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Post-Column Photochemical Reaction Enhances Detection in HPLC Assays of Tamoxifen or Aflatoxins

T. Ascah, Liquid Separations, Supelco, Bellefonte, PA, USA
H. Joshua, Aura Industries, Inc., Staten Island, NY, USA

Some pharmaceutical and biological analytes exhibit UV absorption maxima at wavelengths at which impurities also absorb. Serum and urine components, for example, can interfere with detection at shorter wavelengths (e.g., 220nm). Post-column photochemical reactions can be used to detect analytes at longer UV wavelengths (1) and, potentially, to increase UV, fluorescence, electrochemical, and other detector sensitivity for compounds that must be monitored at low concentrations.

Aura Industries' Photochemical Reactor Enhancement Detection system (PHRED) can enhance analyte detection under these difficult circumstances. Connected between the HPLC column and the detector, the PHRED unit performs continuous on-line photolytic derivatization. The unit includes a 254nm low pressure mercury lamp, a lamp holder, a knitted PTFE reactor coil in which derivatization takes place, and a holder for

Figure A. Photochemical Reactor Enhancement Detection (PHRED) System



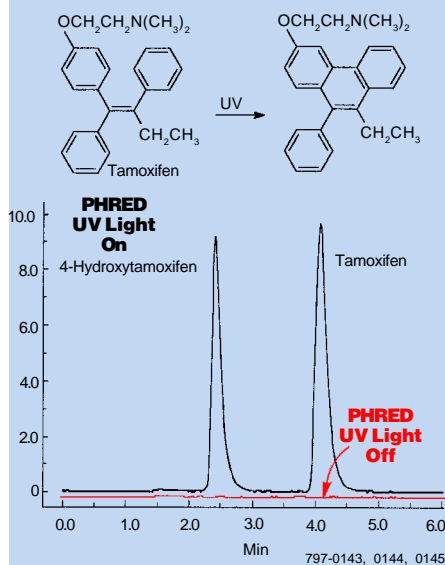
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the reactor coil (Figure A). Analysts at Aura Industries recently developed conditions for using the PHRED system in two important applications: monitoring the pharmaceutical compound tamoxifen and monitoring aflatoxins.

Tamoxifen, a nonsteroid estrogen antagonist, is widely used in treating breast cancer. The underivatized drug and its major metabolite, 4-hydroxytamoxifen, are undetected by fluorescence (Figure B). With post-column photolytic derivatization, however, detection at therapeutic levels is highly reliable.

Figure B. Tamoxifen and Its Major Metabolite

Column: **SUPELCO SILABZ+Plus, 15cm x 4.6mm, 5µm particles**
Cat. No.: **59196**
Mobile Phase: acetonitrile: 0.01M H₃PO₄, 40:60
Flow Rate: 1mL/min
Temp.: 40°C
Det.: fluorescence; 260nm excitation, >418nm emission
Reactor Coil: 5m x 0.25mm ID
Inj.: 20µL mobile phase containing 800ng/mL tamoxifen, 500ng/mL 4-hydroxytamoxifen

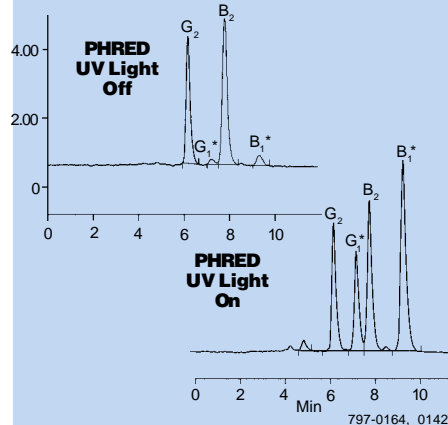


Aflatoxins are of major concern as potentially carcinogenic contaminants in many food products. Figure C shows an analysis of aflatoxin standards, using the PHRED system. Note that the temperature in the reactor coil can be elevated – this minimizes peak spreading and backpressure in the coil. Relative to the peak width with the column connected directly to the detector, the peak width for aflatoxin B₂ is increased by less than 6% when the eluent is passed through the 25-meter coil. PHRED can be used in simultaneous HPLC analysis of aflatoxins, zearalenone, and ochratoxin A (2).

A PHRED unit can effectively improve analyte detection in the presence of interfering matrix components, or when the analytes are present at low concentrations.

Figure C. Aflatoxins

Column: **SUPELCO SIL LC-18, 15cm x 4.6mm, 5µm particles**
Cat. No.: **58230-U**
Mobile Phase: acetonitrile:methanol:water, 20:20:60
Flow Rate: 1mL/min
Temp.: 30°C
Det.: fluorescence; 360nm excitation, >418nm emission
Reactor Coil: 25m x 0.25mm ID (50°C)
Inj.: 20µL (aflatoxin mix*: acetonitrile:water (1:3), 1:4)
*Cat. No. **46304-U**
1µg/mL B₁, G₁,
0.3µg/mL B₂, G₂



*Requires derivatization for fluorescence detection. By replacing chemical derivatization, photochemical derivatization simplifies this procedure.

Ordering Information:

Description	Cat. No.
PHRED Photochemical Reactor	57400-U
HPLC Columns (5µm particles)	
SUPELCO SIL™ LC-18	
15cm x 4.6mm	58230-U
SUPELCO SIL ABZ+Plus	
15cm x 4.6mm	59196

References

- Krull, I.S., C.M. Selavka, M. Lookabaugh, and W.R. Childress, *LC/GC*, **7**: 758 (1989).
- Joshua, H., M.H. Joshua, M.D. Joshua, and J. Joshua., *The Simultaneous HPLC Determination of Six Aflatoxins, Zearalenone and Ochratoxin A Using Gradient Elution, Post-Column Photochemical Derivatization and Fluorescence Detection* presented at the Eastern Analytical Symposium, Somerset, NJ, USA, Nov. 1994.

References not available from Supelco.

Figures B and C provided by Dr. Henry Joshua, Aura Industries, Inc., Staten Island, NY.

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