1) Determination of Azo dyes by Binary HPLC

Experimental

Standard solutions

A standard stock solution ofAzo dyes in acetonitrile, mixed with a stock solution of azo compounds to make up component standard mixture. This mixture was diluted in 0.1% formic acid in methanol/water 10/90 to the appropriate concentration.

Equipment

An Binary HPLC Gradient System with the following configuration was used:

Analytical Technologies Isocratic Pump

Analytical technologies manual injector with automatic start

Analytical Technologies, Gradient Mixer

Analytical technologies Diode Array Detector

Chromatographic Conditions

Method parameters:

Column: **Column C18, 250mm x 4.6mm x 5um**

Mobile phase: A = 20 mM NaH2PO4, pH 4.60

B = methanol/acetonitrile 50/50 v/v

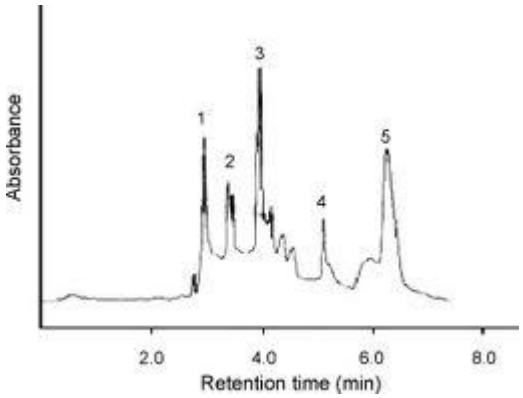
Flow rate: 1.6 mL/min

Gradient: 1–12 min 5-80% B.

12–12.1 min 80-98% B.

12.1-–14 min 98% B

14–15.5 min 5% B (post-time)



Temperature: 36 °C

Injection: 20 µL, needle wash (4 s, flushport, mobile phase B)

Detection DAD:

• Peak width >0.012 min (20 Hz)

• Wavelength

A=Time programmed

0-5.6 min Signal 210/5 nm, Reference off

5.6-8.6 min Signal 262/10 nm, Reference off

8.6-14 min Signal 386/15 nm, Reference off

B = Signal 235/20 nm, Reference off

C = Signal 245/10 nm, Reference off

D = Signal 285/30 nm, Reference off

• Spectra acquisition On, 190–400 nm

2) Determination of Disperse dyes by Binary HPLC

Experimental

Standard solutions

Disperse azo dyestuffs used as a standard during measurements were purchased (Disperse Red 1, Disperse Red 13, Disperse Red 73, Disperse Red 167, Disperse Blue 79).Commercial grade dyes were purified with Soxhlet extraction using toluene solvent for 24h before using then 100 mg/ml

stock standards were prepared in methanol.

Equipment

An Binary HPLC Gradient System with the following configuration was used:

Analytical Technologies Isocratic Pump

Analytical technologies manual injector with automatic start

Analytical Technologies, Gradient Mixer

Analytical technologies UV Detector

Chromatographic Conditions

Method parameters:

Column: **Column C18, 250mm x 4.6mm x 5um**

Mobile phase: Methanol: Water(60:40)

Flow rate: 0.8 mL/min

Temperature: 36 °C

Injection: 20 µL, needle wash Detection UV detector:

•Detection Wavelength: 254 nm

. Disperse Red 73 standards at concentration 3 ppm and 10 ppm from stock solutions (Fig. 2) and (Fig.3), water sample (Fig.4) HPLC chromatograms were obtained. To avoid solvent peak overlap in chromatograms, methanol chromatogram was also obtained. (Fig. 1). Concentration of Disperse Red 73 in wastewater sample was determined with major peak area calculation that ranged at Rt (retention time) 3.750-3.770 min for 10 ppm, 22Determination of disperse… 3.452-3.732 min for 3 ppm and 3.753-3.769 min for sample. Using area under peak centered at Rt 3.70 min, Disperse Red 73 concentration was calculated 0.091 mg/L.

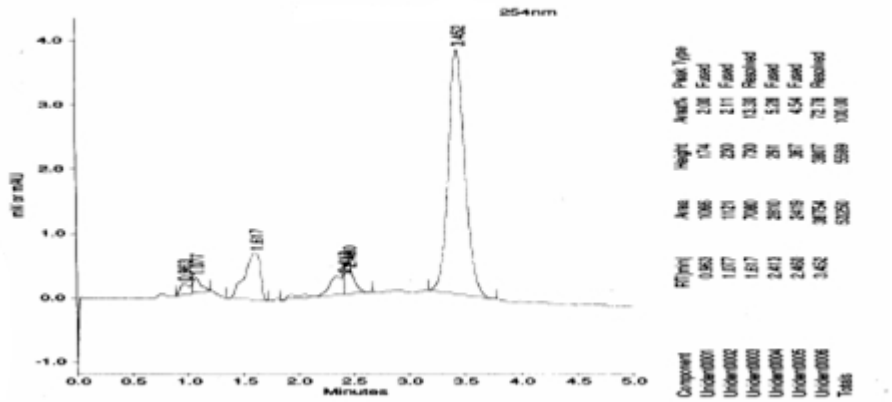


Figure 1. *Solvent (CH_3OH) HPLC-UV chromatogram*

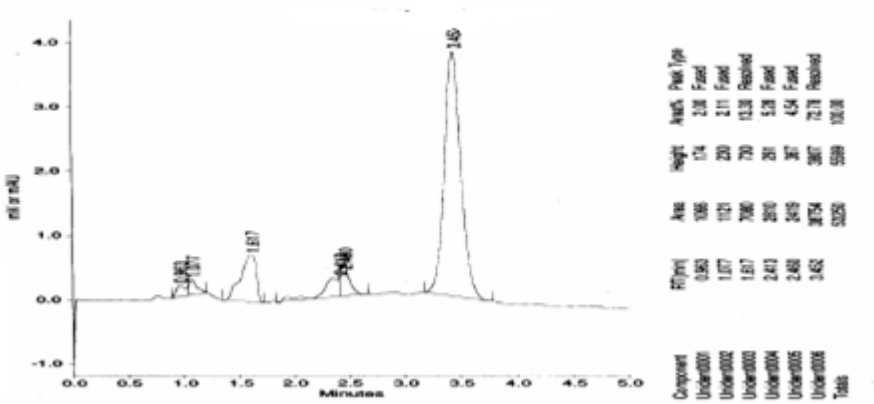


Figure 2.*Standart Disperse Red 73 (3 ppm) HPLC-UV chromatogram*

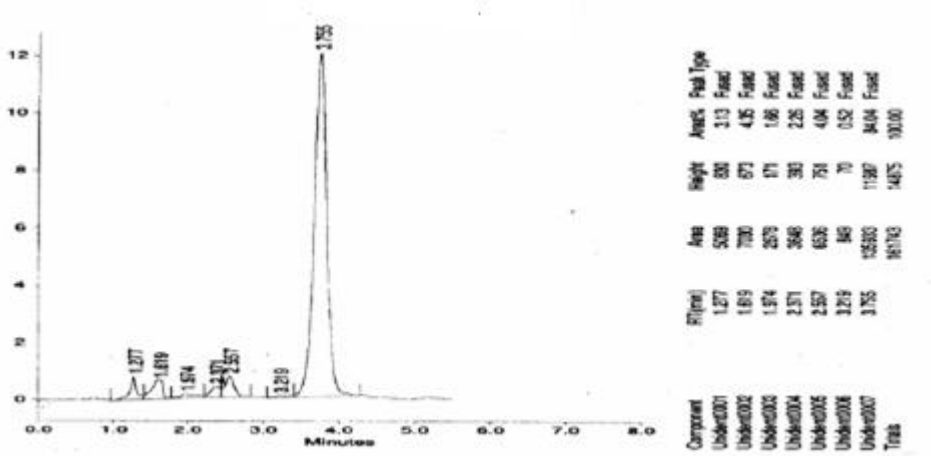


Figure 3. *Standart Disperse Red 73 (10 ppm) HPLC-UV chromatogram*

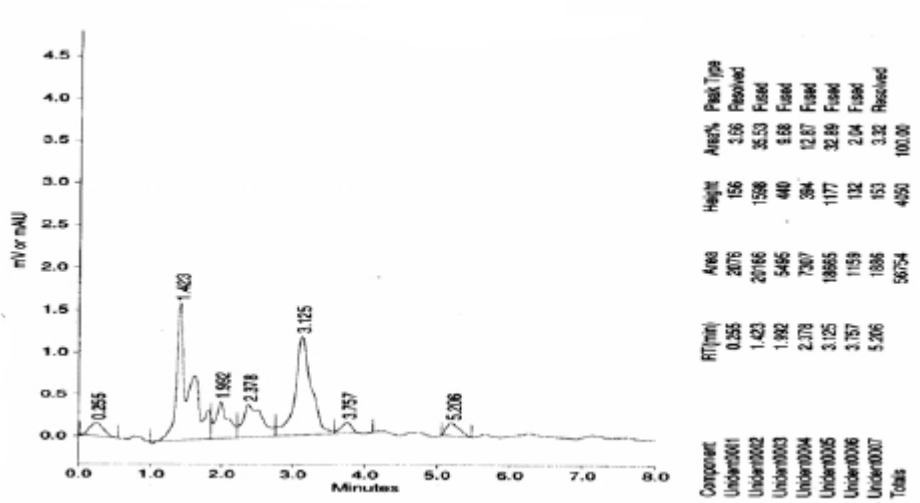


Figure 4. *Water sample HPLC-UV chromatogram*