SHIMADZU

Application Notes

High Performance Liquid Chromatography

Analysis of Azo Dyes by HPLC with PDA Detector

-- Transferring a Conventional HPLC Method to an UFLC Method

Azo compounds are compounds with R-N=N-R' functional group where R and R' represent any aryl and alkyl group. They are often used as synthetic dyes and pigments and are widely used in textile fibres, leather, plastics, papers, hair, mineral oils, waxes, foodstuffs and cosmetics. The reductive cleavage of such dyes at the Azo group may release aromatic amines which are carcinogenic or potentially carcinogenic to humans. Hence, the analysis of such compounds are often important.

This Application News introduces an example of analysis of Azo dyes using an UFLC method which reduces the analysis time and made it more convenient for routine analysis.

Conventional HPLC Method

Sample Preparation

Standard amines solutions were prepared using methanol as solvent. The Azo dye sample consisted of mixture of 7 amines.

Fig 1. shows the LC time program of the conventional HPLC method used while Fig 2. shows the chromatogram of Azo dye sample. The analytical conditions are shown in Table 1

Gradient program

No.	Time	A Conc.	B Conc.
1:	0.01 min	85 %	15 %
2:	15 min	55 %	45 %
3:	16 min	60 %	40 %
4:	30 min	50 %	50 %
5:	45 min	25 %	75 %
6:	45.5 min	85 %	15 %
7:	50 min	Controller	Stop

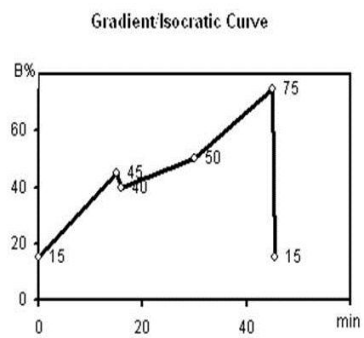

Fig. 1 LC time program

Column	Shim- pack VP- ODS 250 x 4.6mm
Flow rate	0.7ml/ min
Column temperature	40 ° C
Injection volume	5µl
Mobile phase	A- phosphate buffer pH 6.9
	B- methanol
Detection:	PDA (240, 280 and 305 nm)
	Temperature: 40 °C

Table 1 Analytical Conditions

SHIMADZU (sia Pacific) Pte. Ltd.
79 Science Park Drive, #02-01 Cintech IV
Singapore Science Park I, Singapore 118264
www.shimadzu.com.sg
Tel: +65-6778 6280 Fax: +65-6778 2050

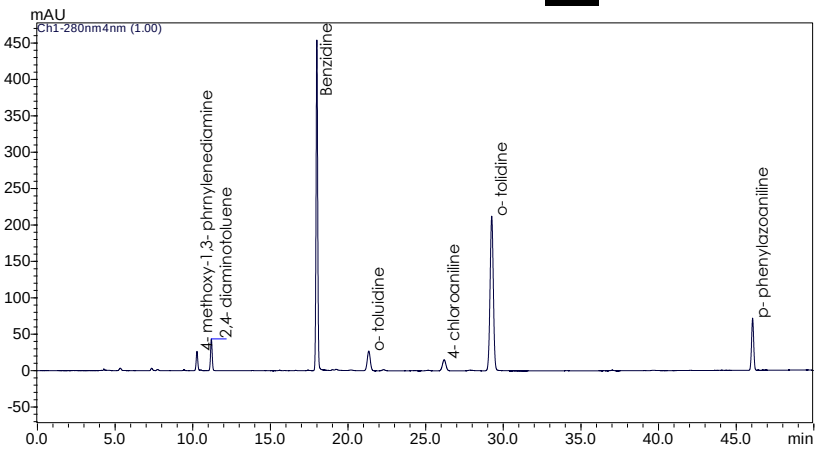

Fig. 2 Chromatogram of Azo dye sample.

UFLC Method Transferred by a 2- way Method Transfer Program

Fig 3. shows the LC time program of the UFLC method used while Fig 4. shows the chromatogram of Azo dye sample. The analytical conditions are shown in Table 2

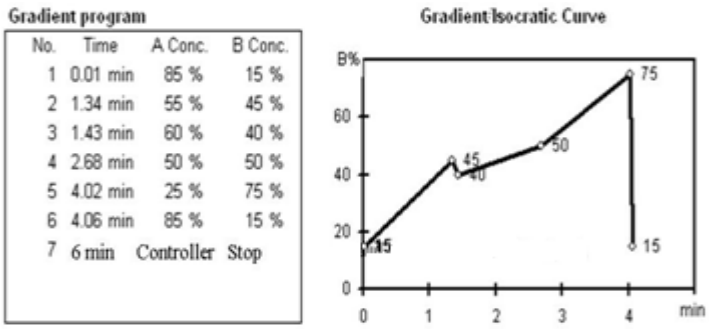

Fig. 3 LC time program

Column	Shim- pack XR-ODSII 75 x 3.0 mm
Flow rate	1.2ml/ min
Column temperature	40 °C
Injection volume	5µl
Mobile phase	A- phosphate buffer pH 6.9
	B- methanol
Detection:	PDA (240, 280 and 305 nm)
	Temperature: 40 °C

Table 2 Analytical Conditions

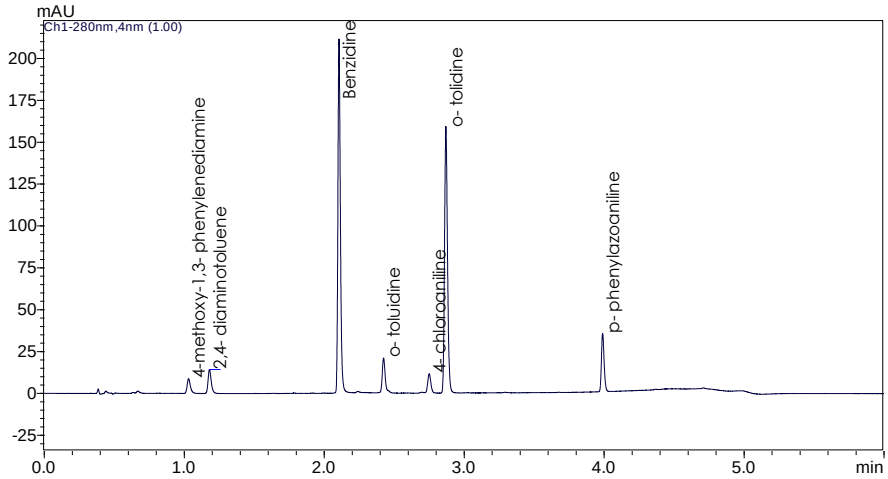

Fig. 4 Chromatogram of Azo dye sample.

Authors: Xing Jie Sun Zhe

SHIMADZU (sia Pacific) Pte. Ltd.
79 Science Park Drive, #02-01 Cintech IV
Singapore Science Park I, Singapore 118264
www.shimadzu.com.sg
Tel: +65-6778 6280 Fax: +65-6778 2050