A Method for the Determination of Maleic Hydrazide and Its β-D-Glucoside in Foods by High-Pressure Anion-Exchange Liquid Chromatography

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A method was developed for the determination of maleic hydrazide and its β-D-glucoside in foods by high-pressure liquid chromatography with detection by light absorption at 313 nm. Both maleic hydrazide and the glucoside were extracted from plant tissue with methanol, and the extract was concentrated. Maleic hydrazide was cleaned up by passage through Amberlite XAD-2 and Dowex 50 resins, while cleanup of the glucoside was effected with a column consisting of Amberlite XAD-2, Dowex 1, and Dowex 50 resins. Maleic hydrazide glucoside was then hydrolyzed with β-glucosidase and the maleic hydrazide was determined by liquid chromatography. Recoveries of free maleic hydrazide added to four commodities from 1 to 50 ppm averaged 87%. The same average recovery was obtained with the glucoside fortified at levels from 2 to 50 ppm.

Maleic hydrazide is registered for use as a growth regulator on some food crops where it functions as a sprout inhibitor for stored produce. Analytical methods for determining maleic hydrazide in foods involve reduction with zinc and hydrolysis in hot alkali to hydrazine (Lane et al., 1958; Ihnat et al., 1973). The hydrazine is isolated by distillation and determined colorimetrically as the azine by the addition of *p*-dimethylaminobenzaldehyde. While these procedures are useful for determining total maleic hydrazide, they are unspecific and susceptible to interferences. More specific gas–liquid chromatographic methods have been developed for maleic hydrazide in tobacco and tobacco smoke (Liu and Hoffmann, 1973; Haeberer and Chortyk, 1979). These methods require derivatization of maleic hydrazide to form either the 4-chlorobenzyl or trimethylsilyl derivatives.

The present method was developed to determine specifically maleic hydrazide and its glucoside in food crops by high-pressure liquid chromatography, avoiding the necessity for derivatization and employing relatively rapid sample preparation.

EXPERIMENTAL SECTION

Materials. Dowex 50 W x 8 (100–200 mesh) cation-exchange resin and Dowex 1 x 8 (100–200 mesh) anion-exchange resin were purchased from Sigma Chemical Co., St. Louis, MO. Dowex 50W x 8 was purified before use by washing with acid and alkali as previously described (Newsome, 1974). Dowex 1 was purified by settling the resin (500 g) in 1 N NaOH (2 L) and decanting the supernatant. The resin was rinsed with water (8 L) and converted to the acetate form by suspension in 1 N acetic acid (2 L). After decanting the supernatant, excess acid was removed by settling the resin in water (8 L) and the resin was again converted to the OH^- form by suspension in 1 N NaOH (2 L). The alkali was removed by decantation and washing with water and the resin was converted back to the acetate form by settling in 1 N acetic acid. The resin was washed twice with 0.2 N acetic acid (4 L), the settled volume measured, and an equal volume of 0.2 N acetic acid added.

Amberlite XAD-2 resin was purchased from Applied Science Laboratories, Inc., State College, PA, and was used without further treatment.

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β-Glucosidase (from almonds, 6.0 units/mg) and acetobromo-α-D-glucose were purchased from Sigma Chemical.

Maleic hydrazide (3.6-dihydroxypyridazine) was supplied by Aldrich Chemical Co. Inc., Milwaukee, WI.

Preparation of Maleic Hydrazide Glucoside Tetraacetate. Maleic hydrazide (4.48 g, 0.04 mol) was suspended in a solution of acetobromo-α-D-glucose (8.22 g, 0.02 mol) in acetonitrile (50 mL). Freshly precipitated silver oxide (4.64 g, 0.02 mol) and anhydrous calcium sulfate (5.5 g) were added, and the mixture was stirred for 20 h with the exclusion of light. The mixture was then filtered through filter paper on a Buchner funnel and the filtrate was evaporated to a syrup on a rotary evaporator. The syrup was dissolved in dichloromethane and applied to a bed of silicic acid (36 g, Woelm activity I, 70–150 mesh) which was prepared in dichloromethane in a chromatography tube. After washing the column with dichloromethane (25 mL), elution was commenced with 15% acetone in dichloromethane. The first 120 mL containing acetobromo-α-D-glucose plus a small amount of maleic hydrazide glucoside tetraacetate were discarded. The next 150 mL contained only maleic hydrazide glucoside tetraacetate and when evaporated to dryness yielded 4.82 g or 55% of crystalline material, mp 183–185 °C.

Preparation of Maleic Hydrazide Glucoside. Maleic hydrazide glucoside tetraacetate (2.21 g, 5 mmol) was dissolved in anhydrous methanol (150 mL) and a solution of sodium methoxide in methanol (6.2 mL, 0.0800 mequi/mL) was added. After 20 h, the methoxide was neutralized with an exact equivalent of standard H_2SO_4. The slightly turbid solution was filtered and the solvent removed on a rotary evaporator to give a white powder. The powder was suspended in hot methanol and after filtration yielded 0.81 g (59%) of the glucoside, mp 185–186 °C.

Ion-Exchange Columns. Cation-exchange columns were prepared by adding 6.0 mL of a rapidly stirred 50% suspension of Dowex 50W x 8 in 0.2 N NaOH to a 12 x 200 mm glass tube fitted with a 2-mm Teflon stopcock and glass wool plug. The resin was converted to the H^+ form by eluting with 1 N HCl (15 mL) and the acid was removed by washing with water until the effluent was neutral. Amberlite XAD-2 (1.0 g) was placed on top of the Dowex 50 bed and washed with water (20 mL).

Mixed bed columns were prepared by adding 4.0 mL of a rapidly stirred 50% suspension of Dowex 1 to the top of a previously prepared 3 mL bed of Dowex 50. The Dowex 1 was rinsed with water until the effluent was neutral. Amberlite XAD-2 (1.0 g) was then placed on top

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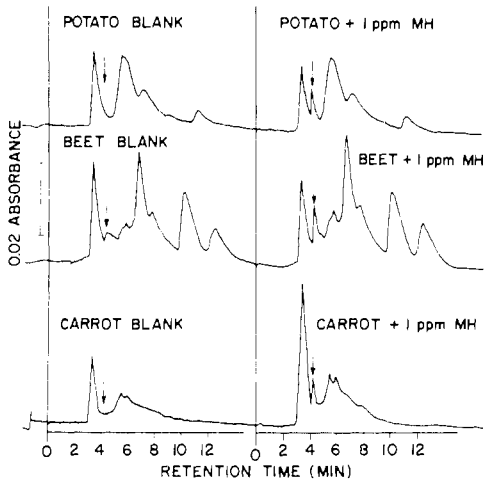

Figure 1. High-pressure liquid chromatograms of extracts of three commodities fortified and unfortified with maleic hydrazide. Arrows indicate the retention time of maleic hydrazide. Detection is by absorption at 313 nm.

of the Dowex 1 and washed with water (20 mL).

ANALYTICAL PROCEDURE

Extraction. A sample of crop (5.0 g) was extracted by homogenizing with methanol (50 mL) in a Sorvall Omni Mixer for 1 min. The homogenate was filtered through Whatman No. 1 paper on a 5-cm Buchner funnel using vacuum and the filtrate was transferred to a 100-mL, round-bottomed flask. After concentration to 2–3 mL on a rotary evaporator, the extract was transferred to a 4-mL volumetric flask and diluted to the mark with water.

Maleic Hydrazide. An aliquot (2.0 mL) of the concentrated extract was placed on a cation-exchange column and permitted to run into the resin. The resin was eluted with water until 14 mL was collected in a graduated tube. The pH of the eluate was adjusted to 4 with NaOH and the volume made to 15 mL with water. An aliquot was analyzed by high-pressure liquid chromatography.

Maleic Hydrazide Glucoside. An aliquot (2.0 mL) of the concentrated extract was passed through a mixed bed resin column and the resin washed with water until a volume of 14 mL was collected. The pH of the eluate was adjusted to 5.0 with NaOH and the volume brought to 15 mL with water. A portion (2.0 mL) of the resulting solution was incubated at 37 °C for 30 min with β-glucosidase solution (0.1 mL, 5 mg/mL in 0.1 M acetate pH 5.0). After incubation, the hydrolysate was placed in a boiling water bath for 5 min, then cooled. Precipitated protein was removed by filtration through a plug of glass wool in a Pasteur pipet and the filtrate analyzed for maleic hydrazide by high-pressure liquid chromatography.

High-Pressure Liquid Chromatography. Separations were carried out at ambient temperature on a 4.6 mm i.d. × 25 cm Whatman Partisil SAX column using 0.1 M acetic acid adjusted to pH 4.8 with tetramethylammonium hydroxide as a mobile phase. Solvent was delivered to the column by an Altex model 110 pump at a rate of 1 mL/min. The column was coupled to a Waters Model 440 absorbance detector containing a 313-nm wavelength filter. Chromatograms were recorded on a 1-mV recorder. Samples were introduced by a Valco injector containing a 100-μL sample loop.

Quantitation of the sample was carried out by comparison of the peak height with that obtained by a 0.5 μg/mL of solution of maleic hydrazide in pH 4.8 buffer. A peak with approximately 40% full-scale deflection was obtained from a 0.5 μg/mL standard with a 0.05 absorbance setting on the detector.

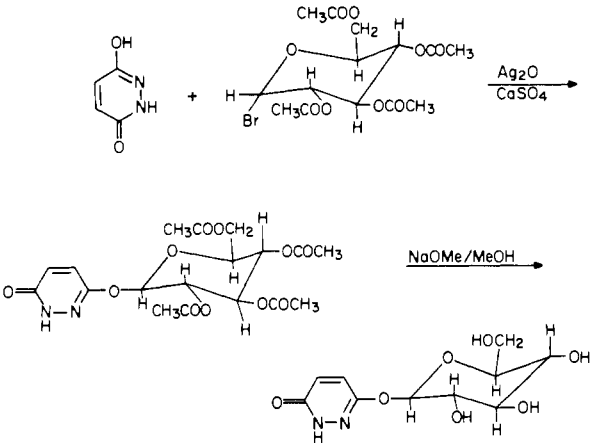

Figure 2. Reaction scheme for the synthesis of maleic hydrazide β-D-glucoside from maleic hydrazide and acetobromo-α-D-glucose.

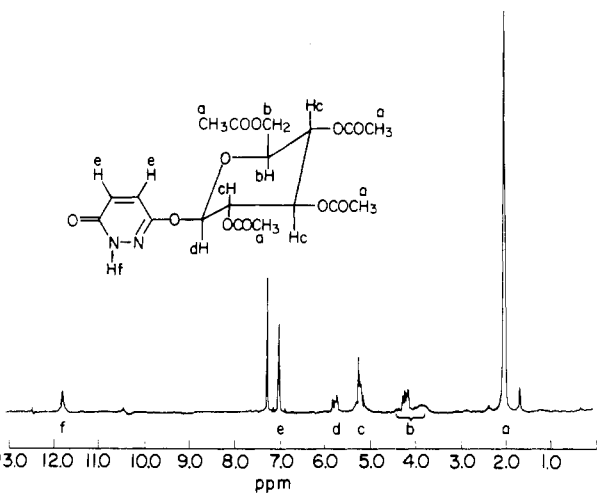

Figure 3. An 80-MHz NMR spectrum of maleic hydrazide glucoside tetraacetate in deuteriochloroform. Tetramethylsilane was used as internal standard.

RESULTS AND DISCUSSION

The weakly acidic nature of maleic hydrazide [pK_a value of 5.65; Miller, (1955)] permits its separation from coextractives by chromatography on a anion-exchange resin. Preliminary passage of the extract through Amberlite XAD-2 and Dowex 50 was found necessary to remove neutral colored material and cationic compounds which resulted in large peaks at the void volume of the liquid chromatograph and obscurred the maleic hydrazide peak. Typical chromatograms are shown in Figure 1. The recovery of maleic hydrazide added to four commodities at levels of from 1 to 50 ppm is given in Table I. Recoveries were generally greater than 80% and were similar for each of the substrates examined.

Development of an analytical method for maleic hydrazide glucoside necessitated its synthesis to provide a standard. The β-glucoside was synthesized since this anomer has been shown to occur in tobacco plants (Frear and Swanson, 1978) and in other species, including apple (Towers et al., 1958). Satisfactory yields were obtained using the Koenigs–Knorr reaction with a silver oxide catalyst as shown by the reaction scheme in Figure 2. Synthesis from β-D-glucose pentaacetate using a boron trifluoride catalyst as described for the glucosides of hydroxycarbaryl (Cardona and Dorough, 1973) gave a much reduced yield. The 80-MHz NMR spectrum of the maleic

Table I. Recoveries[a] of Maleic Hydrazide (MH) and Maleic Hydrazide Glucoside (MHG) from Various Commodities

fortifn level, ppm	potato		turnip		beet		carrot	
	MH	MHG	MH	MHG	MH[b]	MHG	MH	MHG
1	84		95		88		83	
2	89	84	92	72	100	84	79	72
5	91	89	83	71	87	89	83	95
10	88	92	80	81	85	94	88	87
25	85	94	86	88	91	90	85	91
50	89	93	82	81	97	89	87	93

[a] Values are the average of duplicate determinations. [b] Corrected for 0.33 ppm blank value.

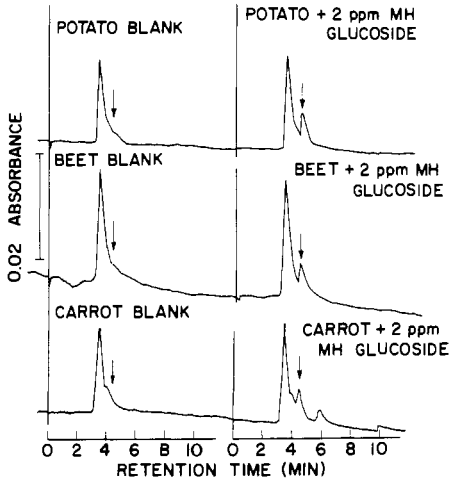

Figure 4. High-pressure liquid chromatogram of β-glucosidase treated extracts of three commodities fortified and unfortified with maleic hydrazide glucoside. Arrows indicate the retention time of maleic hydrazide.

hydrazide glucoside tetraacetate in $CDCl_3$ is shown in Figure 3 and is consistent with the assigned structure. In D_2O, the spectrum of the deacetylated glucoside showed an anomeric hydrogen doublet (δ 5.58) with a coupling constant of J = 6.0 Hz, indicating a β-glucopyranoside. Treatment of the glucoside with β-glucosidase gave the theoretical yield of maleic hydrazide, as determined by liquid chromatography providing further evidence as to the anomeric purity of the product.

For the analysis of maleic hydrazide glucoside in foods, a mixed bed ion-exchange resin was used to remove both interferences and free maleic hydrazide. No maleic hydrazide was detected in the effluent from the columns when samples fortified with it were extracted and chromatographed. Chromatograms of commodities fortified and unfortified with maleic hydrazide glucoside are shown in Figure 4. Although the chromatograms are cleaner than those in the analysis of free maleic hydrazide, the limit of detection is higher due to the higher molecular weight of the glucoside. Recoveries of the glucoside from four commodities are given in Table I. With turnip, a decrease in recovery of approximately 10% was observed at fortification levels below 10 ppm and may be attributable to β-glucosidase inhibitors in the turnip.

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