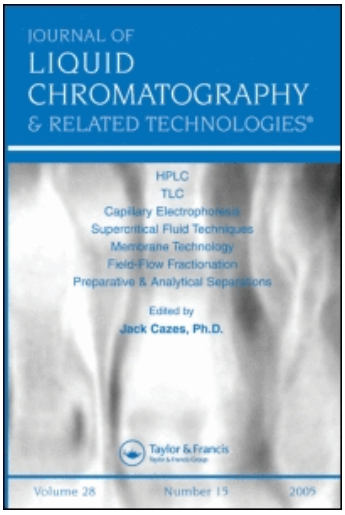

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DETERMINATION OF MALEIC HYDRAZIDE IN POTATOES AND ONIONS BY FLUORESCENCE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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DETERMINATION OF MALEIC HYDRAZIDE IN POTATOES AND ONIONS BY FLUORESCENCE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A fluorescence high performance liquid chromatographic (HPLC) method was developed to separate and quantify maleic hydrazide (MH) in potatoes and onions. Methanol was used as the extraction solvent. A 3-mL aliquot of the extract was dried under nitrogen and the residue was reconstituted in 3-mL of water by sonication. The water fraction was passed through a C_{18} solid phase extraction (SPE) tube to remove interfering compounds. Onions and potatoes fortified from 2.5 to 20 ppm showed percent recoveries ranging from 66 to 94. Within-day and between-day reproducibility studies run at 2.5, 5.0, 10.0, 15.0, and 20.0 ppm indicated the procedure was reproducible yielding percent coefficients of variation (%CV's) ranging from 1.3 to 10. The limit of detection was 0.5 ppm. A comparison was made between this HPLC method and a capillary electrophoresis (CE) method. The linear regression for MH was $y = 0.816x + 1.071$ with a correlation coefficient of 0.878.

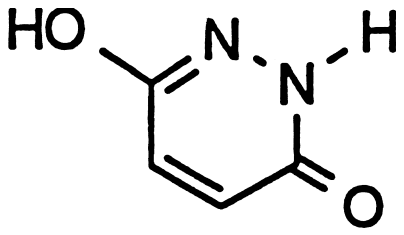

Figure 1. Chemical structure of maleic hydrazide.

INTRODUCTION

Maleic hydrazide (MH) (Fig. 1) is a plant growth regulator with some herbicidal activity whose mode of action is to inhibit cell division. Maleic hydrazide is an isomer of uracil and may be incorporated into the RNA molecule interfering with mitosis.[1] It was first synthesized in 1895 but its ability to regulate plant growth was not discovered until 1949. The USDA registered maleic hydrazide in 1952 for use as a plant growth regulator. Currently, MH is registered for use on tobacco, potatoes, onions, non-bearing citrus, turf, utility and highway rights-of-way, airports, industrial land, lawns, and recreational areas.[2] The majority of MH is applied to tobacco.

Methods to determine maleic hydrazide in potatoes and onions have focused on using either high performance liquid chromatography (HPLC) or gas chromatography (GC). The majority of methods need a clean up or concentrating step to recover maleic hydrazide. Vadukul[3] determined MH in potatoes and onions with an ion-exchange solid phase cartridge followed by anion exchange HPLC. The recoveries ranged from 101 to 104% with a detection limit of 3 ng. Newsome was able to quantify MH and its β-D-Glucoside in potatoes, turnips, beets, and carrots with an anion-exchange HPLC method.[4] Maleic hydrazide has also been determined in potato chips[5] and potato crisps,[6] tobacco, and garlic bulbs[7] by HPLC.

Gas chromatographic methods have also been developed to quantify MH in tobacco and potatoes. Renaud[8] analyzed MH in tobacco by derivatizing it with dimethyl sulphate. The dimethyl derivative was then analyzed by capillary gas chromatography using a nitrogen-phosphorous detector. Haeberer[9] also determined MH in tobacco but derivatized it to the bis(trimethtylsilyl) form that is measurable by flame ionization gas chromatography. Only one GC paper has analyzed potatoes. King[10] converted the MH found in potatoes to a volatile Diels-Alder adduct and measured the amounts by electron capture detection.

This paper describes a fluorescence high performance liquid chromatographic method for the analysis of MH in potatoes and onions. The advantage to this method is that it is rapid, with MH eluting off the column in 2.8 minutes.

EXPERIMENTAL

Chemicals and Reagents

Methanol and acetonitrile (HPLC grade) was purchased from EM Science (Gibbstown, NJ), phosphoric acid was from Fisher Scientific (Fair Lawn, N.J.). Maleic hydrazide (6-hydroxy-2H-pyridazin-3-one) was purchased from Riedel-de Haën (Germany) with a purity of 99%.

Standard Preparation

A stock standard of maleic hydrazide (880 μg/mL) was prepared by dissolving appropriate amounts of MH into methanol. A working standard (8.8 μg/mL) was made by diluting the stock standard.

Sample Extraction

Two potatoes from a control and treated batch were placed into a food processor to create a homogenous sample. A 10 gram aliquot was removed and placed into a conical centrifuge tube with 20 mL of methanol. This mixture was homogenized for four minutes with a polytron tissue homogenizer followed by centrifugation at 5000 x g for 10 minutes.

A 3 mL aliquot of the supernatant was placed into a glass vial and the methanol was evaporated under a stream of nitrogen. The residue was reconstituted in 3 mL of water by sonicating for 30 seconds.

A tC18 solid phase extraction (SPE) tube from Waters (Milford, MA) was activated with 5-mL of methanol followed by 5 mL of HPLC grade water.

The sample was loaded onto the SPE tube with the first two mLs going to waste and the last mL was collected into a glass vial for analysis. Onion samples were extracted and analyzed by the same process.

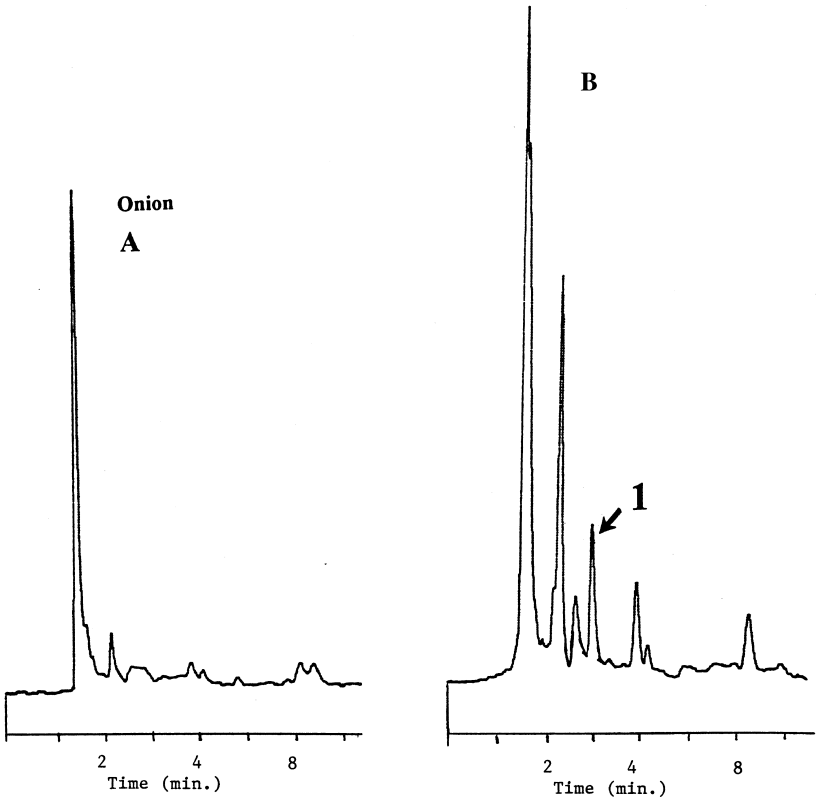

Figure 2. Chromatogram of a potato sample with A: No maleic hydrazide; B: Treated with maleic hydrazide. Peak 1 = maleic hydrazide.

HPLC Analysis

The liquid chromatograph consisted of a Waters 510 pump (Milford, MA) and VICI sample injection valve (Valco Instruments Co. Inc., Houston, TX) with a 10 μL sample loop. The column was a LUNA C_{18} 150 x 4.6 mm with 3μ particles (Phenomenex, Torrance,CA). Mobile phase consisted of 3% acetonitrile in water with 0.15 mL phosphoric acid. Detection was monitored by fluorescence with an excitation wavelength of 305 nm and emission of 400 nm using a Waters 470 (Milford, MA) fluorescence detector. A Hewlett Packard (Avondale, PA) 3396A integrator was used for data collection. Peak height was used for quantitation of MH.

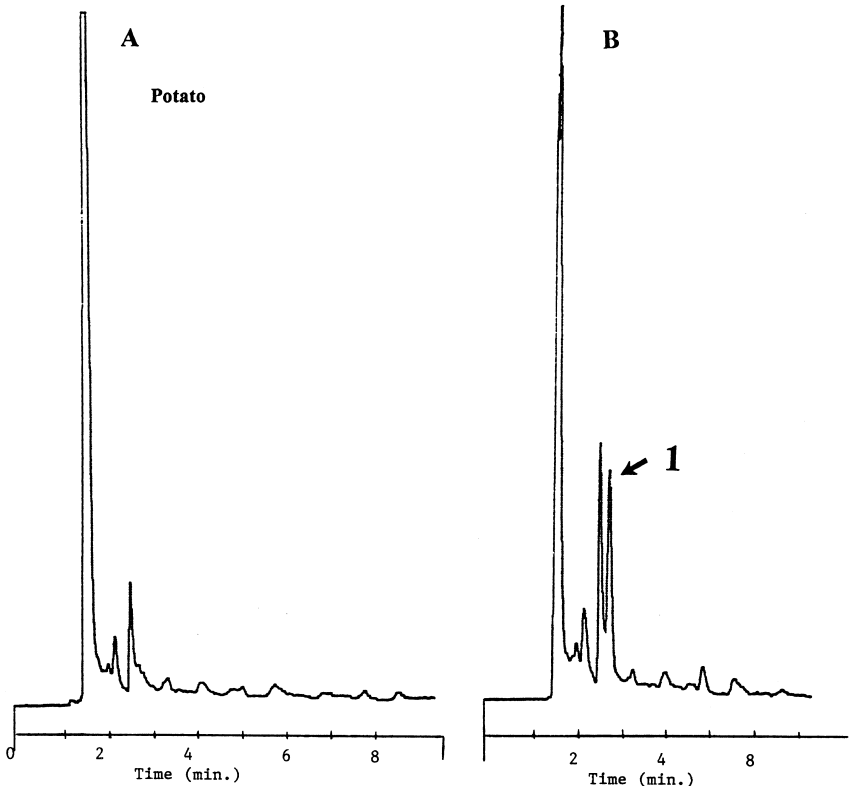

Figure 3. Chromatogram of an onion sample with A: No maleic hydrazide; B: Treated with maleic hydrazide. Peak 1 = maleic hydrazide.

RESULTS AND DISCUSSION

Although maleic hydrazide is not a strong fluorescing compound, it is sufficient to be able to determine MH at the ppm level. Since MH is applied on potatoes and onions in the ppm range because of its function to prevent sprouting, this fluorescence HPLC method is adequate for residue analysis. By employing fluorescence along with the use of a simple clean-up, MH was eluted in 2.8 minutes with no interferences in either onions or potatoes (Figs. 2 and 3).

The clean up step is simple in the fact that MH is so polar that it does not bind to the C_{18} Sep Pak but much of the other constituents found in onions and potatoes do. Therefore, a 3 mL reconstituted sample can be loaded onto the SPE and the MH will remain in solution. Only the final mL is collected for analysis because the water in the activated SPE must be removed.

Table 1

Percent Recovery of MH in Fortified Potato and Onion Samples

	Fortification Level (ppm)				
Sample	**2.5**	**5.0**	**10.0**	**15.0**	**2.0**
Potato	94 (9.5)[a]	75 (11)	75 (5.5)	73 (2.2)	71 (9.8)
Onion	70 (8.1)	68 (10)	68 (7.6)	66 (7.1)	66 (4.4)

[a] Coefficient of variation (%) values based on the extraction of five separate spiked samples.

Table 2

Reproducibility of MH in Spiked Potates and Onions

Samples	Spike (ppm)	Within-Day[a] (ppm)	Between-Day[b] (ppm)
Potato	2.5	104 (3.9)	86 (2.7)
Potato	5.0	83 (8.4)	75 (10)
Potato	10.0	88 (3.7)	74 (9.1)
Potato	15.0	84 (6.3)	70 (5.2)
Potato	20.0	72 (2.8)	68 (2.2)
Onion	2.5	70 (4.8)	70 (8.1)
Onion	5.0	74 (4.2)	68 (10)
Onion	10.0	63 (9.2)	68 (7.6)
Onion	15.0	64 (2.0)	66 (7.6)
Onion	20.0	65 (1.3)	65 (4.1)

[a] Within-day values based on ten determinations in one day (%CV's).
[b] Between-day values based on determinations performed on five different days (%CV's).

To determine the percent recovery, potatoes and onions were spiked at levels of 2.5, 5.0, 10.0, 15.0 and 20.0 ppm. Five separate samples at each level were extracted and analyzed (Table 1). The % recoveries for potatoes ranged from 73 at a spiking level of 15.0 ppm to a high of 94 at 2.5 ppm. The %CV's ranged from 2.2 to 11. The % recoveries for onions ranged from 66 to 70 with %CV's from 4.4 to 10. The recoveries for potatoes and onions were adequate considering a concentration step was not used for this method.

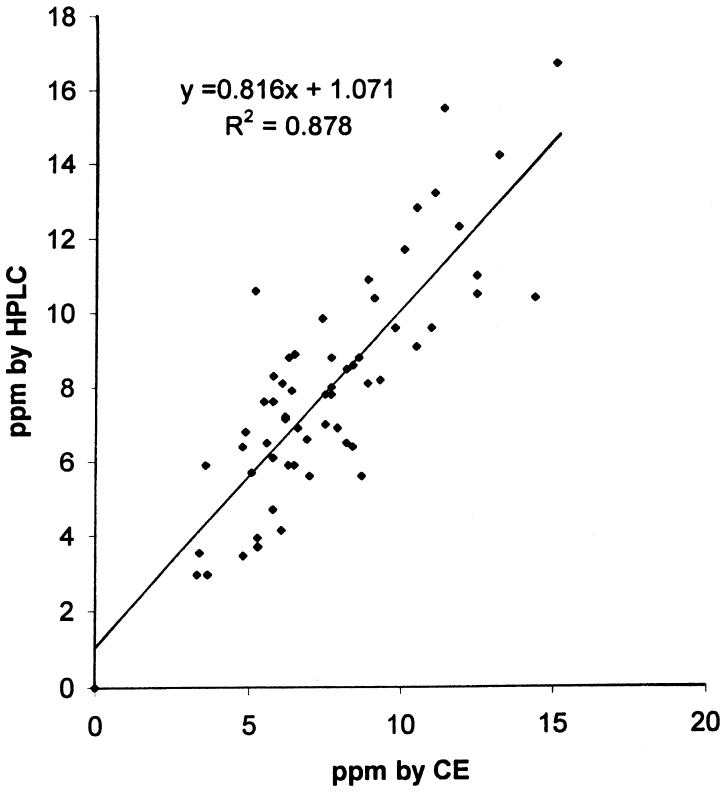

Figure 4. Correlation of maleic hydrazide in potato and onion samples. HPLC versus CE.

Reproducibility

To determine the ruggedness of the method, within-day and between-day reproducibility studies were conducted (Table 2). Within-day determinations were conducted by spiking a sample at each level (2.5, 5.0, 10.0, 15.0, and 20.0 ppm) and analyzing each sample ten times in one day.

For potato samples, the %CV's ranged from 2.8 to 8.4. Onion samples had %CV's between 1.3 and 9.2. Between-day reproducibility was based on determinations of each spike level on five separate days. Potato samples had %CV's from 2.2 to 10 and onion %CV's ranged from 4.1 to 10. The method proved to be very reproducible at each level spiked.

Linearity

From a stock standard of MH various working standards were prepared for linearity testing. The concentrations ranged from 0.5 - 40 ppm. Maleic hydrazide was found to be linear when comparing response to peak height.

CONCLUSIONS

A comparison was made between HPLC and CE for quantitation of MH in potatoes and onions (Fig. 4). The regression equation was $y = 0.816x + 1.071$ with a Pearsons correlation of 0.878. There were 64 total samples included in the comparison. Fifty nine were positive for MH and 5 had no detectable amount of MH. Of the 59 positive samples, 58 were potato samples with levels ranging from 3.3 to 15 ppm. The only positive onion contained 6.1 ppm of MH. The tolerances established for MH in potatoes is 50 ppm and 15 ppm for onions. All of the samples tested were below the established tolerances.

ACKNOWLEDGMENT

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