

Monitor Dinitroaniline Herbicides in Wastewater by HPLC

A single HPLC column can be used to quantify nanogram amounts of five herbicides. The results of this analysis, using a 7.5cm x 4.6mm column filled with 3µm SUPELCO SIL LC-18 packing, are superior to those done by GC following US EPA methodology.

Key Words:

- herbicides
- dinitroaniline
- HPLC
- SUPELCO SIL LC-18

US Environmental Protection Agency (EPA) Method 627 describes the monitoring of five dinitroaniline herbicides in industrial and municipal wastewaters, using gas chromatography with electron capture detection (1). Although the two GC packings used in the EPA method separate profluralin and isopropalin, trifluralin, benfluralin and ethafluralin elute from either column as a single peak. A SUPELCO SIL™ LC-18 column and two mobile phases, however, will permit better determination of these five herbicides by HPLC.

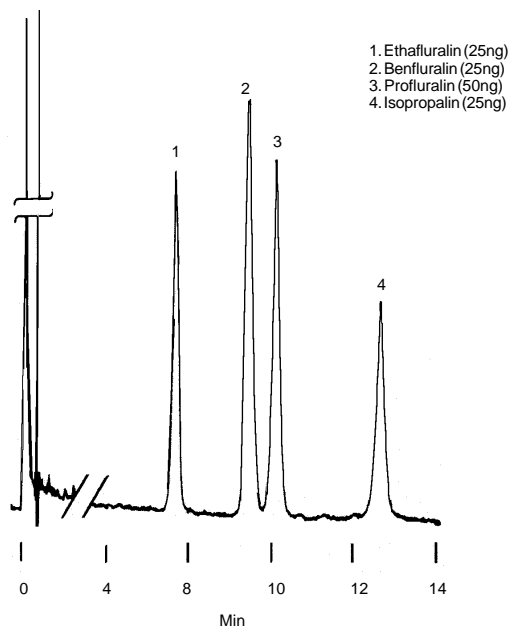
Because the five dinitroaniline herbicides have very similar structures and molecular weights (Figure A), separating them is difficult. Organic solvents from different groups in Snyder's selectivity triangle (2) (methanol from group II, tetrahydrofuran from group III, dioxane from group VIa, and acetonitrile from group VIb) were combined with water into binary mobile phases which resolved the five compounds into 4, 4, 2, and 3 peaks respectively. The elution order was the same for methanol:water, acetonitrile:water, and dioxane:water mobile phases (ethafluralin [E], benfluralin [B], trifluralin [T], profluralin [P], isopropalin [I]), but different compounds coeluted. The elution patterns were E, B + T, P, I for methanol:water; E, B + T + P, I for acetonitrile:water; and E, B + T + P + I for dioxane:water mobile phases. The elution order in tetrahydrofuran:water (P, B + I, T, E) was unique.

Figure A. Structure of Dinitroaniline Herbicides

Compound	Functional Group R	Functional Group R'	Functional Group R''	Molecular Weight
Benfluralin	CF ₃	C ₂ H ₅	nC ₄ H ₉	335.3
Ethafluralin	CF ₃	C ₂ H ₅	CH ₂ =C(CH ₃)CH ₂	333.3
Profluralin	CF ₃	C ₃ H ₇	CH ₂ CH ₂ CHCH ₂	347.3
Trifluralin	CF ₃	C ₃ H ₇	C ₃ H ₇	335.3
Isopropalin	iC ₃ H ₇	C ₃ H ₇	C ₃ H ₇	309.4

Figure B. Dinitroaniline Herbicides in Methanol: Water Mobile Phase

Column: SUPELCO SIL LC-18, 7.5cm x 4.6mm ID, 3µm particles
 Cat. No.: 58984
 Mobile Phase: methanol:water, 70:30
 Col. Temp.: 30°C
 Flow Rate: 1.5mL/min
 Det.: UV, 254nm
 Inj.: 10µL, analyte quantities shown on figure



With a 70:30 methanol:water mobile phase, sharp, well resolved peaks are obtained for ethafluralin, profluralin, and isopropalin (Figure B).

If both benfluralin and trifluralin are present, however, they will coelute in this system. In a tetrahydrofuran:methanol:water mobile phase, the elution order is drastically different (Figure C). Trifluralin is well resolved from benfluralin, but benfluralin now coelutes with isopropalin. When necessary, isopropalin and trifluralin can be quantified by using the methanol:water or the tetrahydrofuran:methanol:water mobile phase, respectively. To quantify benfluralin in the presence of both isopropalin and trifluralin, it is necessary to use both mobile phases and combine the data from the separate analyses.

Figure C. Dinitroaniline Herbicides in a Tetrahydrofuran:Methanol:Water Mobile Phase

Column: **SUPELCOSIL LC-18, 7.5cm x 4.6mm ID, 3µm particles**
 Cat. No.: **58984**
 Mobile Phase: tetrahydrofuran:methanol:water, 50:10:40
 Col. Temp.: 30°C
 Flow Rate: 1.5mL/min
 Det.: UV, 254nm
 Inj.: 10µL mobile phase, analyte quantities listed on figure

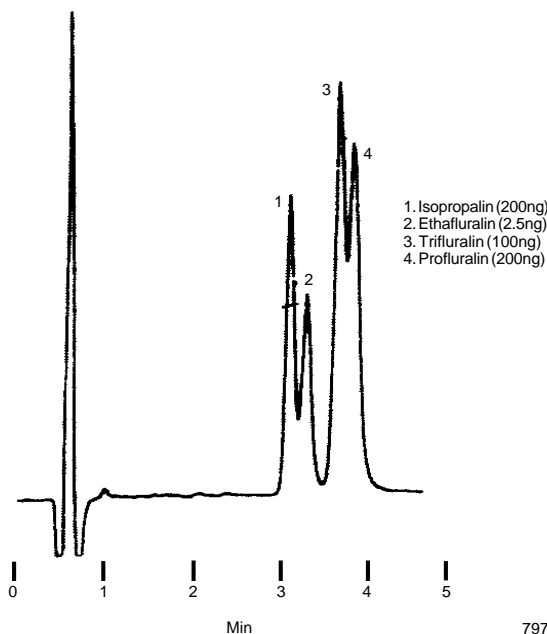
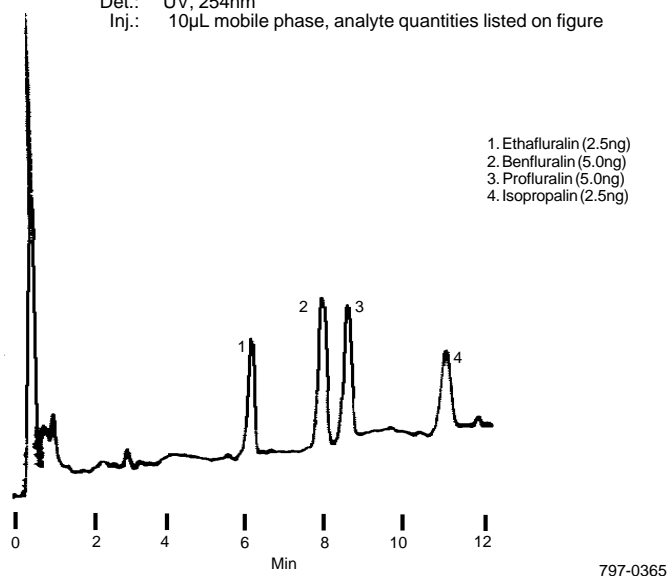


Figure D. Dinitroaniline Herbicides at High Detector Sensitivity

Column: **SUPELCOSIL LC-18, 7.5cm x 4.6mm ID, 3µm particles**
 Cat. No.: **58984**
 Mobile Phase: methanol:water, 70:30
 Col. Temp.: 35°C
 Flow Rate: 1.5mL/min
 Det.: UV, 254nm
 Inj.: 10µL mobile phase, analyte quantities listed on figure



The value of this HPLC analysis depends on the detection level required for the herbicides. In EPA Method 627, a 10mL extract is obtained from one liter of water. Subsequently 1-5µL aliquots are injected into the GC column. This provides detection limits of 20-140ng/L (or parts per trillion) with electron capture detection, although accuracy and precision determinations included in the EPA method suggest that the range of practical interest is 0.5-2.0µL/L (or parts per billion).

The HPLC analysis shown in Figure B was obtained using a Spectra-Physics 773 UV detector with a 12µL cell at a sensitivity of 0.04 AUFS, and injecting 25-50ng of each herbicide onto the column. A reasonably stable baseline can be maintained at 0.01 AUFS; consequently the on-column amount of each herbicide could be reduced at least 10- to 20-fold (Figure D). Thus parts per billion sensitivity can be achieved by HPLC, using 0.1 to 1.0% aliquots of the 10mL extract.

The separations described here were produced with a 7.5cm x 4.6mm column filled with 3µm SUPELCOSIL LC-18 packing. Equivalent results can be achieved with a 15cm x 4.6mm column filled with 5µm SUPELCOSIL LC-18.

References

1. Federal Register, **48**: 6250-6266 (1983).
 2. Snyder, L.R., *J. Chromatogr.*, **92**: 223 (1974).
- References not available from Supelco.

Trademarks

SUPELCOSIL and Supelguard are trademarks of Sigma-Aldrich Co.

Ordering Information:

Description	Cat. No.
SUPELCOSIL LC-18 Columns	
7.5cm x 4.6mm, 3µm particles	58984
15cm x 4.6mm, 5µm particles	58230-U
Supelguard LC-18 Guard Column Kit	
2cm column with 5µm particles, plus column holder and connecting hardware.	59554
Supelguard LC-18 Guard Columns, 2cm	
Pk. of 2	59564

Contact our Technical Service Department (phone 800-359-3041 or 814-359-3041, FAX 814-359-5468) for expert answers to your questions.

Note 134

For more information, or current prices, contact your nearest Supelco subsidiary listed below. To obtain further contact information, visit our website (www.sigma-aldrich.com), see the Supelco catalog, or contact Supelco, Bellefonte, PA 16823-0048 USA.

ARGENTINA · Sigma-Aldrich de Argentina, S.A. · Buenos Aires 1119 **AUSTRALIA** · Sigma-Aldrich Pty. Ltd. · Castle Hill NSW 2154 **AUSTRIA** · Sigma-Aldrich Handels GmbH · A-1110 Wien
BELGIUM · Sigma-Aldrich N.V./S.A. · B-2880 Bornem **BRAZIL** · Sigma-Aldrich Quimica Brasil Ltda. · 01239-010 São Paulo, SP **CANADA** · Sigma-Aldrich Canada, Ltd. · 2149 Winston Park Dr. · Oakville, ON L6H 6J8
CZECH REPUBLIC · Sigma-Aldrich s.r.o. · 186 00 Praha 8 **DENMARK** · Sigma-Aldrich Denmark A/S · DK-2665 Vallensbaek Strand **FINLAND** · Sigma-Aldrich Finland/YA-Kemia Oy · FIN-00700 Helsinki
FRANCE · Sigma-Aldrich Chimie · 38297 Saint-Quentin-Fallavier Cedex **GERMANY** · Sigma-Aldrich Chemie GmbH · D-82041 Deisenhofen **GREECE** · Sigma-Aldrich (o.m.) Ltd. · Ilioupoli 16346, Athens
HUNGARY · Sigma-Aldrich Kft. · H-1067 Budapest **INDIA** · Sigma-Aldrich Co. · Bangalore 560 048 **IRELAND** · Sigma-Aldrich Ireland Ltd. · Dublin 24 **ISRAEL** · Sigma Israel Chemicals Ltd. · Rehovot 76100
ITALY · Sigma-Aldrich s.r.l. · 20151 Milano **JAPAN** · Sigma-Aldrich Japan K.K. · Chuo-ku, Tokyo 103 **KOREA** · Sigma-Aldrich Korea · Seoul **MALAYSIA** · Sigma-Aldrich (M) Sdn. Bhd. · Selangor
MEXICO · Sigma-Aldrich Química S.A. de C.V. · 50200 Toluca **NETHERLANDS** · Sigma-Aldrich Chemie BV · 3330 AA Zwijndrecht **NORWAY** · Sigma-Aldrich Norway · Torshov · N-0401 Oslo
POLAND · Sigma-Aldrich Sp. z o.o. · 61-663 Poznań **PORTUGAL** · Sigma-Aldrich Química, S.A. · Sintra 2710 **RUSSIA** · Sigma-Aldrich Russia · Moscow 103062 **SINGAPORE** · Sigma-Aldrich Pte. Ltd.
SOUTH AFRICA · Sigma-Aldrich (pty) Ltd. · Jet Park 1459 **SPAIN** · Sigma-Aldrich Química, S.A. · 28100 Alcobendas, Madrid **SWEDEN** · Sigma-Aldrich Sweden AB · 135 70 Stockholm
SWITZERLAND · Supelco · CH-9471 Buchs **UNITED KINGDOM** · Sigma-Aldrich Company Ltd. · Poole, Dorset BH12 4QH
UNITED STATES · Supelco · Supelco Park · Bellefonte, PA 16823-0048 · Phone 800-247-6628 or 814-359-3441 · Fax 800-447-3044 or 814-359-3044 · email: supelco@sial.com

H

Supelco is a member of the Sigma-Aldrich family. Supelco products are sold through Sigma-Aldrich, Inc. Sigma-Aldrich warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product for a particular use. Additional terms and conditions may apply. Please see the reverse side of the invoice or packing slip.

BII