Determination of Maleic Hydrazide in Potatoes and Onions by Capillary Electrophoresis‡

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A micellar electrokinetic chromatographic method (MEKC) 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 tC18 solid-phase extraction tube to remove interferences. The last milliliter of the water sample was collected and injected into the capillary electrophoresis (CE) system. 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. Potato samples treated with MH were obtained from Aroostock Experimental Farm in Presque Isle, Maine and local supermarkets, while onion samples were purchased from local stores. A comparison was made between CE and a high performance liquid chromatography (HPLC) method. The linear regression for maleic hydrazide was $y = 0.816x + 1.071$ with a correlation coefficient of 0.878. Detection limit was 2.0 ppm.

Keywords: *Environmental analysis; maleic hydrazide; pesticides; capillary electrophoresis*

INTRODUCTION

Maleic hydrazide (Figure 1) is a plant growth regulator (sprout inhibitor) 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 (Royal Society of Chemistry, 1987). Although MH was first synthesized in 1895 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, nonbearing citrus, turf, utility and highway rights-of-way, airports, industrial land, lawns, and recreational areas (EPA, 1994). 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). King describes a GC method where MH in potato tubers are oxidized with an aqueous solution of lead dioxide to 3,6-pyridazinedione in the presence of cyclopentadiene. The reaction product, a volatile Diels−Alder adduct, could then be measured by electron capture detection (King, 1983). Haeberer derivatized MH to form the bis(trimethylsilyl) derivative and measured MH levels by flame ionization GC (Haeberer et al., 1974). This method has been used on tobacco but should be applicable to other plant and plant extracts. Renaud determined MH in tobacco by GC with nitrogen phosphorus detection of the dimethyl derivative (Renaud, 1992). Even though GC methods are very sensitive they can be time-consuming due to the derivatization process.

HPLC methods have also been published for MH in potatoes and onions. Newsome determines MH levels in potatoes, beets, and carrots by ion exchange (Newsome, 1980). Vadukul determines MH in potatoes and onions by ion-exchange SPE followed by anion exchange HPLC (Vadukul, 1991). Only one CE paper has been published dealing with growth regulators, but MH was not determined (Yeo S. K. et al., 1992). One of the main advantages of CE over HPLC is the decreased amount of organic solvent used. This method is also simple and unique since the SPE is used as a cleaning stage rather than to concentrate the analyte. Typically, HPLC methods employ some form of ion exchange.

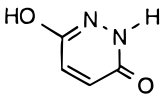

Figure 1. Chemical structure of maleic hydrazide.

Capillary electrophoresis is most commonly used for pharmaceutical and biochemical analysis but can easily be applied to pesticide analysis. Its use in this area has significantly increased over the years and most likely will continue to do so. CE is a very efficient separation technique that achieves high resolution. Capillary electrophoresis does have some disadvantages as a separation technique though. The greatest limitation to CE is the detection sensitivity, especially with UV detectors. Since MH is found in ppm amounts in vegetables this did not pose a problem, which makes CE ideal for its analysis. One mode of CE, micellar electrokinetic chromatography, is based on the partitioning of compounds distributed between an aqueous and micellar phase (Lindeberg, 1996) improving the separation of charged and neutral compounds. A surfactant is added to the running buffer above its critical micellar concentration (cmc). The micelles formed by the surfactants have the hydrophobic tails pointed inward and the charged heads orientated toward the buffer-forming spheres. Compounds in the sample will

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then interact with the micelle to enhance separation. Surfactants can be classified as anionic, cationic, nonionic, or zwitterionic. Sodium dodecyl sulfate (SDS) is the most commonly used anionic surfactant. Other surfactants though, such as cholic acid, are being employed with more frequency in method development.

This paper describes a MEKC method for the analysis of maleic hydrazide in potatoes and onions. One advantage to this method is that the sample preparation is quick with only a simple cleanup step.

EXPERIMENTAL PROCEDURES

Chemicals. All chemicals used were analytical grade. Sodium phosphate dibasic and cholic acid were obtained from Sigma (St. Louis, MO). Methanol (HPLC grade) was obtained from EM Science (Gibbstown, NJ). Maleic hydrazide (6-hydroxy-2*H*-pyridazin-3-one) was obtained 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. Prior to injection an aliquot of the working standard was evaporated under nitrogen and reconstituted in water for analysis.

Sample Extraction. Two potatoes from each batch, control and treated, were placed into a food processor to prepare a homogeneous sample. A 10-g aliquot was removed and placed into a conical centrifuge tube followed by 20 mL of methanol. This mixture was homogenized for approximately 4 min with a polytron tissue homogenizer. The sample was then centrifuged for 10 min at 5000*g*. A 3-mL aliquot of the supernatant was placed into a glass vial. The methanol was evaporated under nitrogen with the residue being reconstituted in 3 mL of HPLC grade water by sonicating for 30 s.

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 2-mL going to waste and the last milliliter collected into a glass vial for analysis. This was filtered through a 0.2 μm filter before adding a 100 μL aliquot to the injection vial. Samples were dissolved in water to promote stacking.

Onion samples were extracted and analyzed by the same process.

CE Analysis. Quantitation of maleic hydrazide was performed on a Hewlett-Packard (Avondale, PA) 3^D CE capillary electrophoretic system equipped with a photodiode array detector and an extended light path capillary. The wavelength monitored was 220 nm.

The capillary column had an i.d. of 75 μm with a bubble factor of 2.7 and a total length of 48.5 cm (effective length 40 cm). Prior to injection the capillary was conditioned by flushing for 2 min with 0.1 M NaOH followed for 2 min with the running buffer (10 mM sodium phosphate with 40 mM cholic acid pH 7.0). When not in use, the capillary was stored in the running buffer.

Rinsing, sample introduction, and separation were all controlled by a HP Vectra XM2 with Chemstation software. Sample introduction into the system was performed hydrodynamically for 1 s at 5 mbar. The system was run at a constant current of 30 μA using positive polarity. Current was determined by generating an Ohms plot for the running buffer. The capillary temperature was maintained at 30 °C.

Fortification Studies. To ascertain the percent recovery, 10 g of potato and onion samples were spiked at concentrations of 2.5, 5.0, 10.0, 15.0, and 20.0 ppm, respectively. At each concentration level five separate samples were extracted and analyzed.

Reproducibility Studies. To determine within-day reproducibility potato and onion samples were spiked at 2.5, 5.0, 10.0, 15.0, and 20.0 ppm with maleic hydrazide. The samples were injected a total of 10 times in 1 day. Between-day reproducibility was determined by preparing five potato and five onion samples which were spiked at levels of 2.5, 5.0, 10.0, 15.0, and 20.0 ppm. These were analyzed on five separate days.

Linearity Studies. From a stock standard of maleic hydrazide various working standards were prepared for linearity testing. The concentrations ranged from 0.25 to 20.0 ppm. Maleic hydrazide was found to be linear when comparing response to peak height.

Table 1. Percent Recovery of MH Fortified Potato and Onion Samples

	fortification level (ppm)				
compd	2.5	5.0	10.0	15.0	20.0
potato	78 (19)[a]	79 (11)	89 (5.1)	85 (11)	74 (6.2)
onion	89 (3.4)	80 (11)	86 (14)	82 (17)	90 (14)

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

Table 2. Reproducibility of MH in Spiked Potatoes and Onions

compd	spike (ppm)	within-day[a] (ppm)	between-day[b] (ppm)
potato	2.5	1.8 (10)	1.9 (13)
potato	5.0	3.4 (18)	4.0 (20)
potato	10.0	9.6 (10)	8.3 (3.4)
potato	15.0	12.3 (14)	12.7 (6.3)
potato	20.0	12.0 (13)	14.4 (2.8)
onion	2.5	2.0 (14)	1.8 (16)
onion	5.0	3.3 (8.0)	2.9 (9.4)
onion	10.0	7.9 (14)	6.8 (15)
onion	15.0	10.0 (6.3)	10.8 (5.0)
onion	20.0	14.6 (7.1)	15.0 (8.7)

[a] Within-day values based on ten determinations of one sample in one day (%CVs). [b] Between-day values based on determinations performed on five different days (%CVs).

RESULTS AND DISCUSSION

Under ideal conditions, standard only, maleic hydrazide can be quantitated by free zone capillary electrophoresis (CZE). However, with actual samples this was not the case. A method using micellar electrokinetic chromatography (MEKC) had to be developed along with a cleanup step because of interfering substances in potatoes and onions.

The cleanup step consisted of passing a 3-mL sample of MH extract in water through an activated C18 cartridge. MH is very polar and thus very water soluble which makes for a simple cleanup procedure on C18. The MH will not bind to the C18 even in water, but this is not the case for the majority of other substances in vegetables. By letting the first 2 mL go to waste, the final milliliter should have the same MH concentration as the extract since the water trapped in the cartridge after conditioning will be gone.

As for the MEKC development, sodium dodecyl sulfate (SDS), the most common surfactant used in micellar systems, was not adequate in resolving MH from interfering constituents after cleanup. But another surfactant, cholic acid, did provide baseline separation of MH in potatoes and onions (Figure 2). Cholic acid is one of many bile acids that are classified as anionic surfactants (Shahab et al., 1997).

Quantitation was the external standard method based on peak heights. Peak heights were chosen over peak area due to the variability in migration time, which causes peak area changes. Peak area increases as the migration time increases since solutes which have a

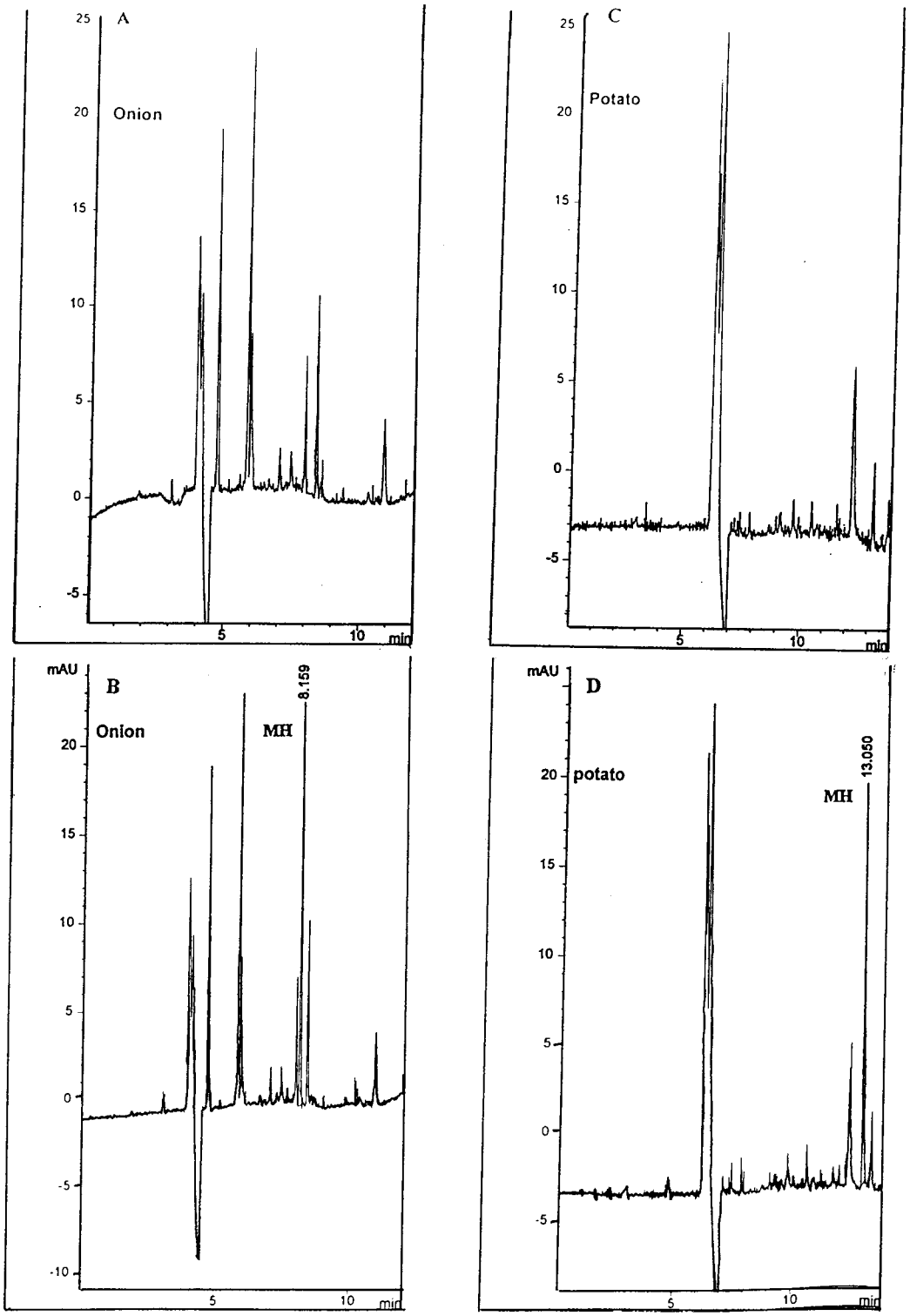

Figure 2. (A) Onion sample without maleic hydrazide; (B) onion sample spiked with 15.0 ppm maleic hydrazide (MT = 8.159 min); (C) potato sample without maleic hydrazide; (D) potato sample spiked with 15.0 ppm maleic hydrazide (MT = 13.050). Running buffer was 10 mM sodium phosphate and 40 mM cholic acid pH 7.0.

lower velocity tend to remain in the detection window for a longer time when compared to solutes with a higher velocity. This can artificially inflate values.

Also, potatoes contain a high amount of protein and starch. These compounds may coat the capillary and cause changes in the electroosmotic flow (EOF) leading to migration time fluctuations which was often observed. Thus, peak heights were used for calculations since they tended to fluctuate to a smaller extent when compared to peak areas.

Table 1 summarizes the results of fortifying potato and onion samples at concentrations of 2.5, 5.0, 10.0, 15.0, and 20.0 ppm. Percent recoveries ranged from 74 to 89 for potatoes with percent coefficients of variation (%CVs) ranging from 6.2 to 19. For onions, the percent recoveries ranged from 80 to 90 with %CVs from 3.4 to 17. Recoveries for both vegetables were more than adequate.

The reproducibility results are given in Table 2. For within-day reproducibility the %CVs ranged from 10 to

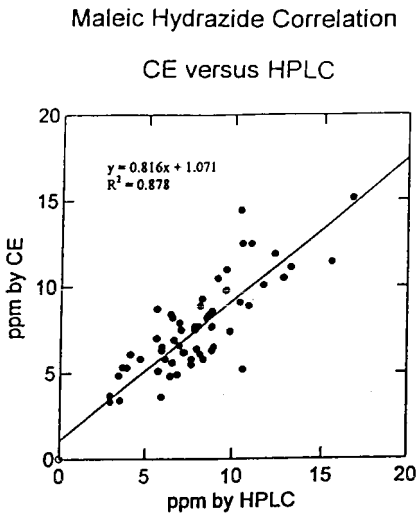

Figure 3. Correlation between HPLC and CE in potato and onions.

19 for potatoes and 6.3−15 for onions. The between-day %CVs varied from 3.4 to 20 for potatoes and 5.0−16.0 for onions.

A comparison was made between HPLC and CE for quantitation of MH in potatoes and onions (Figure 3). The regression equation was $y = 0.816x + 1.071$ with a Pearsons correlation of 0.878. There were 64 samples included in this comparison. Fifty-nine were positive and five were below the detection limit of 2 ppm. Of the 59, 58 were potatoes ranging from 3.3 to 15 ppm MH. The only positive onion contained 6.1 ppm MH. Given that the MH tolerance for potatoes is 50 and 15 ppm for onions, all samples were well below the tolerances.

ABBREVIATIONS USED

MEKC, micellar electrokinetic chromatography; CE, capillary electrophoresis; MH, maleic hydrazide; HPLC, high performance liquid chromatography; GC, gas chromatography; SDS, sodium dodecyl sulfate; SPE, solid-phase extraction; CZE, capillary zone electrophoresis; EOF, electroosmotic flow.

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