Determination of anthraquinone in crude tall oil by HPLC

1. Scope

1.1 The purpose of this method is to determine the amount of anthraquinone (AQ) in crude tall oil. It has been demonstrated at levels of approximately 100 - 2000 ppm AQ.

2. Applicable Documents

2.1 ASTM Standards
D804 standard definition of terms relating to Naval Stores and Related Products

3. Summary of Methods

3.1 The method is based on external standard calibration of high pressure liquid chromatography, utilizing a UV-VIS detector.

4. Purity of Reagents

4.1 Tetrahydrofuran, HPLC grade
4.2 Anthraquinone (AQ), 99+%
4.3 Water "Baker Analyzed" HPLC grade, filtered before use. Bacterial growth must be avoided.

5. Apparatus

5.1 Liquid chromatograph, Hewlett-Packard 1090 or equivalent, equipped with a gradient pumping system, flow rate controller and a diode array detector.
5.2 Guard column, Zorbax, Rx C-18, 4.0 mm x 12.5 mm - available from Mac Mod Analytical, Inc., 127 Commons Court, Chadds Ford, PA 19317, Part Number 820674-914, or equivalent.
5.3 Column Zorbax Rx C-18, 4.6 mm x 250 mm - Ibid., Part Number 880967-902, data system, or strip chart recorder with integrator suitable for determining peak areas, or equivalent.
5.4 Injection valve, equipped with a 5 μ L loop - Rheodyne Model 7125 or equivalent.
5.5 Volumetric flasks, 10 mL and 100 mL.
5.6 Balance, capable of weighing to 0.0001 gram
5.7 Vortex mixer - available from VWR Scientific, Bridgeport, NJ, Cat. No. 58815-178 or equivalent.
5.8 Filters, Nylon-66,0.2 Fm, disposable - available from Rainin Instruments Co., Woburn, Mack Rd., MA, Cat. No. 38-151.
5.9 Syringe, 3 mL disposable - available from VWR, Cat. No 9585 or equivale
5.10 Autosampler vials, 2 mL.

6. Procedure

Chromatographic Operating Conditions

Pump	Hewlett Packard 1090
Detector	HP 1090 Diode Array, Wavelength Setting, 254, 220, 275, 330 NM, Reference 550, 100, Bandwidth 4.
Injection Volume	5 .μL
Temperature	40°C
Column	Zorbax RX-C-18; 5 .μm particle size, 4.6 mm x 250 mm + 4 mm x 12.5 mm guard column.

Mobile Phase Gradient Composition

Time (Min.)	%A (Methanol)	%B (Water)	%C (THF)
Initial	2	98	0
1.0	2	98	0
40.0	100	0	0
40.1	0	0	100
60.0	0	0	100

20 minute post run equilibration time

A. Preparation of Standard

6.1 Weigh , to the nearest 0.0001 g, 0.05 g of anthraquinone into a tared 100 mL volumetric flask. Record the weight of anthraquinone.

6.2 Fill to the mark with THF, and shake until dissolved.

6.3 Pipet 10 mL of the solution into a 100 mL volumetric flask, and fill to the mark with THF. Shake to mix.

6.4 Filter the solution through a 0.2 .μm -filter, known to be resistant to THF, into an autosampling vial.

6.5 With the chromatograph equilibrated at the operating conditions, inject 5 μ L of the filtered anthraquinone standard solution and obtain the chromatogram. Obtain the area of the anthraquinone peak.

B. Preparation of Sample

6.6 Weigh, to the nearest 0.0001 g, 0.5 to 0.6 g of sample into a 10 mL volumetric flask.

6.7 Fill to the mark with tetrahydrofuran and shake until dissolved.

6.8 Filter the solution through a 0.2 μm filter known to be resistant to THF, into an autosampler vial.

6.9 With the chromatograph equilibrated at the operating conditions, inject 5 μL of the filtered sample

solution and obtain the chromatogram. Obtain the area of the anthraquinone peak. A typical chromatogram of a CTO sample is shown in Figure 2.

NOTE: There are two ways to confirm the identity of the anthraquinone peak:
(1) Compare the spectrum of the anthraquinone standard to the spectrum for the peak eluting in the time frame for anthraquinone. A significant signal (minimum of 20 mAU) is needed to generate a UV spectrum. The spectrum for the standard should match that of the peak in the sample.
(2) If the signal for the peak of interest is not high enough in the method above to obtain spectral information, then a wavelength ratio technique can be used. Select several wavelengths that represent peaks and valleys for the anthraquinone. The highest signal is 254 nm, the second highest is 275 nm and the third is 330 nm. This relationship (ratio) should be the same for the sample and the anthraquinone standard. If the ratio is different, then it indicates either that the peak is not due to anthraquinone or that an interfering peak is present. In these cases, the peak can be used to calculate the anthraquinone content, but it should be stated that this represents a maximum value.

6.10 Calculate the anthraquinone content (ppm) in the sample using Equation 1.

Calculation

$$\frac{As \times Cstd \times P \times 1{,}000{,}000}{Astd \times Cs} = \text{anthraquinone (ppm)} \qquad \text{Eq (1)}$$

Where:

As = area of the anthraquinone peak in the sample
Cstd = concentration of the AQ standard, mg/mL
P = Purity of AQ standard , expressed as a fraction (i.e.if the AQ purity is 97%, then the P = 0.97)
Astd = area of the AQ peak in the standard
Cs = sample concentration, mg/mL

7. **Report**

7.1 Report the anthraquinone as parts per million (ppm) of the CTO sample.

8. Precision and Bias

8.1 The standard deviation of the method was found to be 0.89 ppm with 10 degrees of freedom at the 91 ppm level in a single laboratory. Duplicate determination from one laboratory should agree within 2.5 ppm (95% confidence). Such values are suitable for averaging. There is only limited data available from interlaboratory studies.