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Registry No. Lignin, 9005-53-2; arabinose, 10323-20-3; xylose, 58-86-6; galactose, 59-23-4; glucose, 50-99-7; sodium sulfite, 7757-83-7; nitrogen, 7727-37-9; cellulose, 9004-34-6.

Analysis for Daminozide in Apple Juice by Anion-Exchange Chromatography–Particle Beam Mass Spectrometry

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Daminozide [Alar, succinic acid mono(2,2-dimethylhydrazide)] is measured directly as the parent compound in 11 commercially obtained apple juice samples at levels ranging from 0 to 280 ppb (average 160 ppb). Sample preparation involves only evaporation and treatment with methanol and acetone to precipitate sugars and inorganic salts. Analysis is via anion-exchange chromatography particle beam mass spectrometry, with positive chemical ionization using isobutane as a reagent gas. A method detection limit of 25 ppb is achieved in part by a signal enhancement resulting from the constant addition of 0.4 mM malic acid to the mobile phase. Malic acid also modifies the nature of the mass spectrometer response to daminozide, changing it from a nearly cubic to a linear relationship and also changing relative ion intensities, between the base ion of M + 1 − H_2O without and M + 1 with 0.4 mM malic acid.

The use of daminozide [Alar, succinic acid mono(2,2-dimethylhydrazide)] as a plant growth regulator has generated controversy because of its reported animal carcinogenicity (Toth et al., 1977; National Cancer Institute, 1978) and because of a recent suggestion that children may be at risk from eating apples treated with this compound (Natural Resources Defense Council, 1989). Average daminozide residues in apples, applesauce, and apple juice have been reported to range from 4.9 ppm (Environmental Protection Agency, 1985) to 0.48 ppm (Conditt et al., 1988) and will presumably decline after the manufacturer withdraws the compound from the market (Thayer, 1989).

The highly hydrophilic nature of daminozide (solubility, grams/100 g of solvent: water 10 g; methanol, 5 g; acetone, 2.5 g; acetonitrile, 0.2 g; xylene, insoluble (Environmental Protection Agency, 1985)) suggests that conventional liquid/liquid based extraction procedures and gas chromatography based methods will not be appropriate for direct analysis of this compound. Existing analytical methods detect daminozide only directly via alkaline hydrolysis of the parent compound to the easily oxidized 1,1-dimethylhydrazine (UDMH) followed by extraction and derivatization to yield a stable, usually volatile adduct that can be easily analyzed by gas chromatography. Differences in these methods are only in the means of detection of the adduct, utilizing colorimetry (Edgerton et al., 1967), electron capture (Newsome, 1980), and mass spectrometry (Conditt et al., 1988). Typical detection limits are from 0.1 to 2 ppm.

Although risk and exposure assessments of daminozide emphasize its hydrolysis product UDMH, both the succinyl and *N*-methyl moieties of the intact parent compound bind covalently to both liver DNA and protein of treated mice and to human hemoglobin in vitro via oxidation with hydrogen peroxide (Brown and Casida,

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Table I. Daminozide Concentrations Detected in Eleven Samples of Commercially Available Apple Juice

apple juice source[a]	daminozide concn,[b] ppb ± SD
Organic Grocery Store	
A	31 ± 10
B	202 ± 4
C	209 ± 21
Commercial Grocery Store	
A	101 ± 10
B	250 ± 22
C	198 ± 26
D	136 ± 16
E	BMD[c]
School Cafeteria	
A	278 ± 6
B	236 ± 16
C	109 ± 9

[a] Based on analysis of 10-mL apple juice samples. [b] Analysis in triplicate. [c] Below method detection limit (25 ppb).

1988a). The parent compound is also photochemically oxidized to form the potent carcinogen *N*,*N*-dimethylnitrosamine via singlet oxygen oxidation (Brown and Casida, 1988b). The present study reports a sensitive analytical method for direct detection and quantification of daminozide utilizing anion-exchange chromatography–particle beam mass spectrometry.

MATERIALS AND METHODS

Apple Juice Preparation. The extraction procedure concentrates the target compound in commercially available apple juice and removes most of the principal interferences, sugars and inorganic salts. Eight different commercial brands of apple juice were purchased from two conventional grocery store chains (five samples) and from a local "organic" food store (three samples) in California. Three additional samples were obtained from local public elementary school cafeterias.

Apple juice (10 mL, pH ≈3.8) is centrifuged (2000*g* for 10 min) and the supernatant passed through a reversed-phase solid-phase extraction cartridge (CH cartridge, 0.5 g each) (Analytichem International, Frampton City, CA) conditioned with methanol (2 mL) followed by distilled water (pH 3.8, acidified with acetic acid, 2 mL). Acetonitrile (40 mL) is added to the filtrate and removed under vacuum at room temperature. After the addition is repeated and acetonitrile evaporated, the dry residue is dissolved in methanol (6 mL), followed by addition of acetone (30 mL). The resulting precipitate is removed by decanting and the supernatant evaporated under reduced pressure. After the methanol/acetone precipitation process is repeated, the residue is redissolved in methanol (0.5 mL) for analysis.

Anion-Exchange Chromatography–Particle Beam Mass Spectrometry Analysis of Daminozide. Confirmation and quantification of daminozide is via anion-exchange liquid chromatography–particle beam mass spectrometry. Conditions for anion-exchange chromatography: retention time, 10.24 ± 0.8 min [SD]; column, SGE Model 250GL, 25 cm × 2 mm, Ringwood, Australia. The solvent system is water–aqueous malic acid (2.0 mM, 35% acetonitrile)–ammonium acetate (0.10 M, pH 6.0) (60:20:20) with a flow rate of 0.25 mL/min on a Hewlett-Packard 1090 HPLC equipped with a variable-volume (0.1–25-μL) automatic sample injector, a ternary solvent delivery system, and a column output switching valve. Injected volumes are between 10 and 20 μL.

The particle beam mass spectrometer detector consists of a Hewlett-Packard 5988A mass spectrometer equipped with a Hewlett-Packard 59980A particle beam liquid chromatography interface and a Hewlett-Packard HP 1000 computer with RTE-A software. Optimal conditions for the particle beam interface: nebulizer setting, 13; nebulizer helium pressure, 50 psi; desolvation chamber temperature, 45 °C; pressure, 200 Torr. The mass spectrometer system is regularly tuned with perfluorotributylamine (PFTBA) on ions *m*/*z* 69, 214, and 502 in electron impact and positive chemical ionization modes. System performance is routinely checked by injecting 2 μL of a 10 ng/μL caffeine solution and 4 μL of a 10 ng/μL daminozide standard solution.

Table II. Recovery of a Daminozide Standard Spiked into Apple Juice at 100 and 1000 ppb

spike concn,[a] ppb	recovery, % ± SD
0	BMD[b]
100	84.4 ± 3.6
1000	98.1 ± 17.4

[a] Spiked in triplicate 10-mL samples. [b] Below method detection limit.

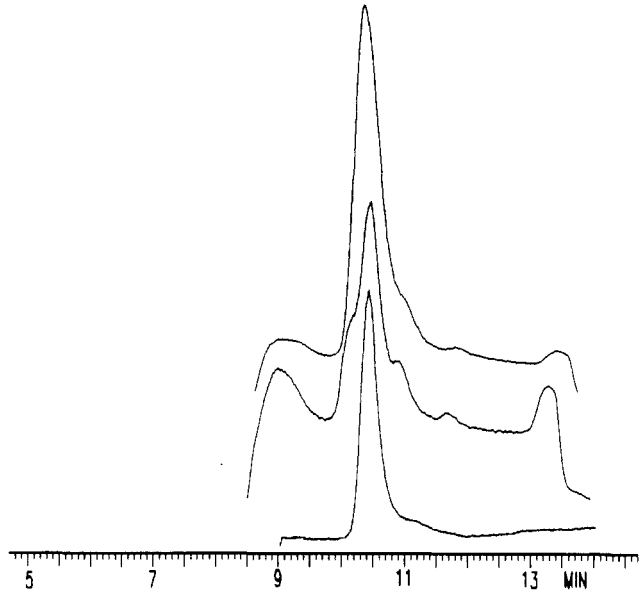

Figure 1. Anion-exchange chromatography with particle beam mass spectrometry detection (malic acid enhancement, selected ion monitoring) of a daminozide standard alone (bottom trace), an apple juice sample containing 31 ppb daminozide (middle trace), and the same apple juice sample spiked with an equivalent amount of daminozide (top trace, shown with the same vertical scale).

Eluents from the first 8 min are diverted from the mass spectrometer particle beam liquid chromatography interface to waste. Diverting the early eluting materials that probably include large amounts of residual sugars minimized clogging problems with the nozzle and the skimmer cones in the particle beam momentum separator. Particle beam mass spectra are obtained with selective ion monitoring in positive chemical ionization mode with isobutane as a reagent gas (ion source manifold pressure 1.5×10^{-4} Torr). Standard calibration curves and minimum detection values are established for this analyte both with and without the presence of malic acid. Chromatograms are obtained 8–13 min after sample injection, and daminozide was confirmed by both retention time and presence of characteristic mass ions (*m*/*z*, ion, relative abundance): 161, M + 1, 100%; 162, M + 2, 7%; 143, M + 1 − H_2O, 4%.

Quantification is based on one-point calibration assuming linearity, by injecting a daminozide standard after every three sample injections. The amount of injected standard is selected based upon the peak size of the preceding three sample injections (approximately 0–50 ng of standard daminozide). Reanalysis after storage at −20 °C for 3 weeks indicates that daminozide is stable in the extract during this period.

Efficiency of Recovery of Daminozide Spiked into Apple Juice. A commercial brand of apple juice with below the method detection limit for daminozide is used for a spike recovery experiment. Three 10-mL replicates are spiked at 100 and 1000 ppb each. A third group of three 10-mL samples is also prepared as a blank.

RESULTS AND DISCUSSION

Detection of Daminozide in Commercially Available Apple Juice via Anion-Exchange Chromatog-

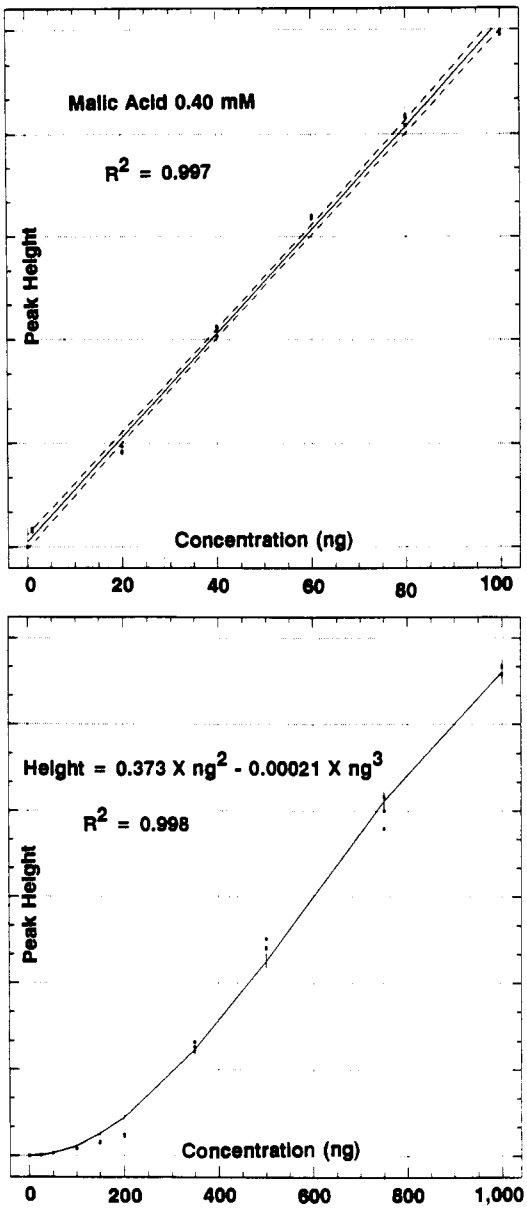

Figure 2. Calibration curves (peak height versus amount injected) for positive chemical ionization particle beam mass spectrometry quantitation of daminozide, with (top figure) and without (bottom figure) 0.4 mM malic acid in the mobile phase, monitoring characteristic mass ions (m/z, ion): 161, M + 1; 162, M + 2; 143, M + 1 - H_2O.

raphy–Particle Beam Mass Spectrometry. Table I shows that daminozide is detected in 10 of the 11 samples tested in concentrations ranging from 31 to 278 ppb. The average concentration of 160 ppb (relative standard deviation 56%) is approximately 31- and 3-fold less than values reported in 1985 and 1988, respectively (Environmental Protection Agency, 1985; Conditt et al., 1988), possibly reflecting a reduction in use of this compound by North American apple growers during this period. Interestingly, two of the samples obtained from organic suppliers had daminozide levels comparable to those obtained from conventional grocery stores.

Conventional reversed-phase liquid chromatography columns give essentially no retention of daminozide over a wide range of solvent and pH conditions. Conversely, anion-exchange chromatography of daminozide gives a retention time of 10.64 ± 0.24 min (SD) with 20 mM ammonium acetate (conditions described in Materials and Methods). Decreasing the ammonium acetate concentration to 10 mM (with constant water and acetonitrile concentrations) gives an increase in retention time to 15.0 ± 0.21 min, indicating that retention time is sensitive to buffer concentration in the mobile phase under these conditions.

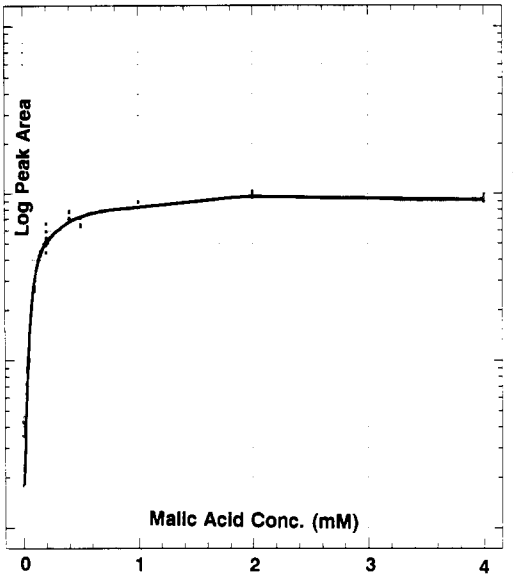

Figure 3. Signal enhancement of 40 ng of daminozide via addition of 0–4.0 mM malic acid to the mobile phase, with particle beam mass spectrometry detection in positive chemical ionization selected ion monitoring mode, monitoring M + 1, M + 2, and M + 1 - H_2O ions.

Recovery of Daminozide from Spiked Apple Juice. Results of the analysis of a daminozide standard spiked into apple juice that had been determined to contain less than the method detection limit of daminozide at 100 and 1000 ppb are shown in Table II. The method detection limit (the 99% confidence limit for the 100 ppb spike recovery replicates) is determined to be 25 ppb. Figure 1 shows an anion-exchange chromatogram of a daminozide standard alone (bottom trace), an apple juice sample determined to contain 31 ppb daminozide (middle trace), and the same sample spiked with an equivalent amount of daminozide (top trace, shown on the same scale).

Signal Enhancement by the Constant Addition of Malic Acid. The constant addition of malic acid to the chromatography solvent system in concentrations from >0 to 4 mM yields a signal enhancement in positive chemical ionization mode (both in full scan and selected ion monitoring modes) for daminozide (Figure 2). A calibration curve (peak height versus amount injected) (Figure 2 bottom) for daminozide via direct injection (water–acetonitrile–ammonium acetate (0.1 M, pH 6), 73:7:20) (0.25 mL/min) with positive chemical ionization (isobutane) and selected ion monitoring (M + 1 and M + 1 - H_2O ions) conditions shows a nonlinear response with a reduction in the response factor at low concentrations that is typical of particle beam liquid chromatography mass spectrometry (Brown et al., 1989). The instrument detection limit under these conditions is 40 ng. The corresponding reduction in response factor at lower concentrations has been suggested to be due to a reduction in particle size with a corresponding reduction in analyte transfer efficiency in the particle beam mass spectrometry interface.

Maximal signal enhancement is attained with malic acid at approximately 0.4 mM (Figure 3). The corresponding calibration curve (Figure 2, top) under conditions of constant addition of malic acid (0.4 mM) response becomes linear. In selected ion mode with malic acid enhance-

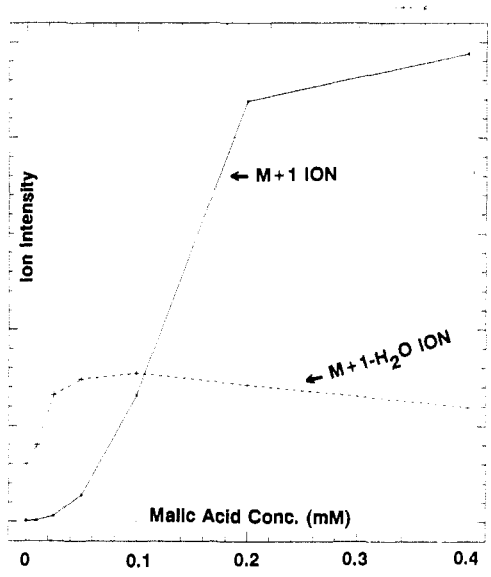

Figure 4. Ratio of M + 1 to M + 1 − H_2O ions of daminozide as a function of malic acid concentration from 0 to 0.4 mM in the mobile phase with particle beam mass spectrometry detection in positive chemical ionization mode.

ment (0.4 mM), the instrument detection limit is 1.3 ng (calculated as the x intercept of the 95% prediction limits of the linear regression calibration curve; Figure 2, top) for an enhancement factor of 31-fold. Anion-exchange chromatography particle beam mass spectrometry under these conditions is a sensitive method for the direct detection of daminozide residues in apple juice without requiring hydrolysis and derivatization steps.

The large increase in linearity and sensitivity from the constant addition of malic acid to the mobile phase may be due in part to the ability of these relatively nonvolatile low molecular weight acids to act as transfer agents or carriers for any concentration of an analyte by forming a constant particle size. However, ionization of the analyte within the mass spectrometer ion source is also affected by addition of malic acid. Thus, the ratio of M + 1 to M + 1 − H_2O ions is dramatically affected by addition of malic acid; at low malic acid concentrations the M + 1 − H_2O ion predominates whereas at malic acid concentrations giving maximum signal enhancement this ion is actually slightly suppressed and the M + 1 ion predominates (Figure 4). Malic acid enhancement, which may be due to multiple factors, enhances daminozide sensitivity by 31-fold, transforms the response to a linear relationship, and under the positive chemical ionization conditions used increases the proportion of the pseudo molecular ion (M + 1) to the fragment ion (M + 1 − H_2O).

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Registry No. Daminozide, 1596-84-5.