

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

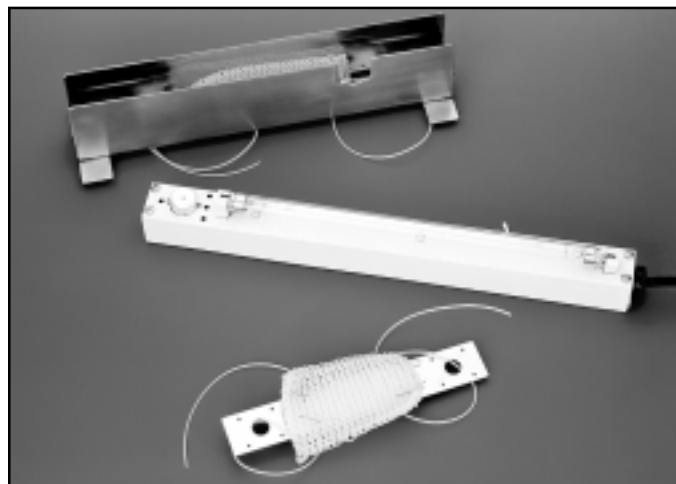
A post-column photochemical reactor enhancement detection system (PHRED) can improve analyte detection in the presence of interfering matrix components, or when the analytes are present at low concentrations. Tamoxifen, a nonsteroid estrogen antagonist widely used in treating breast cancer, and its major metabolite, 4-hydroxytamoxifen, are literally undetected at 260nm. With post-column photolysis, however, detection is highly reliable. Use of in-line photochemical derivatization to screen for aflatoxins is simple, reliable, reproducible, and sensitive. A PHRED system can be used in simultaneous HPLC analysis of aflatoxins, zearalenone, and ochratoxin A.

Key Words:

- tamoxifen • aflatoxins • post-column derivatization

Some pharmaceutical and biological analytes exhibit UV absorption maxima at wavelengths at which impurities also are absorptive. Serum and urine components, for example, can interfere with detection at shorter wavelengths (e.g., 220nm). Post-column photochemical reaction can be used to detect analytes at longer UV wavelengths (1) and, potentially, to increase detector sensitivity for compounds that must be monitored at unsatisfactorily low concentrations (e.g., because they have limited solubility in a desired solvent).

Figure A. Photochemical Reactor Enhancement Detection (PHRED) System



913-0355

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

Data in Table 1 show that post-column photochemical reaction generally increased barbital peaks at both 220nm and 270nm. At 270nm, the amobarbital/pentobarbital peak showed a more than 30-fold increase (these analytes coeluted under the chromatographic conditions used). Note that there was some loss of detection sensitivity in changing from detection at 220nm without enhancement to detection at 270nm with enhancement. Thus, relative detection sensitivities of sample components at different wavelengths must be kept in mind. If matrix components do not interfere with an analysis, it may be preferable to use a lower wavelength, with or without enhancement.

Table 1. Post-Column Photochemical Reaction Enhances Detection

Analyte	Peak Area (x 10 ³) without / with PHRED		% Enhancement w/PHRED	
	220nm	270nm	220nm	270nm
Amobarbital/ Pentobarbital	202 / 237	5 / 171	18	3103
Secobarbital	133 / 173	3 / 72	30	2034
Butethal	138 / 162	3 / 78	17	2507
Barbital	203 / 195	4 / 53	-4	1198
Means for 3 analyses. C60 Fullerene	1020 / 1155 (254nm)		13	
Single analysis.				
Column:	SUPELCOSIL LC-18, 25cm x 4.6mm (5µm packing)			
Cat. No.:	58298			
Mobile Phase:	0.02M NaH ₂ PO ₄ ; acetonitrile, 1:1 (pH 7 with 10N NaOH)			
Flow Rate:	barbitals - 1mL/min; fullerene - 2mL/min			
Temp.:	ambient			
Reactor Coil:	15m x 0.25mm ID			
Inj.:	10µL mobile phase containing 10µg each barbital or 10µL toluene containing 1µg fullerene			

Table 1 also shows that detection was enhanced for C60 fullerene, a minor impurity in C70 product. This indicates the PHRED system can increase sensitivity for analytes at low concentrations.

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 (Figure B), is widely used in treating breast cancer. The drug and its major metabolite, 4-hydroxytamoxifen, are literally undetected at 260nm (Figure B). With post-column photolysis, however, detection at therapeutic levels is highly reliable.

Aflatoxins are of major concern as potentially carcinogenic contaminants in many food products. Use of in-line photochemical derivatization to screen for these contaminants is superior to other methods in several ways, including simplicity, reliability, reproducibility, and sensitivity (2-4). The equipment offers both lower initial cost and lower cost per analysis, and requires a very small amount of bench space. 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% (from 0.202 min to 0.214 min) when the eluent is passed through the 25-meter coil. PHRED can be used in simultaneous HPLC analysis of aflatoxins, zearalenone, and ochratoxin A (4).

A PHRED unit can effectively improve analyte detection in the presence of interfering matrix components, or when the analytes are present at low concentrations. For more information about this highly useful tool, request Bulletin 844.

Figure B. Tamoxifen and Its Major Metabolite

Column: **SUPELCOSIL ABZ⁺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

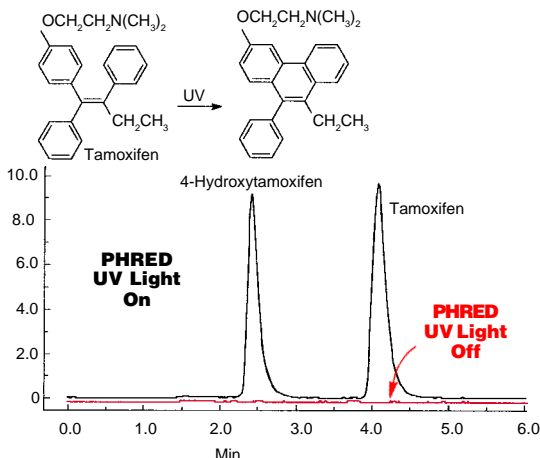


Figure provided by H. Joshua, Aura Industries, Inc., Staten Island, NY. 797-0143, 0144, 0145

Acknowledgment

The information and chromatograms in this application note were provided by Dr. Henry Joshua, Aura Industries, Inc., Staten Island, NY, USA

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Figure C. Aflatoxins with Post-Column Photolysis

Column: **SUPELCOSIL 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. **4-6304**
 1µg/mL B₁, G₁, 0.3µg/mL B₂, G₂

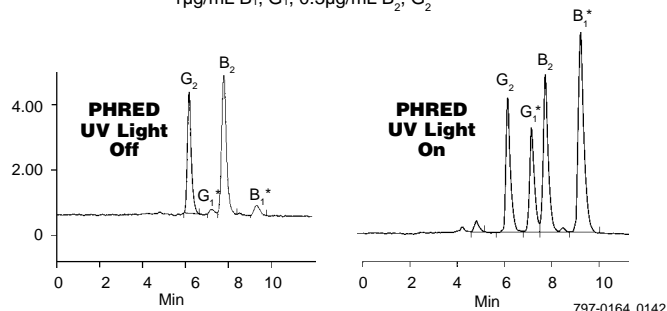


Figure provided by H. Joshua, Aura Industries, Inc., Staten Island, NY. 797-0164, 0142

Ordering Information:

Description	Cat. No.
PHRED Photochemical Reactor	57400-U
Knitted Reactor Coils	
5m x 0.25mm ID (0.25mL)	57402
10m x 0.25mm ID (0.5mL)	57403
15m x 0.25mm ID (0.75mL)	57404
5m x 0.50mm ID (1.0mL)	57405
10m x 0.50mm ID (2.0mL)	57406
15m x 0.50mm ID (3.0mL)	57407
Reflective Support Plate (stainless steel)	57408
SUPELCOSIL™ HPLC Columns (5µm particles)	
SUPELCOSIL LC-18, 15cm x 4.6mm	58230-U
SUPELCOSIL LC-18, 25cm x 4.6mm	58298
SUPELCOSIL ABZ ⁺ Plus, 15cm x 4.6mm	59196
Columns of other dimensions are available – please inquire.	
Aflatoxin Mix	
1µg/mL B ₁ , G ₁ , 0.3µg/mL B ₂ , G ₂ in methanol. 5 x 1 mL	46304-U
For many other mycotoxin standards, please refer to our general catalog.	

References

- Krull, I.S., C.M. Selavka, M. Lookabaugh, and W.R. Childress, *LC/GC*, 7: 758 (1989).
- Joshua, H., *J. Chromatogr.*, **654**: 247 (1993).
- Joshua, H., *Analysis of Aflatoxins in Naturally Contaminated Corn by HPLC, Post-Column Photochemical Derivatization and Fluorescence Detection* presented at the Pittsburgh Conference and Exhibition, Chicago, IL, USA, March 1994; published in *American Laboratory*, p.36J-36M, April 1995.
- 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, November 1994.

References not available from Supelco.

Note 120

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