

Separating Atrazine and Its Metabolites by Reversed Phase HPLC, Using Discovery Columns

The herbicide atrazine and its four metabolites were separated by reversed phase HPLC on Discovery C18, Discovery C8, and Discovery RP-AmideC16 columns. The C18 and the C8 columns yielded similar results, offering excellent resolution and unique selectivity.

Key Words

- atrazine • atrazine metabolites • herbicides
- reversed phase HPLC • Discovery columns

Atrazine is a selective triazine herbicide used to control broadleaf and grassy weeds in corn, sorghum, sugarcane, pineapple, Christmas trees, and other crops. It is also used as a nonselective herbicide on non-cropped industrial lands and on fallow lands. Atrazine has been classified as a Restricted Use Pesticide (RUP) due to its potential for groundwater contamination (1,2).

HPLC has been shown to be effective for separating and identifying herbicides and their metabolites (3,4). We analyzed atrazine and its four metabolites – atrazine desethyl, atrazine desisopropyl, atrazine-2-hydroxyl, and 2-chloro-4,6-diamino-1,3,5-triazine. We first dissolved these materials in methanol, then diluted them to appropriate concentrations using deionized water.

We separated a mixture of atrazine and the four metabolites by reversed phase HPLC, using Discovery™ C18, Discovery C8, and Discovery RP-AmideC16 columns, each 15cm x 4.6mm ID, 5µm particles. The compounds generally were well separated by gradient elution (Figure A).

We also developed an isocratic elution method to separate the four atrazine metabolites. Figure B shows the results using a Discovery C18 column and a Discovery RP-AmideC16 column. Note that the selectivity differs on the RP-AmideC16 column. This selectivity difference possibly can be attributed to the hydrogen bonding between the amide functionality of the phase and the hydroxy group of the analyte. An isocratic separation using the Discovery C8 column yielded results similar to those for the Discovery C18 column.

The DT50 (time for 50% loss) for atrazine in soil is 60-150 days (5). In situations in which atrazine application has not been recent, it may be advantageous to use the isocratic conditions to analyze samples for the breakdown products, rather than follow the longer gradient elution procedure.

Discovery columns provide excellent resolution, peak shape, and reproducibility in the analysis of herbicides. Selectivity is unique among the three Discovery columns.

Figure A. Atrazine and Its Metabolites by Gradient Elution

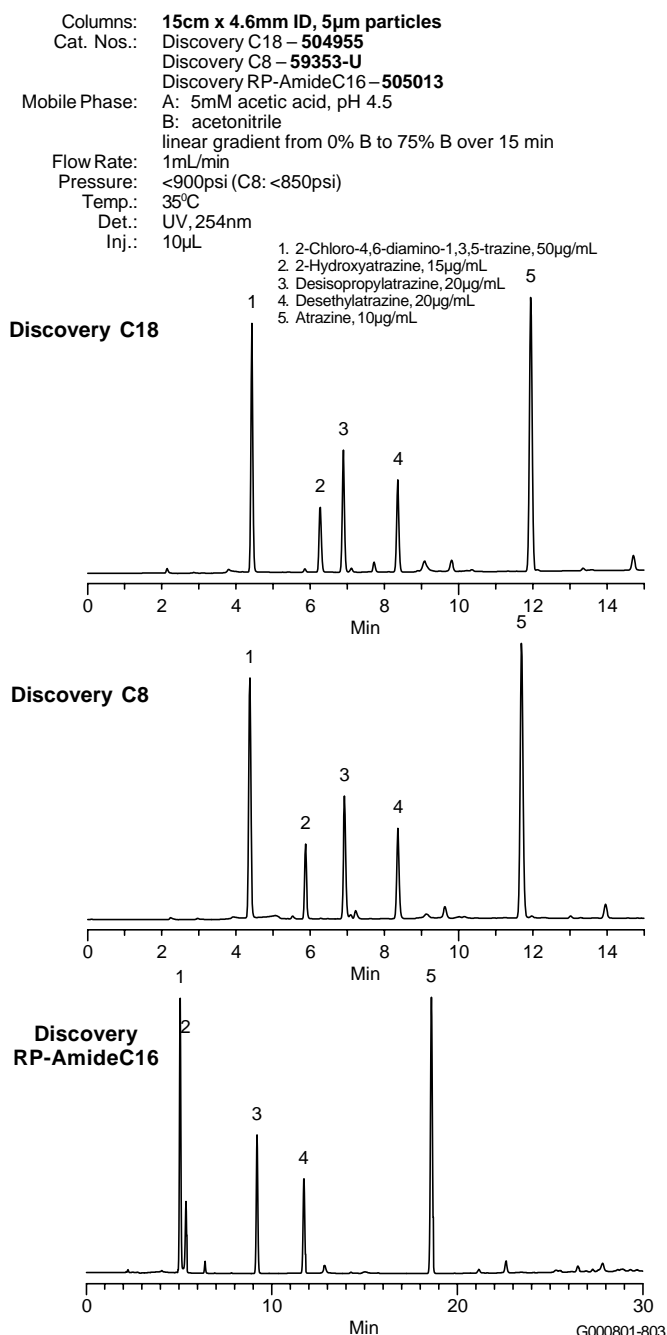
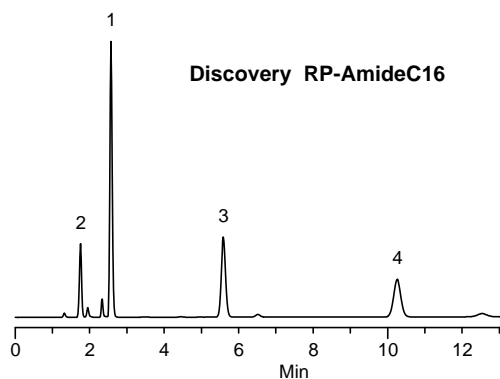
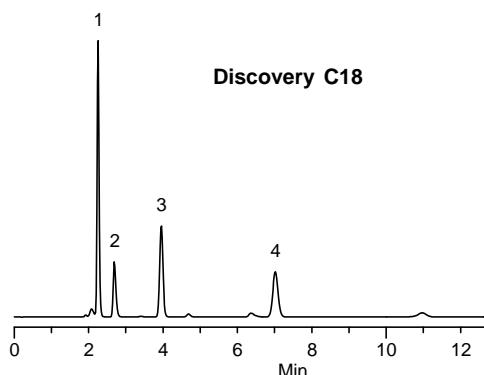


Figure B. Atrazine Metabolites by Isocratic Elution

Columns: **15cm x 4.6mm ID, 5µm particles**
 Cat. Nos.: Discovery C18 – **504955**
 Discovery RP-AmideC16 – **505013**
 Mobile Phase: acetonitrile:5mM acetic acid, pH 4.5, 17:83
 Flow Rate: 1mL/min
 Pressure: 800psi
 Temp.: 35°C
 Det.: UV, 254nm
 Inj.: 10µL

- 2-Chloro-4,6-diamino-1,3,5-triazine, 50µg/mL
- 2-Hydroxyatrazine, 15µg/mL
- Desisopropylatrazine, 20µg/mL
- Desethylatrazine, 20µg/mL



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References 1, 2, and 5 not available from Supelco.

Ordering Information:

| Description | Cat. No. |
|--|------------------|
| Discovery HPLC Columns | |
| 15cm x 4.6mm ID, 5µm particles | |
| Discovery C18 | 504955 |
| Discovery C8 | 59353-U |
| Discovery RP-AmideC16 | 505013 |
| Discovery Selectivity Packs¹ | |
| 5cm x 2.1mm ID columns | 55720-U21 |
| 15cm x 2.1mm ID columns | 55722-U21 |
| 5cm x 4.6mm ID columns | 55720-U |
| 15cm x 4.6mm ID columns | 55722-U |
| 25cm x 4.6mm ID columns | 55724-U |

¹Four columns of equal dimensions, one of each Discovery phase (C18, RP-AmideC16, C8, Cyano).

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