

DIQUAT SALT SOLUBLE CONCENTRATES 55/SL/M/-

As for diquat salt soluble concentrate 55/SL/M/-, CIPAC H, *p*, 161, together with:

5 Terpyridine Isomers

OUTLINE OF METHOD Terpyridine isomers are extracted from the sample with ethylacetate. The impurities are selectively removed from the ethylacetate extractant using a solid phase extraction (SPE) procedure prior to determination by liquid chromatography coupled to a tandem quadrupole mass spectrometer. Multiple reaction monitoring (MRM) of four different ion transitions is performed. Using the response from one ion transition channel, the amount of terpyridine is quantified using single point calibration. Confirmation of identity of terpyridine isomers is obtained via peak area ratio matching of the four monitored ion transition channels.

HAZARDS AND SAFETY PRECAUTIONS Terpyridines are highly toxic by ingestion, inhalation and in particular dermal/eye absorption. Measures to prevent exposure or potential exposure to these impurities must be taken at all times.

REAGENTS

Ethylacetate HPLC Grade

Acetonitrile HPLC Grade

Water HPLC Grade

Diethylamine

Trifluoroacetic acid

2,2':6',2''-terpyridine; standard of known purity

Sodium Hydroxide $c(\text{NaOH}) = 1 \text{ mol/l}$

Sodium Chloride $c(\text{NaCl}) = \text{saturated at room temperature } (\sim 6 \text{ mol/l})$

Ethylacetate-trifluoroacetic acid solution; Add ethylacetate (100 ml) to a clean bottle, add trifluoroacetic acid (5 ml) and mix well.

Acetonitrile-water-trifluoroacetic acid solution; Add acetonitrile (70 ml) to water (30 ml) in a clean bottle, add trifluoroacetic acid (1 ml) and mix well.

Acetonitrile-water-diethylamine solution; Add acetonitrile (70 ml) to water (30 ml) in a clean bottle, add diethylamine (5 ml) and mix well.

Mobile Phase A; Add diethylamine (2ml) to water (1000 ml) in a suitable flask. Mix well and degas before use.

Mobile Phase B; Add diethylamine (2ml) to acetonitrile (1000 ml) in a suitable flask. Mix well and degas before use.

2,2':6',2''-terpyridine standard stock solution: Weigh (to the nearest 0.1 mg) 12.5 ± 0.5 mg 2,2':6',2''-terpyridine standard into a volumetric flask (25 ml) and dilute to volume with acetonitrile. Perform in duplicate.

Intermediate standard stock solution; Accurately pipette 2,2':6',2''-terpyridine standard stock solution (1.0 ml) into a volumetric flask (10 ml) and dilute to volume with acetonitrile. Perform for both standard stock solutions.

Calibration solution; Accurately pipette intermediate stock solution (0.5 ml) into a volumetric flask (50 ml) and dilute to volume with acetonitrile-water-diethylamine solution. Perform for both intermediate stock solutions.

APPARATUS

Liquid Chromatograph capable of generating a binary gradient at a pressure of at least 30 MPa (about 300 Bar, 4500 psi), equipped with automatic liquid sampler capable of injecting 20 μ l and temperature controlled column compartment capable of maintaining 40 ± 3 °C coupled to a tandem quadrupole mass spectrometer fitted with and electrospray ionization source operating in positive ion mode.

Chromatographic column 150 x 3 mm (i.d.), stainless steel, packed with 3 μ m particle diameter silica with C₈ bonded stationary phase (ACE 3 C8 or equivalent).

Electronic data system

Laboratory centrifuge capable of spinning double contained samples at 2500 rpm (~1250 RCF)

Solid phase extraction (SPE) cartridges packed with 60 mg of a 30 μ m particle diameter polymeric support modified with strong cation exchange functionality (Oasis MCX or equivalent).

Solid phase extraction manifold

PROCEDURE

(a) *Operating Conditions (typical):*

<i>Flow rate:</i>	0.5 ml/min		
<i>Gradient:</i>	Time (mins)	%A	%B
	0	35	65
	3	35	65
	3.5	0	100
	7.5	0	100
	7.6	35	65
	12	35	65
<i>Column temperature:</i>	40 °C		

<i>Injection volume:</i>	20 μ l
<i>MS split ratio:</i>	2:3 MS:waste
<i>ESI conditions:</i>	optimized for 234.1 m/z
<i>CID conditions:</i>	optimized for MRM transition 3
<i>MRM Transition 1:</i>	234 \rightarrow 78 m/z
<i>MRM Transition 2:</i>	234 \rightarrow 130 m/z
<i>MRM Transition 3:</i>	234 \rightarrow 155 m/z
<i>MRM Transition 4:</i>	234 \rightarrow 207 m/z
<i>Retention times:</i>	2,2':6',2"-terpyridine: about 3.6 min Other isomers: variable dependent on sample

(b) *System suitability check:* Inject onto the liquid chromatograph 20 μ l portions of one calibration solution until the response factor (*f*) of 2,2':6',2"-terpyridine for three consecutive injections differ by no more than $\pm 2\%$ of the mean and the retention times differ by no more than ± 0.1 min of the mean. Depending on the previous history of the column and instrument, a significant number of injections (between 10 and 100) may be required to obtain stability of response.

(c) *Preparation of Sample:* Weigh (to the nearest 0.01 g) in duplicate an amount of sample (2.3 g) containing an unknown amount of impurity into glass vials (min. 14 ml). Add diethylamine (0.1 ml) and sodium hydroxide solution (2 ml). Accurately add ethyl acetate (10.0 ml) and tightly cap the vial. Invert the vial a number of times to ensure complete mixing and leave to stand for 5 mins (± 30 s). Place the vial inside second sealable container, such as a plastic bottle, seal and shake vigorously (min. 15 s). Centrifuge the sample inside the secondary container at 2500 rpm (2 min).

Perform the following SPE clean up procedure for each sample extracted: Condition an SPE cartridge with acetonitrile (~ 3 ml), followed by water (~ 2 ml), saturated NaCl solution (~ 3 ml), water (~ 3 ml) and further acetonitrile (~ 3 ml). Add accurately half of the ethylacetate extractant (2 x 2.5 ml) produced above (upper layer following centrifugation) and draw through the conditioned SPE cartridge using a vacuum (in ~ 10 s), collecting the eluate in a clean dry glass vial. Draw fresh ethylacetate (~ 2 ml) through the SPE cartridge under vacuum (in ~ 10 s) collecting the eluate in the same vial. Draw air through the cartridge for 10 s under maximum vacuum to dry the cartridge. Acidify the eluate from the SPE cartridge with trifluoroacetic acid (0.75 ml) and mix. Condition a further SPE cartridge as before and transfer the acidified eluate to the second conditioned SPE cartridge. Allow the acidified ethylacetate mixture to pass through the SPE cartridge under gravity

without collecting the eluate. Rinse the vial that contained the acidified ethylacetate eluate from the original SPE cartridge with ethylacetate-trifluoroacetic acid solution (~2 ml) and transfer to the second SPE cartridge. Slowly draw the washings through the cartridge using a vacuum (in ~10 s). Draw through the cartridge under vacuum acetonitrile-water-trifluoroacetic acid solution (~2 ml) without collecting the eluate. Dry the cartridge for ~20 s under maximum vacuum. Elute the terpyridines from the SPE cartridge into a clean dry glass vial using acetonitrile-water-diethylamine solution (2.0 ml accurately) under gravity and then dry the cartridge for ~20 s under maximum vacuum.

(d) Determination. Following successful completion of the system suitability checks, make an injection of the first calibration solution, triplicate injections of the eluate from the second SPE cartridge followed by an injection of the second calibration solution. Repeat this pattern for the duplicate sample taken. In all instances the injection volume should be fixed (20 µl). Determine the average response factor for 2,2':6',2"-terpyridine in the calibration solutions following and proceeding the sample injections in the 234→155 m/z MRM transition channel. Average the peak areas for each individual peak determined in the same MRM channel for each sample injection.

(e) Confirmation of identity of terpyridine isomers. Calculate the MRM response ratio detected for 2,2':6',2"-terpyridine in the calibration solutions using

$$R^{MRM} = \frac{(H_{s_{234>78}} + H_{s_{234>155}})}{(H_{s_{234>207}} + H_{s_{234>130}})}$$

where:

$H_{s_{234>78}}$ = area of peak for 2,2':6',2"-terpyridine in 234→78 m/z MRM channel in the calibration solution

$H_{s_{234>155}}$ = area of peak for 2,2':6',2"-terpyridine in 234→155 m/z MRM channel in the calibration solution

$H_{s_{234>207}}$ = area of peak for 2,2':6',2"-terpyridine in 234→207 m/z MRM channel in the calibration solution

$H_{s_{234>130}}$ = area of peak for 2,2':6',2"-terpyridine in 234→130 m/z MRM channel in the calibration solution and

R^{MRM} = MRM response ratio

For each individual peak detected in the 234→155 m/z MRM channel for the sample injections, confirm that the MRM response ratio (calculated in an identical fashion as above) differs by no more than ±50% of the mean MRM

response ratio calculated for the calibration solutions. Any peak in a sample that has an MRM response ratio that lies outside this range is disregarded.

(f) *Calculation of Content.* For each peak in the 234→155 m/z MRM channel that passes the confirmation of identity test, calculate the content of terpyridine using:

$$f = \frac{s \times P}{25000 \times H_s}$$

$$\text{Content of terpyridine isomer} = \frac{H_w \times f \times 4}{w} \text{ mg / kg}$$

where:

f = average response factor

H_s = area of peak for 2,2':6',2"-terpyridine in 234→155 m/z MRM channel in the calibration solution

H_w = area of individual peak in 234→155 m/z MRM channel in the sample solution

s = mass of 2,2':6',2"-terpyridine in calibration solution (mg)

w = mass of sample taken (g)

P = purity of 2,2':6',2"-terpyridine reference substance (g/kg)

An assumption is made that the average response factor calculated for the 2,2':6',2"-terpyridine isomer is close to the response factor that would be obtained for all other terpyridine isomers.

Sum the results for all peaks and report the total content of terpyridine isomers.

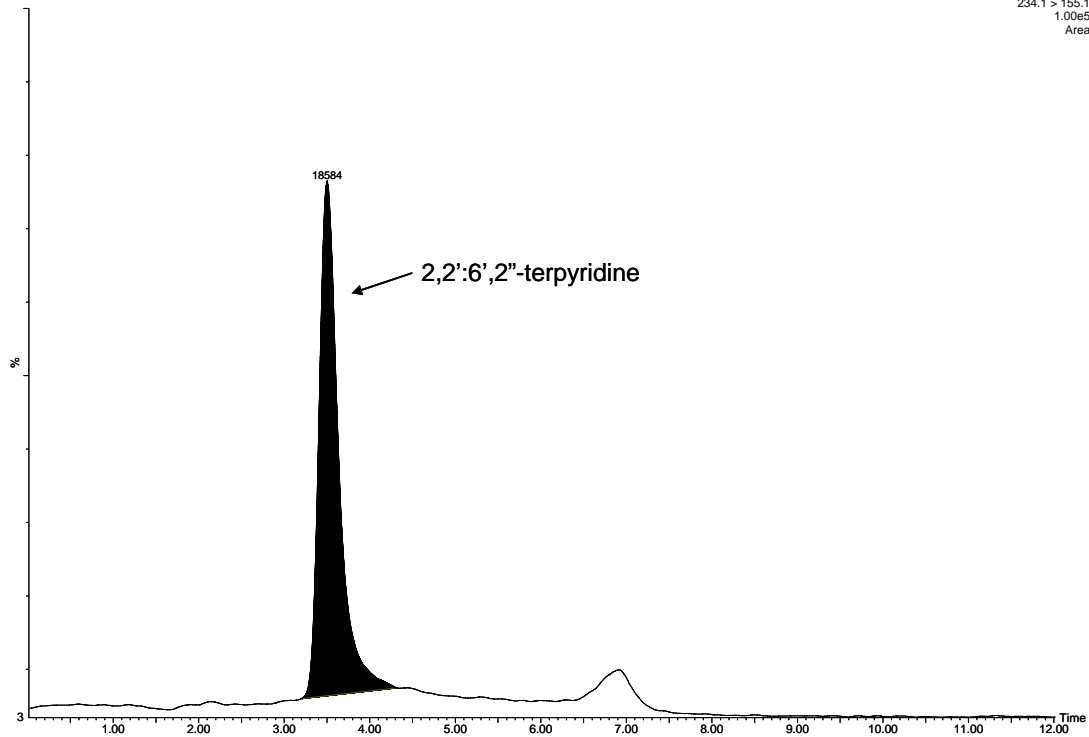


Figure 1: 2,2':6',2''-terpyridine calibration solution - 0.5µg/ml [Scaling Factor: x 1]

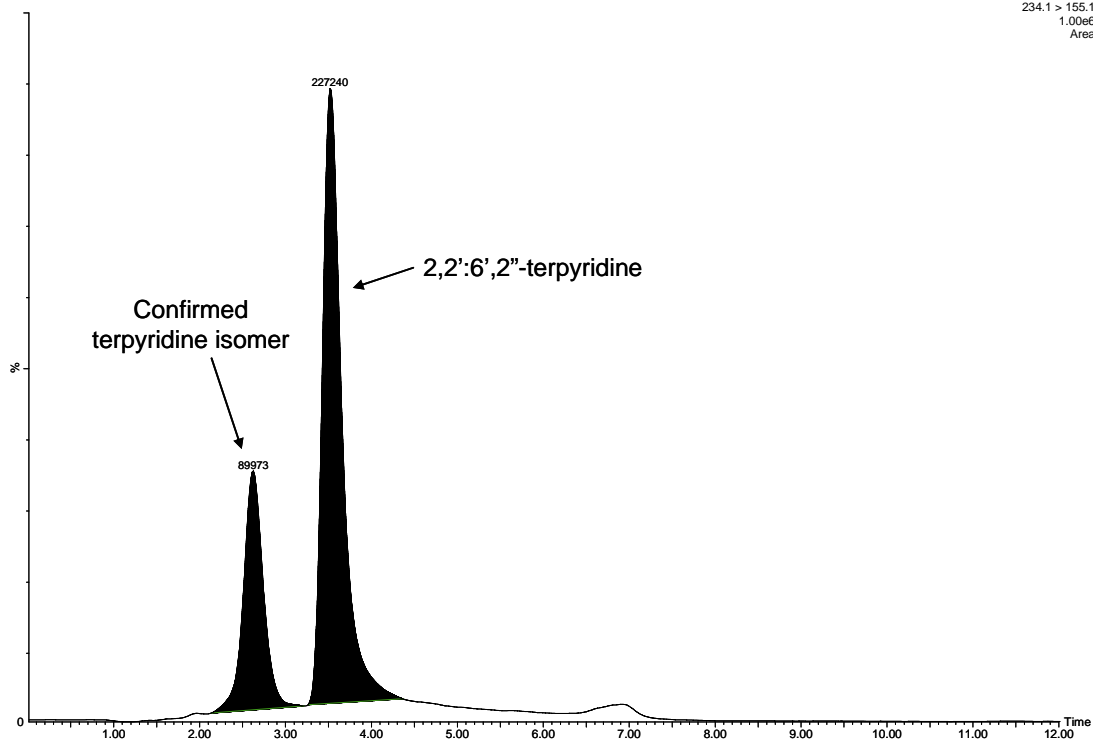
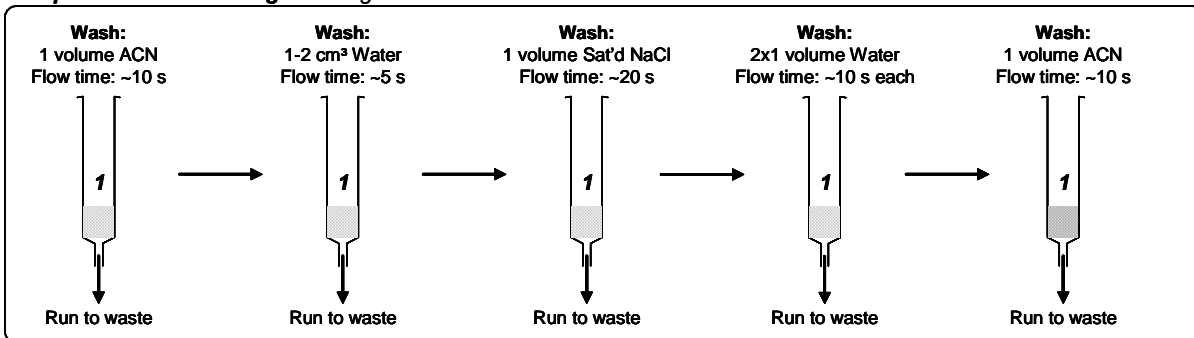
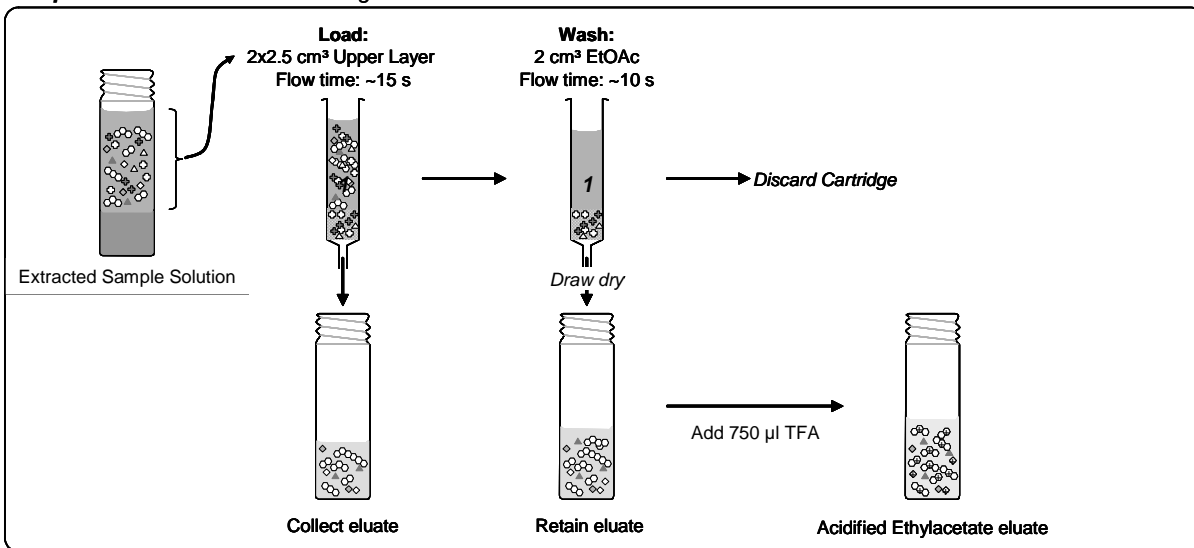


Figure 2: Diquat SL Formulation - Sample concentration 0.55 g/ml; 2,2':6',2''-terpyridine concentration = 8.2 mg/kg, terpyridine isomer concentration = 3.4 mg/kg [Scaling factor: x 0.1]

Step 1 – Preconditioning: Cartridge 1: Oasis® MCX



Step 2 – Cation Removal: Cartridge 1: Oasis® MCX



Step 3 – Preconditioning: Cartridge 2: Oasis® MCX

Repeat Step 1 (preconditioning) with fresh cartridges

Step 4 – Phase Change: Cartridge 2: Oasis® MCX

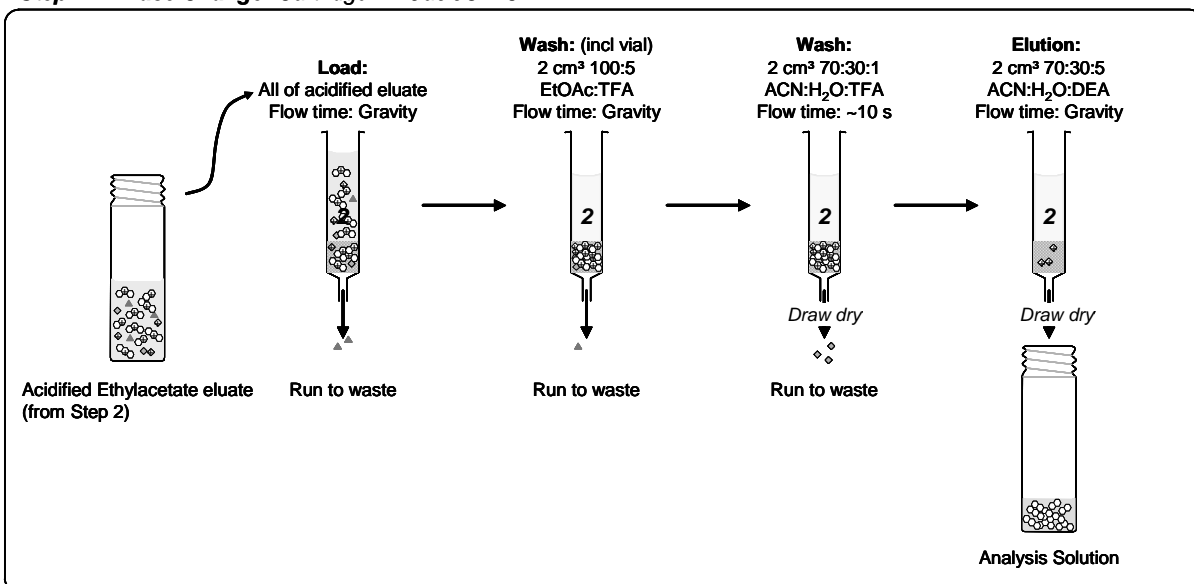


Fig. 1 Schematic representation of SPE Clean-up procedure

DIQUAT AQUEOUS SOLUTIONS

55/SL/M/-

As for diquat aqueous solutions 55/SL/M/-, CIPAC E, *p.* 74, together with:

5 Terpyridine Isomers

As for diquat soluble concentrate 55/SL/M/5.

DIQUAT + PARAQUAT AQUEOUS SOLUTIONS

55 + 56/SL/M/-

As for diquat + paraquat aqueous solutions 55 + 56/SL/M/-, CIPAC H, *p.* 163, together with:

6 Terpyridine Isomers

As for diquat soluble concentrate 55/SL/M/5.

PROCEDURE As for diquat soluble salt concentrate 55/SL/M/5 except that diethylamine should not be added to the sample prior to extraction.