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NEW HPLC-DAD METHOD FOR DETECTION OF SYNTHETIC DYES IN WASTEWATER SAMPLES

Alina Tatarus[1,2], Vasile Ion Iancu[1], Laura Florentina Chiriac[1], Roxana Scutariu[1], Claudia Maria Simonescu[2], Gheorghe Nechifor[2]

[1]National Research and Development Institute for Industrial Ecology-ECOIND, 57-73 Drumul Podu Dambovitei, district 6, 060652, Bucharest, alina.tatarus@yahoo.com, Romania
[2]University Politehnica of Bucharest, Faculty of Applied Chemistry and Materials Science, Department of Analytical Chemistry and Environmental Engineering, 1-7 Polizu Street, Bucharest, alina.tatarus@yahoo.com, Romania

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Introduction

This study explores the development of a new sensitive and selective High Performance Liquid Chromatography with Diode Array Detector (HPLC-DAD) method for the quantification of two synthetic dyes: Congo Red (CR) and Methyl Orange (MO) synthetics dyes from wastewater. Dyes may be classified as cationic (basic dyes), anionic (acid and colour reagents), and non-ionic (dispersion dyes) dyes. The separation of dyes from mixture solutions or wastewater samples, can led to higher sensitivity and reliable quantification should be allowed. The main innovation of the HPLC method is the possibility to analyze a wide variety of dyes (direct dispersed, reactive, acid and basic dyes) with a single chromatographic method.

Materials and methods

Dyes used, Methyl Orange (purity > 95%) and Congo Red (purity > 97%), were purchased from Sigma-Aldrich (Germany), acetonitrile, methanol and ammonium acetate were acquired from Merck (Germany). The basic standard solutions (CR and MO), with a concentration of 1000 mg/L, were prepared in methanol. Individual dilutions and mixed standard solutions of analytes were prepared in ultrapure water. All solutions have been refrigerated at 4°C and were protected against light in amber flask.

Experiments to establish optimal conditions for chromatographic separation and detection were carried out using an Agilent 1200 HPLC system consisting of: solvent cabinet and membrane degasser; quaternary pump, capable of providing in the system a mobile phase with up to 4 components with variable flow; autosampler with a capacity of 100 positions and variable injection volume (0.1-100 µL); thermostat for constant temperature maintenance in the chromatographic column; the Acclaim Surfactant Plus chromatographic column with a length of 15 cm, an inner diameter of 3 mm and the diameter of 3 µm stationary phase particles; variable wavelength DAD detector and ability to record up to 8 different wavelengths simultaneously; Agilent ChemStation software for data acquisition, processing and

reporting. In order to simultaneously determine the two compounds, it was attempted to establish the conditions of chromatographic separation using gradient elution program. Different compositions of mobile phase were tested using different proportions of acetonitrile and ammonium acetate. The composition of mobile phase is 100 mM ammonium acetate was ultrapure water with a pH value of 5.0 (solvent A) and acetonitrile (solvent B). Experiments were performed in gradient elution using different proportions between aqueous and organic solvents (0-5 min. 70% B, 0,5 mL/min.; 5-15 min. 5% B, 0,9 mL/min.). Injection volume was 10 μL and the chromatogram run time was only 15 minutes. Detection of compounds was carried out at optimal wavelengths identified after the maximum absorption in UV-VIS spectra: 506 nm for CR and 428 nm for MO. Figure 1 shows the absorption spectra for each dye: CR and MO.

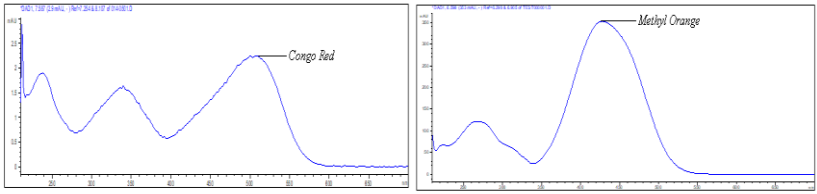

Fig.1. UV-VIS absorption spectra of Congo Red (a) and Methyl Orange (b) obtained by HPLC-DAD (200-700 nm)

Results and conclusions

In this study a newly developed HPLC-DAD method for the simultaneous detection Congo Red and Methyl Orange synthetic dyes was validated and applied to wastewater samples having a run-time of only 12 minutes. All liquid-chromatographic conditions (nature and composition of the mobile phase, injection volume, detection wavelength, column temperature, etc.) have been optimized for the rapid separation of the two analytes with a high sensitivity in order to determine these synthetic dyes at parts per million (mg/L) concentration levels from complex matrices of wastewater samples. The detection (LOD) and instrumental quantification (LOQ) limits were determined by injecting solutions with lower analyte concentrations until the experimentally determined signal-to-noise ratio was equal to 3 (LOD) and 10 (LOQ) respectively. The quantification limits of the method were 0.45 mg/L for CR and 0.55 mg/L for MO. These extremely low values for an HPLC-type method with DAD detection are mainly due to chromophore-rich structures of the analytes that show intense absorption bands (high molar absorption coefficients in the visible field (wavelengths between 506 and 428 nm) which is generally protected from the interferences of most organic compounds present in wastewater samples. The method developed was successfully applied for the determination of dyes from 10 wastewater samples.