Performance PerSPECtives

Waters® CapLC™ System
Fluorescence Detection

Detection Schemes in Capillary Liquid Chromatography

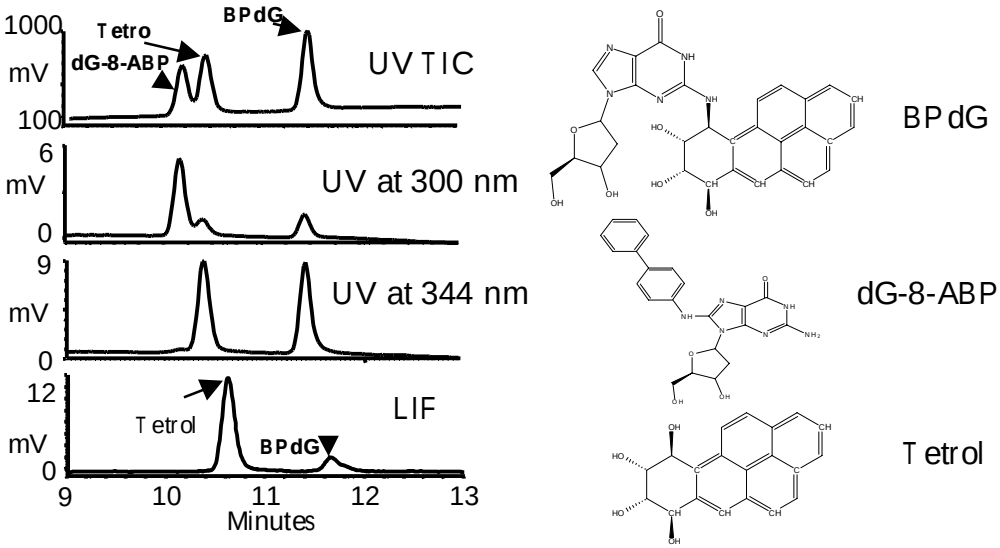
The ability to perform separations with highly sensitive detection is becoming increasingly important in analytical laboratories that work with mass-limited samples. To increase sensitivity, small internal diameter columns and low system volume chromatographic instruments have been developed. The Waters CapLC System is a direct flow capillary LC system specifically designed to provide separations in applications where sample is limited and sensitivity must be maximized. Detection is achieved with an integrated photodiode array (PDA) detector, and a mass spectrometer is easily coupled to the system. Although ultraviolet (UV) detection and mass spectrometry (MS) are commonly used forms of detection in capillary liquid chromatography, some analyses may benefit from the use of an alternative type of detector that can provide additional sensitivity and/or selectivity.

Laser induced fluorescence (LIF) can provide highly sensitive and selective detection of compounds that fluoresce when excited at an appropriate wavelength. The sensitivity of LIF is derived from the direct proportionality between the intensity of the excitation energy and the intensity of the fluorescent signal. By using a powerful laser for excitation, sensitivity may be greatly increased over that achieved in traditional fluorescence detection using an incandescent (xenon or deuterium) lamp. In addition, LIF is selective towards compounds that have excitation and emission characteristics compatible with the system setup.

Flexible Detection Options for Compound Specific Analyses

A sample solution containing DNA adducts was analyzed using ultraviolet, mass spectrometric, and laser induced fluorescence detection. A separation of the 3 components in the mixture using serial UV and LIF detection is shown in Figure 1.

Figure 1. UV and LIF detection for 16 fmol of a DNA adduct sample



Analytical Conditions:
System: CapLC System
Column: 0.32 x 15 cm Waters Symmetry® C_{18}, 100Å, 5 µ particle size, column temperature: 50 ºC
Solvents: A: 0.01% TFA in water, B: 100% MeOH
Flow Rate: 10 µL/min
Gradient: 30-70% B in 20 min
Injection: 1 µL
LIF: Excitation at 325 nm with a LiCONiX® helium cadmium laser; Emission detection at 395 nm using a Picometrics model ZETALIF fluorescence detector

Waters Corporation 34 Maple Street Milford, MA 01757 508 478 2000

The selectivity of fluorescence detection is displayed in Figure 1 where the UV trace shows the coelution of tetrol and dG-8-ABP while the LIF trace shows only the signal from th tetrol molecule. This selectivity is useful in situations where there are interferences with the analyte of interest. In addition, although useful spectral information may be gained using the PDA, the limits of detection for a fluorescent molecule like tetrol may be improved by up to an order of magnitude using LIF. Laser-induced fluorescence of tetrol allowed a detection limit of 1 fmol as shown in Figure 2, while the UV limit of detection for tetrol was 10 fmol. The linearity of the LIF response from 3 fmol/µL to 500 fmol/µL tetrol is displayed in Figure 3. Mass spectrometric analyses of these samples yielded detection limits 10-fold higher than those achieved using LIF detection.

Figure : Limit of Detection for Tetrol Using LIF

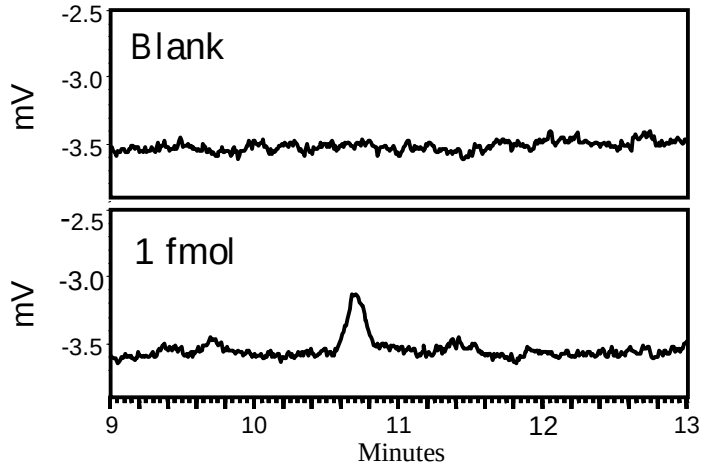
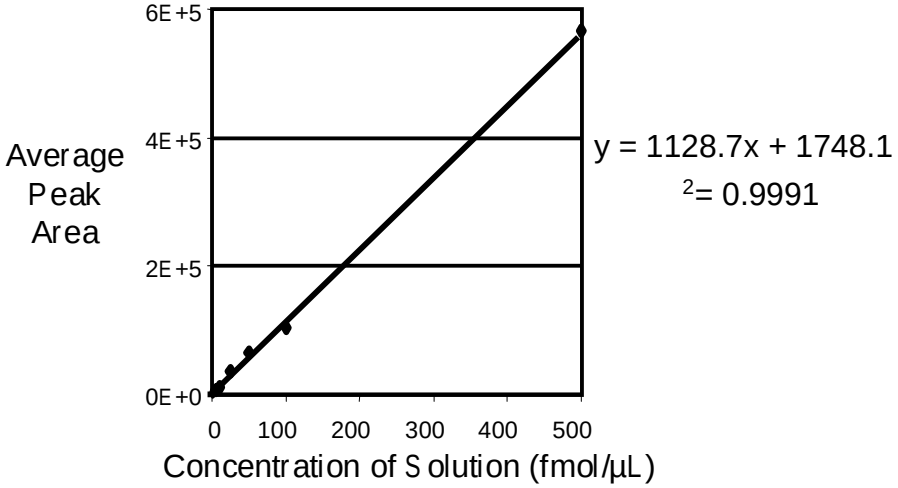


Figure 3: LIF Response Linearity for Tetrol



Summary

As the need for sample limited analyses grows, the detection schemes required for capillary liquid chromatography will increase in scope.

The Waters CapLC System may be easily interfaced with alternative detection schemes, such as fluorescence detection, to provide sample specific sensitivity and selectivity.

The incorporation of a fluorescence detector with the CapLC System allows for increased flexibility in the type of samples analyzed by capillary liquid chromatography.

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