

# Application

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## SUPELCO<sup>SM</sup> HPLC Columns Provide Rapid Analyses of Plant Growth Regulators

*Traditional methods of analysis of plant growth regulators are time consuming and sometimes ineffective. HPLC analyses of PGRs are faster and more accurate than biological assays or TLC. A SUPELCO<sup>SM</sup> LC-18-DB column produces sharp peaks and excellent resolution.*

### Key Words:

- HPLC • ion suppression chromatography • kinetin
- plant growth regulators

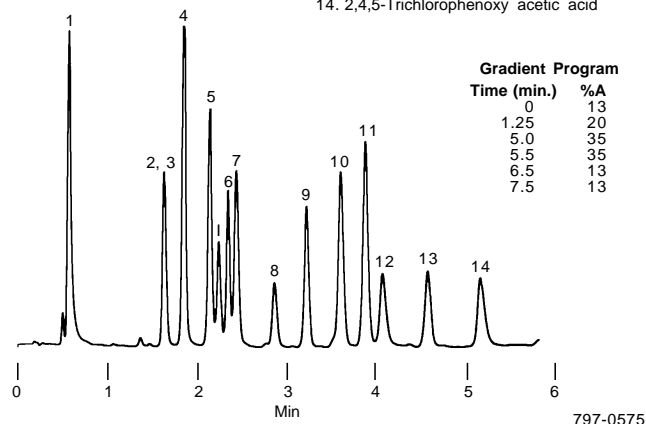
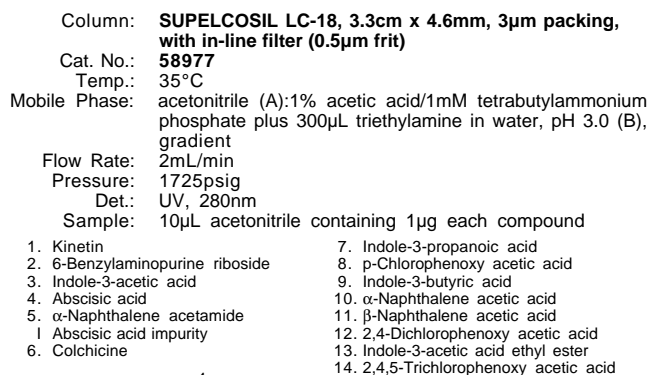
Plant growth regulators (PGRs) are a diverse group of naturally occurring hormones and synthetic compounds that stimulate or inhibit plant growth. Among PGRs produced by plants are auxins (including indole-3-acetic acid and its derivatives), gibberellins, cytokinins, abscisic acid, and ethylene. Because artificial application of natural PGRs is sometimes ineffective, synthetic materials (e.g., naphthalene acetic acid, maleic acid hydrazide) have been developed to mimic or antagonize the actions of natural materials. These compounds must usually be identified and quantified in trace amounts.

Biological assays and TLC are widely used means of analyzing and measuring plant hormones. However, these methods are time consuming, lack precision, and have low sensitivity for certain compounds. \* HPLC analyses on 15cm or 25cm columns are faster and more accurate than bioassays, but still take 30 to 40 minutes. Using SUPELCO<sup>SM</sup> columns (3.3cm x 4.6mm, 3 $\mu$ m packing), PGR analyses can be done much more rapidly, without sacrificing resolution.

Because most PGRs are acidic compounds, they can be eluted from reversed phase HPLC columns by several retention mechanisms. At a slightly acidic, neutral, or basic pH, the carboxylic acid group is ionized, presenting the most polar, and therefore the most rapidly eluting, form of the molecule. For greater separation, retention can be prolonged by running the analysis at a lower pH, at which the acid group is protonated (ion suppression chromatography). Alternatively, retention can be prolonged by performing the analysis at a near neutral pH (acids ionized) and adding a positively charged ion pair reagent, such as tetrabutylammonium phosphate, to the mobile phase (ion pair chromatography).

For the most rapid and convenient analyses, we chose to separate PGRs by ion suppression chromatography, using a SUPELCO<sup>SM</sup> LC-18 column (Figure A). In order to resolve several chlorophenoxy acetic acid herbicides included in the sample, we added an ion pair reagent (TBA) to the mobile phase. In attempts

**Figure A. A Competing Base Provides a Sharp Kinetin Peak, but Reduces Resolution of Other Plant Growth Regulators**



to obtain a symmetrical peak for kinetin, we also added triethylamine (TEA). But as the kinetin peak became symmetrical, resolution of other compounds (compounds 2 and 3 in Figure A) was lost.

We subsequently found that a SUPELCO<sup>SM</sup> LC-18-DB column, deactivated to provide symmetrical peaks for basic compounds, is an excellent alternative to a conventional C18 column used with mobile phase containing TEA. Kinetin elutes as a symmetrical peak from a SUPELCO<sup>SM</sup> LC-18-DB column, yet the other PGRs and the herbicides are well resolved (Figure B).

\* In analyses of plant growth regulators, sample preparation is often prolonged and difficult process. Solid phase extraction on the same bonded phase packing used in the HPLC column may shorten and simplify sample preparation. We encourage investigators to consult the Supelco catalog for information about solid phase extraction products.

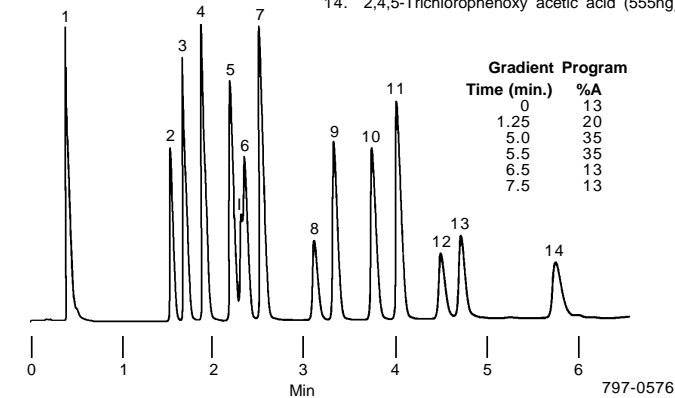
Because three important PGRs (cycloheximide, gibberellic acid, and maleic acid hydrazide) absorb 280nm UV radiation poorly, these were not included in the analyses shown in Figures A and B. These compounds are better detected at 215nm UV. In Figure C, these PGRs were separated by gradient elution on a SUPELCOSIL LC-18-DB column. The mobile phase formulation for this separation was dictated by the strong polar nature of maleic acid hydrazide.

In Figures B and C, a SUPELCOSIL LC-18-DB column resolved the plant growth regulators rapidly and well. Whether a deactivated SUPELCOSIL LC-18-DB or a conventional SUPELCOSIL LC-18 column is used, PGR analyses are reproducible from column to column. For analyses involving kinetin, however, we recommend using a deactivated column.

### Figure B. A Deactivated Column Provides a Sharp Kinetin Peak and Excellent Resolution of Other Growth Regulators

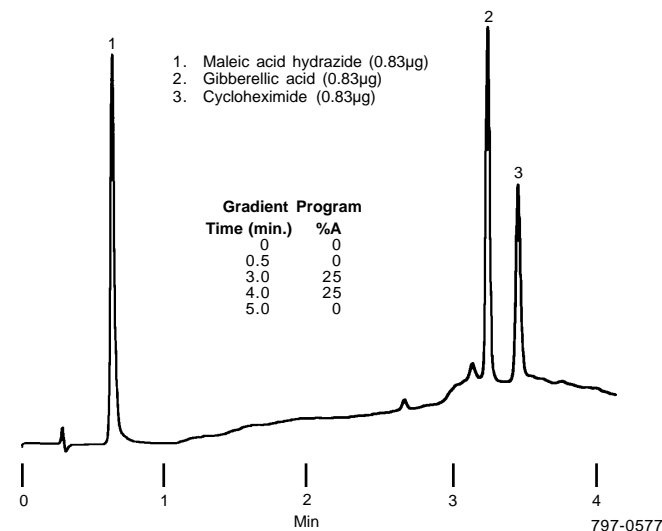
Column: **SUPELCOSIL LC-18-DB, 3.3cm x 4.6mm, 3µm packing, with in-line filter (0.5µm frit)**  
 Cat. No.: **58978**  
 Temp.: 35°C  
 Mobile Phase: acetonitrile (A):water containing 1% acetic acid in 1mM TBA, pH 2.8 (B), gradient  
 Flow Rate: 2mL/min  
 Pressure: 1700psig  
 Det.: UV, 280nm, 0.064 AUFS  
 Sample: 5µL 13% A:87% B, on-column amounts on figure

1. Kinetin (222ng)
2. 6-Benzylaminopurine riboside (445ng)
3. Indole-3-acetic acid (222ng)
4. Abscisic acid (222ng)
5. α-Naphthalene acetamide (222ng)
1. Abscisic acid impurity
2. Colchicine (555ng)
7. Indole-3-propanoic acid (222ng)
8. p-Chlorophenoxy acetic acid (333.5ng)
9. Indole-3-butyric acid (333.5ng)
10. α-Naphthalene acetic acid (333.5ng)
11. β-Naphthalene acetic acid (333.5ng)
12. 2,4-Dichlorophenoxy acetic acid (555ng)
13. Indole-3-acetic acid ethyl ester (445ng)
14. 2,4,5-Trichlorophenoxy acetic acid (555ng)



### Figure C. PGRs that Absorb Short Wavelength UV Must Be Analyzed Separately

Column: **SUPELCOSIL LC-18-DB, 3.3cm x 4.6mm, 3µm packing, with in-line filter (0.5µm frit)**  
 Cat. No.: **58978**  
 Temp.: 35°C  
 Mobile Phase: acetonitrile (A):0.01M KH<sub>2</sub>PO<sub>4</sub> + 0.01M K<sub>2</sub>PO<sub>4</sub> + 3mM TBA, pH 6.0 (B), gradient  
 Flow Rate: 2mL/min  
 Pressure: 1875psig  
 Det.: UV, 215nm, 0.064 AUFS  
 Sample: 5µL 50% A:50% B, on-column amounts on figure



### Ordering Information:

Description	Cat. No.
<b>SUPELCOSIL Columns, 3.3cm x 4.6mm, 3µm packings</b>	
SUPELCOSIL LC-18-DB*	58978
SUPELCOSIL LC-18	58977
<b>In-Line Filters (0.5µm pores) for Column Protection</b>	
Rheodyne® Frit Filter	59124
Replacement Frits (pk. of 5)	59126
Valco® Frit Filter	58420-U
Replacement Frits (pk. of 10)	59037

\* DB — Deactivated for analyses of basic compounds.

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