE.

- Evaluation for consideration as official method

- Application to other matrices (soil, produce)

Conclusions

- The Oasis WCX cartridge is an effective SPE device for determination of paraquat and diquat in aqueous samples
- The paraquat/diquat SPE protocol is effective for enrichment and cleanup from various types of water
- The paraquat/diquat SPE protocol can be used for LC-UV analysis with minor modification
- The UPLC/MS(MS) method is fast, sensitive and requires no ion-pairing reagents
- Method recovery was better than 80 % for all types of water tested with LOQ below 40 ng/L (ppt)