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Detection and quantification of Captan residue on small orchard and farm stand apples in the Finger Lakes region of New York using solid phase microextraction

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Abstract

The goal of this research was to identify and quantify captan residues on apple samples purchased from a variety of orchards from the Finger Lakes region of New York. This was determined by extracting captan from apple peels via solid phase microextraction (SPME), identification via gas chromatography mass spectrometry (GC/MS), and quantification via a captan standard curve. It was found that 9 out of 15 samples contained captan residues or its breakdown component, THPI (tetrahydrophthalimide.) The amount ranged from 0.09886 ppb to 3.424 ppb. Overall, a useful method of detecting and quantifying captan on apple samples was developed. Levels of captan detected on the apples were found to be in very small amounts relative to the maximum allowed.

Keywords: Captan, solid phase microextraction, apples, GC/MS, pesticides

Introduction

New York state is one of the largest apple producing regions in the United States ^[1]. There are a variety of small and large orchards that sell their produce to supermarkets, food companies, and some directly to consumers via local businesses and farm stands. The more rural regions of New York, such as the Finger Lakes, contain many local farms. This allows consumers in the area to buy or pick their own apples from local farmer markets or orchards.

There are currently no food labelling requirements for apples purchased at supermarkets or at farm stands, except for organic produce. Consumers generally do not have access to information about their apples, besides where they are from. This prevents consumers from knowing what pesticides were used on their apples or whether the residues will be present at the time of purchase.

Pesticide use is an integral part of growing apples and other produce because they help to prevent insects and fungal infections from ruining crops ^[2]. Therefore, farmers typically spray pesticides multiple times in a growing season to protect their crops ^[3]. Captan is a common phthalimide fungicide that is used on apples and many other types of produce, including grapes, and strawberries ^[2], to help prevent apple diseases such as apple scab, brown root rot, green root rot, and jacket rot ^[4]. Captan residues can have slow disappearing rates on apples, allowing the residues to persist in the environment ^[5]. According to United States federal regulations, the tolerance (the maximum concentration of pesticide residue that can be on the produce) for captan and its breakdown component, tetrahydrophthalimide (THPI), residues on apple products are 25.0ppm ^[6]. Other commonly used pesticides on apple produce include carbaryl, Malathion, and chlorpyrifos (Figure 1) ^[3].

The goal of our research was to detect and identify the pesticide residues found on apples purchased from a variety of locations around the Finger Lakes in New York State. This allows us to understand what pesticides exist on the apples at the time of purchase. In order to extract samples and examine the compounds collected from the apples, solid-phase microextraction (SPME) and gas chromatography/mass spectrometry (GC/MS) were used. Chromatographic and mass spectra techniques are commonly used in pesticide analysis ^[7]. SPME has shown to be effective in extracting pesticide residues as well ^[8, 9]. Another method that has shown to be effective for extracting pesticides is the QuEChERS method (quick, easy, cheap, effective,

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ugged, and safe) [10, 11]. The QuEChERS method was tested, but required more materials and solvents and gave poorer

results for captan residues, so SPME was implemented for extraction.

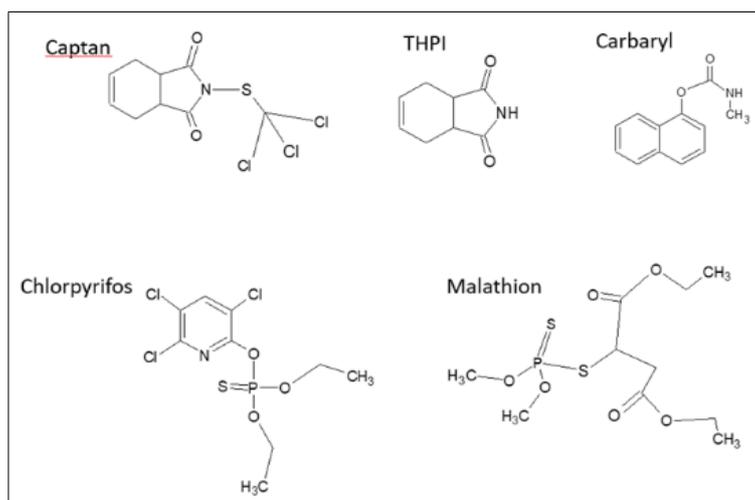


Fig 1: Structures of pesticides commonly used on apple produce in New York

Material and Methods

Apples were purchased in the Finger Lakes region directly from six different orchards and roadside farm stand locations. A variety of apple varieties were sampled, including Cortland, Empire, Macoun, Gala, Suncrip, Honeycrisp, Jona Gold, Ginger Gold, Jersey Mac, Paula Red, Crispin, and Macintosh.

Sample Preparation

Unwashed Apples were peeled and 5.000g of apple peel (which contained approximately half the apple peel) was blended in 45.0 mL of distilled water with a standard kitchen blender. A 14.5 mL portion of blended apple sample was collected and centrifuged at 5000 RPM for 2.5 min using a Model HN-S Centrifuge. Some of the samples were prepped ahead of time and stored at 5°C for up to 5 days. Apples from a tree known to have never been treated with any pesticide were used as a control.

Sample Extraction and Clean Up

The pesticide residues were extracted using the Restek PAL SPME Manual Injection Kit, with a Restek 100 μm "red" polydimethylsiloxane fiber. The fiber was placed in the blended apple samples at room temperature for 25 minutes before injection into the GC/MS. The fiber was cleaned daily by injection into the GC injector port and heated at 270°C for 5 min. In between samples the fiber was cleaned by suspending it in ethanol for 2 min.

Captan Standard

Ampoules of 100 $\mu\text{g}/\text{mL}$ captan in acetonitrile standard solution were purchased from Restek. A standard curve was created using 10 μL , 25 μL , 50 μL , 75 μL , 100 μL , and 125 μL of the captan standard measured by micropipette. The volumes of standard were added to the centrifuge containing blended untreated control apples, vortexed for 30 sec using a Fisher Vortex Genie, centrifuged at 5000 rpm for 2.5 min, and extracted using the same conditions and procedure before being injected into the GC/MS.

Gas Chromatography-Mass Spectroscopy (GC/MS) Analysis

The conditions used were the same for all samples and captan standards. The GC used was a PerkinElmer Clarus 580 gas

chromatograph with a 30.00 m column with a 250 μm diameter using a 10:1 split ratio and a He carrier gas flow rate of 1.00 mL/min. The injector port was set at 250 °C. The oven temperature started at 50 °C, held for 3.00 minutes followed by a 10 °C/min increase to 250 °C, and held for 15 minutes at 250 °C for a total runtime of 38.0 minutes. The injection was done manually into the injection port.

The conditions in the MS were identical in each experiment. The MS used was a Clarus SQ 8 S mass spectrometer with source and inlet line temperatures of 200 °C. A solvent delay of 3.00 minutes was used with a runtime of 38.0 minutes. The mass scan was from 45 amu to 550 amu.

Results and Discussion

Out of the 15 samples tested, 9 out of 15 apples (60%) contained captan residues, and 11 out of 15 apples (73%) contained either captan or THPI residues (Table 1) making captan and its break down product the most common pesticide found on apples in the Finger Lakes. The residues were identified based on peak retention times from 21.94-22.00 and mass spectrum matching with the standard (Figure 2) and NIST library spectra. All measurable chromatogram peaks for each apple sample were characterized by mass spectroscopy to determine if any other pesticides were present. An example chromatogram from a Paula Red apple from orchard 5 shows the large number of molecules extracted by the SPME fiber, the majority of them being natural compounds (Figure 3). The peak that corresponds to the captan residue detected was found at RT=21.94 for the Paula Red sample, which produced a MS that was identical to the standard. THPI residues were detected on the Honeycrisp apple from orchard 1 and the Macoun apple from orchard 6 (Table 1), but it was found at the retention time for captan. This suggests that these samples contained captan, but it began to breakdown while running through the GC/MS. Small amounts of the organophosphate pesticide, Malathion, were detected on 2 out of 15 apple samples (structure shown in Figure 1).

Captan Standard and Quantification

The captan standard results were used in order to approximate the amount of captan found on the apple samples. The captan standard solution used to make the calibration curve included the same amount of spray free blended apple to account for

matrix effects. Trials attempted without a blended apple in the standard led to poor and inconsistent results. The standard curve was based on the peak area and the mass (μg) of standard found. The peak area was determined by the integration process in the GC/MS Turbomass program, while the mass (μg) of standard was found based on the concentration of the standard ($100\mu\text{g}/\text{mL}$) and the amount of standard added to each sample. The curve is linear with an R^2

value of 0.9832 (Fig 4). Figure 5 shows an example of the captan standard chromatogram for $125\mu\text{L}$ of standard, where the captan peak was located at $\text{RT}=21.94$. The standard curve and peak area for each individual apple sample were used to approximate the amount (ppm) of captan pesticide on the apple (Table 1). The amount of captan on the apples ranged from 0.00009886 ppm to 0.003424 ppm for the samples where captan was detected.

Table 1: Captan and THPI residues detection on apple samples with corresponding retention times and amount (ppm) detected. The pesticide residues were extracted via SPME

Sample	Orchard	Captan (RT=21.94-22.00)	THPI (RT=15.45)	ppm captan ($\mu\text{g}/\text{mg}$)
Control		no	no	0
Gala	1	yes	no	0.003424
Jona Gold	1	yes	no	0.002175
Honeycrisp	1	no	yes*	0.0001664
Suncrisp	2	no	no	0
Gala	2	no	no	0
Cortland	3	no	no	0
Cortland	4	yes	no	9.886E-05
Paula Red	5	yes	no	0.001885
Jersey Mac	5	yes	no	0.0004115
Ginger Gold	5	yes	no	0.0008127
Cortland	6	no	no	0
Empire	6	yes	no	0.0003116
Macoun	6	no	yes*	0.0007251
Macintosh	6	yes	no	0.003415
Crispin	6	yes	no	0.003204

*Found at retention time for captan

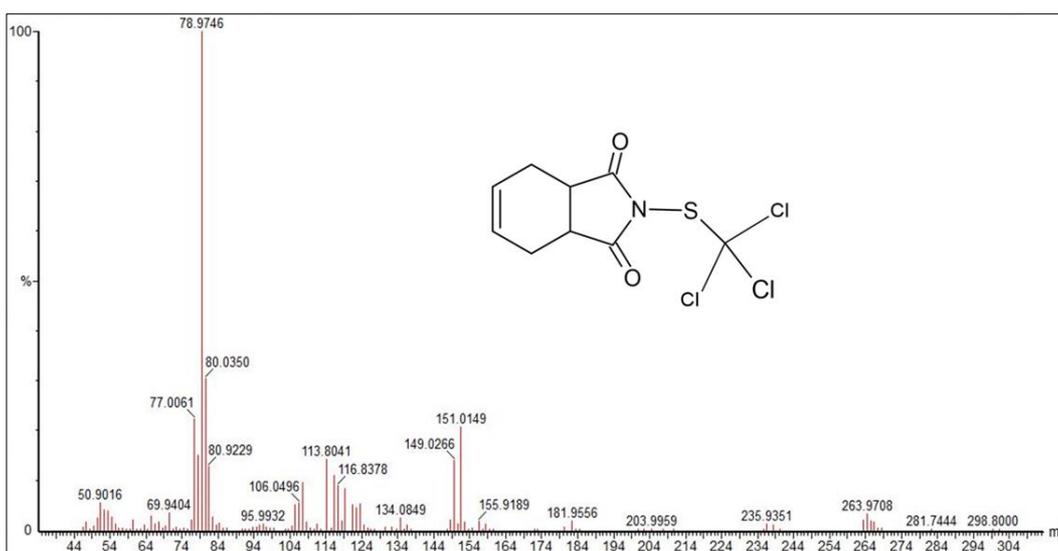


Fig 2: The MS and chemical structure of captan, molecular weight 300.58. The ion fragments at m/z 151 and 79 are part of the typical fragmentation pattern for captan

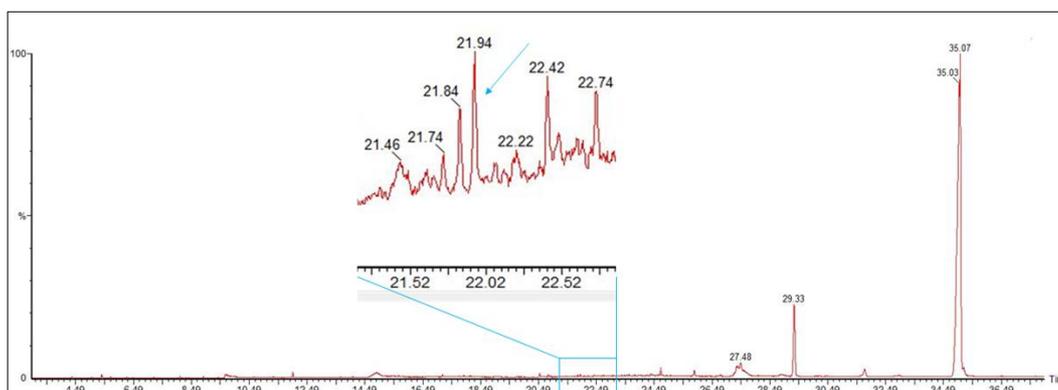


Fig 3: Chromatogram of Paula Red apple purchased from Orchard 6 extracted via SPME. The peak that corresponds to the captan residue found is $\text{RT}=21.94$

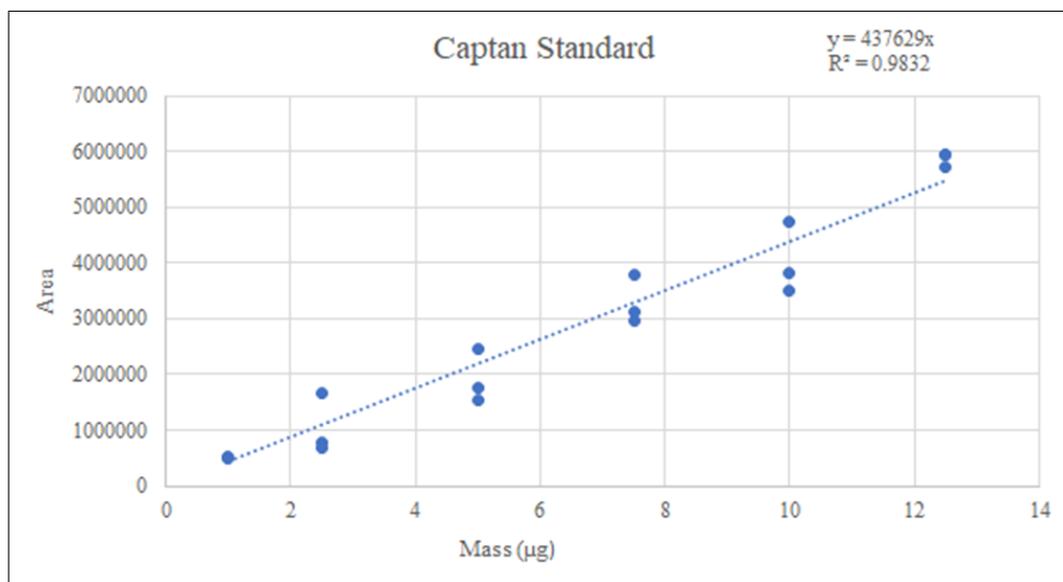


Fig 4: Captan standard curve created by the addition of 10 μ L, 25 μ L, 50 μ L, 75 μ L, 100 μ L, and 125 μ L of standard to the apple samples. The captan residues were extracted via SPME. The curve's axis shows chromatogram peak areas and the mass (μ g) of standard

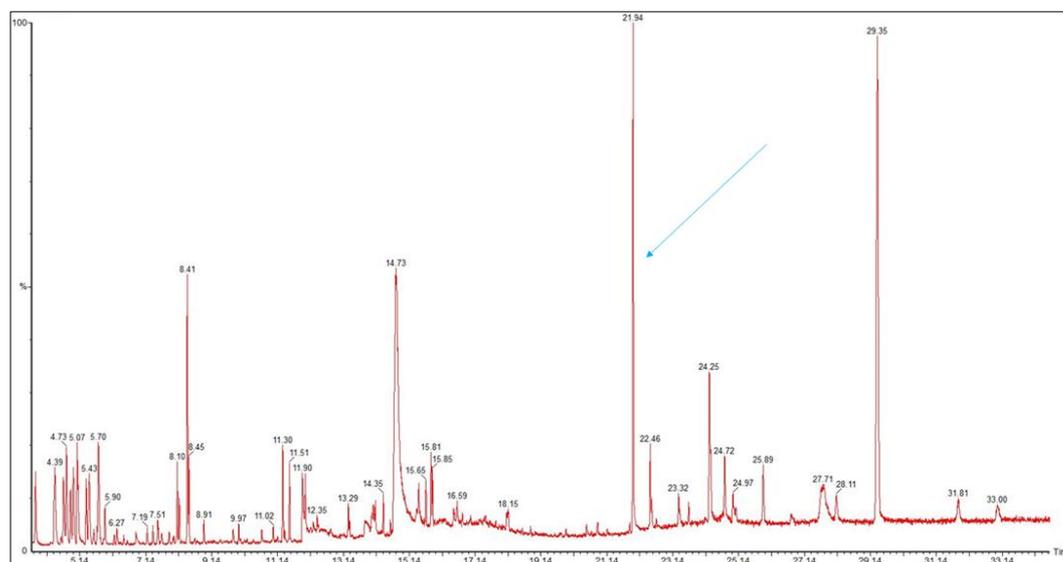


Fig 5: Chromatogram of 125 μ L of captan standard extracted via SPME. The peak corresponding to the standard is found at RT=21.94

Conclusion

This study investigated the levels of the fungicide captan on conventionally grown apples sold at roadside stands and small orchards in the Finger Lakes region of New York, USA. The results indicate that while the majority (73%) of apples did show the presence of captan, the levels present on every sample were far below the standards set by the EPA. This combined with the low human toxicity of captan relative to other pesticides [12] suggests there are little to no health impacts for consumers from captan on apples in the region.

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