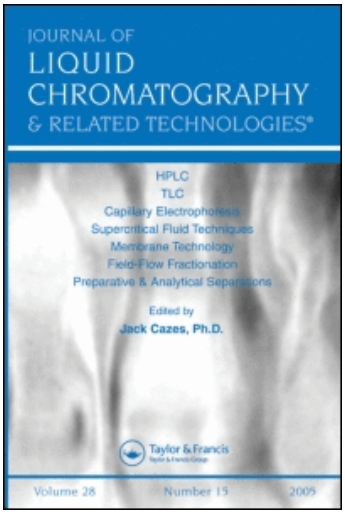

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SOLID PHASE EXTRACTION/HIGH PERFORMANCE LIQUIDCHROMATOGRAPHY METHOD FOR THE DETERMINATION OF METHYL ANTHRANILATE RESIDUES IN BLUEBERRIES

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ABSTRACT

Methyl anthranilate fortified blueberries were extracted with methanol, cleaned up by solid phase extraction and analyzed by reverse phase high performance liquid chromatography. Methyl anthranilate was quantified by UV absorbance and fluorescence detection. Recoveries of 70 ± 13% and 14% were obtained for blueberry samples fortified with methyl anthranilate at 5 and 0.15 ppm respectively. The method limits of detection for ultraviolet and fluorescence detection were 0.04 and 0.009 μg methyl anthranilate/g, respectively.

INTRODUCTION

Methyl Anthranilate (MA) is a methyl ester used as an additive in the food and cosmetic industry. It is used commonly in chewing gum and

beverages as grape flavoring and odor. It is also used frequently as a fragrance in perfumes.

Methyl anthranilate is also an effective feeding deterrent to many species of birds[1-3] and rodents.[4] As MA is on the Food and Drug Administration list of compounds that are generally recognized as safe (GRAS), it has excellent potential as a non-toxic bird repellent. MA is being tested as a bird repellent for a variety of bird management uses.[5] One such application involves using MA to deter bird feeding on blueberries. Bird depredation to ripening blueberries is a major problem for growers in the United States and Canada with annual losses estimated at $8.8 million in 1989 for the United States.[6]

To determine the effectiveness of MA as a bird deterrent on blueberries, microencapsulated MA formulation was mixed with water and applied with air blast sprayers. The field test was conducted at 5 different sites in Washington and Oregon by the Denver Wildlife Research Center. To support efficacy studies, an analytical method utilizing High Performance Liquid Chromatography (HPLC) coupled with ultraviolet detection (uv) was developed to determine the minimum methyl anthranilate residue levels associated with bird repellency. This analytical method had a limit of detection of 0.4 μg MA/g[7] which was sufficient to quantify methyl anthranilate in the spray formulation and the methyl anthranilate residue levels associated with bird repellency. However, a more sensitive analytical method was needed to quantify the lower methyl anthranilate residue levels on blueberries harvested for consumption. For this harvest residue study, a more sensitive method was developed which utilizes solid phase extraction (SPE) and fluorescence detection to achieve the required lower limits of detection for long term residue studies.

MATERIALS

Methanol and acetonitrile (Fisher Scientific, Denver, CO) were liquid chromatography grade. Deionized water was purified using a Milli-Q water purification system (Millipore, Bedford, MA). The solvents were degassed by the Hewlett-Packard 1050 series on line degasser. Concentrated sulfuric acid (Fisher Scientific, Denver, CO) was used to make the 5% sulfuric acid solutions in methanol.

Methyl anthranilate was obtained from Aldrich (Milwaukee, WI) and PMC Specialties (Cincinnati, OH). Concentrated stock solutions of MA were prepared from the commercial products, without further purification, by

dissolving 100 mg in 25 mL of methanol. Working solutions were prepared weekly by dilution with 1% sulfuric acid in 86:13, methanol to water. All standard solutions were stored in the dark at 5°C.

Isolute NH_2 SPE cartridges containing 500 mg aminopropyl sorbent and 10 mL reservoir volume were obtained from Jones Chromatography (Lakewood, CO). SPE cartridges were used with a VacMaster sample processing station (Jones Chromatography).

METHODS

Formulation Application And Sample Collection

Prior to application, control samples were collected from the control and treated plots. A microencapsulated methyl anthranilate solution was applied at a concentration of 2.5 to 3.0% MA with air blast sprayers. Residue samples were carefully collected by randomly selecting blueberry bushes and then clipping a stem with a cluster of blueberries. Each blueberry was clipped and allowed to drop into the sample container until approximately 100 grams of sample were collected. The container was immediately sealed and placed in a cooler with ice, five replicates were taken each sampling day. Within one to two hours the samples were transferred to a -25°C freezer and eventually shipped with dry ice to the Denver Wildlife Research Center for analysis.

Sample Preparation

For quality control and method development samples, blueberries harvested from untreated control plots were spiked with solutions of MA in methanol at levels ranging from 0.15 to 125 ppm. Prior to analysis, the frozen blueberry samples were partially thawed at room temperature for 15 to 20 minutes. The lids were removed, followed by removal of the lid liners which were rinsed into the sample jars with 0.75 mL of 5% sulfuric acid in methanol. Another 0.25 mL portion of the sulfuric acid solution was used to rinse the inside rim of the sample jar. The blueberry samples were then homogenized to a puree with a hand blender. Each homogenized sample was then extracted by weighing 1.50 to 1.60 grams of the puree into a 15.0 mL graduated 15-mL glass screw cap centrifuge tube. Methanol was added to the tube to give a total volume of 15.0 mL. The samples were vortexed for 10 seconds and then sonicated three times for 15 minutes each. Between each sonication period the

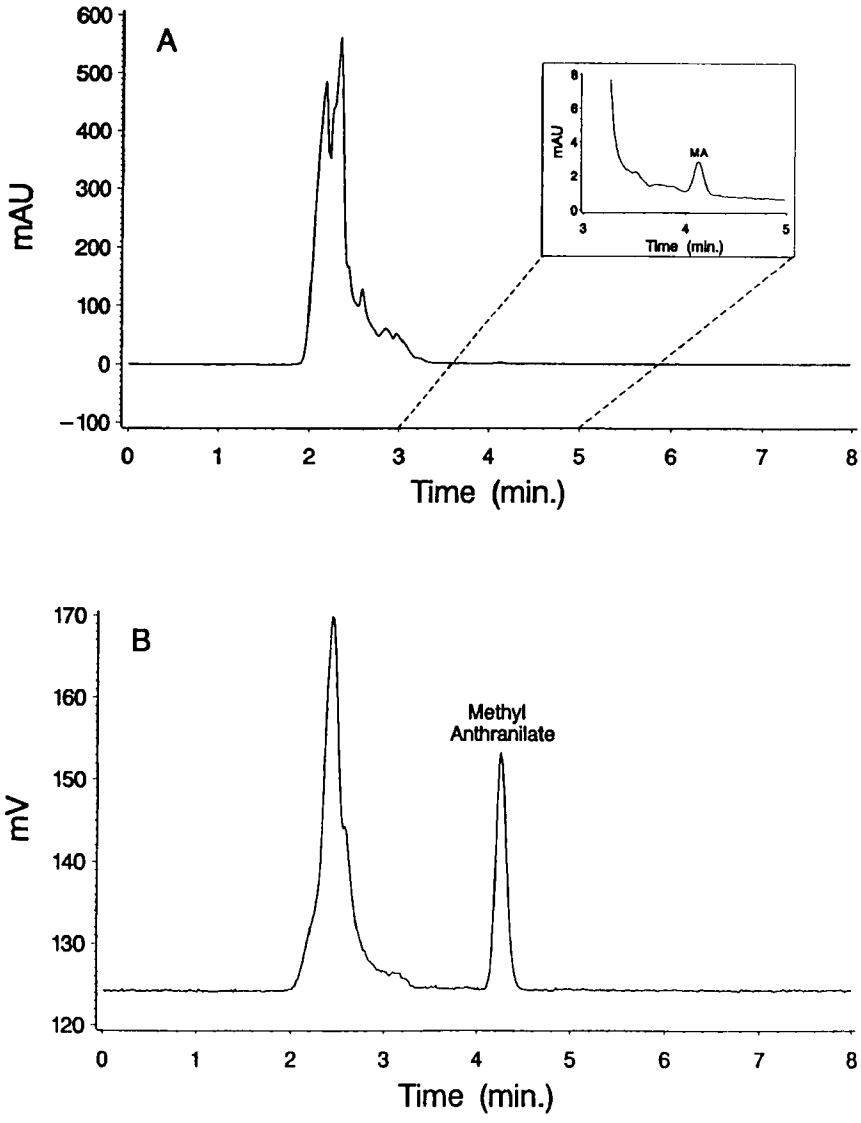

Figure 1. Chromatogram of a 1.0 mg/g MA fortified control blueberry extract with detection:

(A) UV and (B) Fluorescence

samples were shaken by hand for 5 seconds. The samples were then centrifuged for five minutes at approximately 2500 rpm and 5 mL of the supernatant transferred to an NH_2 column which had been preconditioned with 6 mL each of water and methanol. Matrix interferences were retained on the SPE column and the MA containing eluant was collected in a 10 mL centrifuge tube. The volume of the eluant was reduced to approximately 0.5 mL by evaporation at 25°C under a gentle stream of nitrogen. The contents of the tube were then brought to 1.0 mL total volume with HPLC mobile phase which was then transferred to an autosampler vial and analyzed by HPLC.

High Performance Liquid Chromatography

The HPLC system consisted of a Hewlett-Packard 1050 liquid chromatograph (Palo Alto, CA) operated at ambient temperature. A Hewlett-Packard 1050 variable wavelength detector was used at 220 nm to detect MA. A SpectroVision Inc.(Cambridge, MA), dual monochromator fluorescence detector placed serially in-line with the UV detector was used with an excitation wavelength of 338 nm and an emission wavelength of 424 nm. The sample extracts (15 μL) were chromatographed with a acetonitrile:water (70:30) mobile phase at 1.00 mL/min. The MA was separated on a 25-cm x 0.46-cm i.d. stainless steel analytical column packed with 5-μm Alltech Econosil C_{18} (Deerfield, IL). To prolong column life, a 1.5-cm x 0.46 cm i.d. Keystone Octyl-H (Bellefonte, PA) guard column was used. The MA peak was identified and quantified on the fluorescence chromatogram by comparison of retention time and chromatographic response with a MA standard. When possible, qualitative confirmation was also accomplished by comparison of the uv chromatographic response to the uv spectrum and retention time of a standard. A Hewlett-Packard 386 Vectra computer work station with an Epson printer was used to collect, process, store, and print the chromatographic data.

RESULTS AND DISCUSSION

HPLC chromatograms of extracts from blueberries spiked with MA at 1.0 and 0.15 μg/g are presented in Figures 1 and 2, respectively. These extracts were neither cleaned up by SPE nor concentrated. The retention time of MA is 4.18 minutes. Coextracted matrix constituents eluted primarily between 2 and 3.5 minutes. Both figures clearly illustrate the improved sensitivity of fluorescence detection as compared with uv detection. Figures 1A and 2A show that uv detection is sufficient to detect MA in blueberries at a level of 1.0

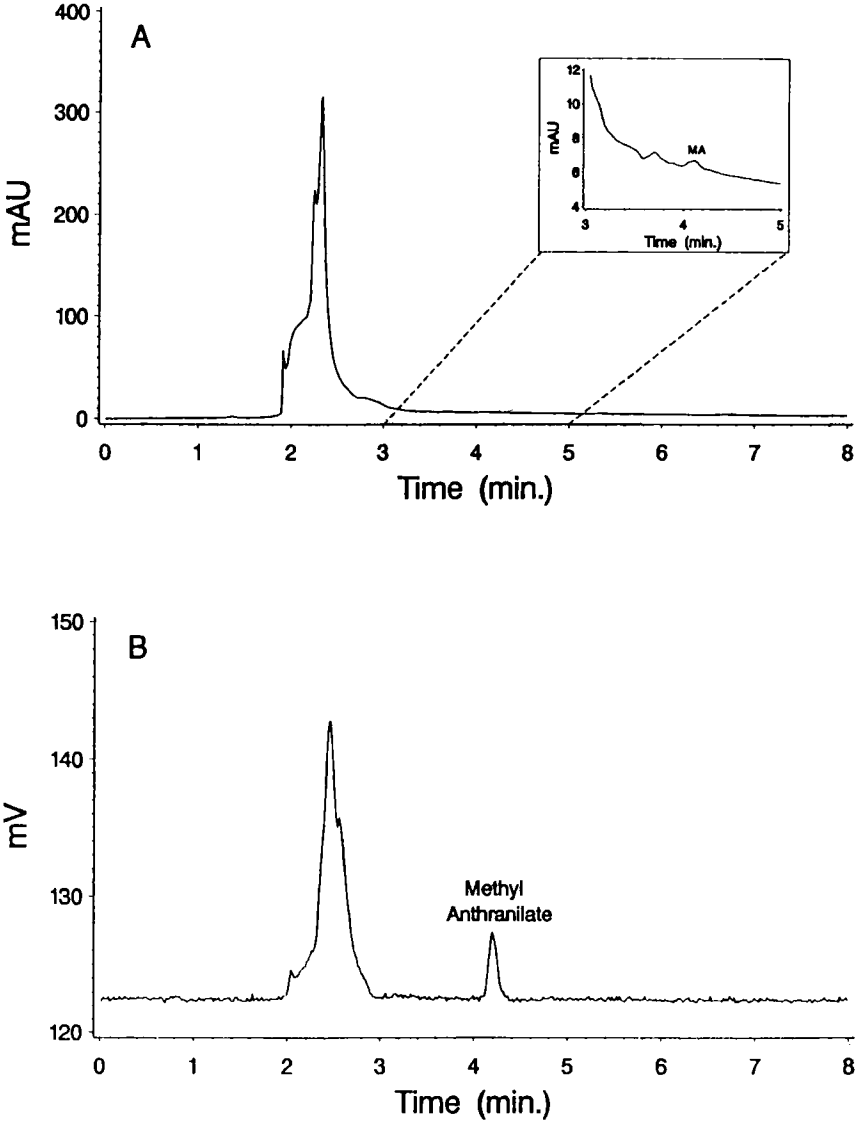

Figure 2. Chromatogram of a 0.15 mg/g MA fortified control blueberry extract with detection:

(A) UV and (B) Fluorescence

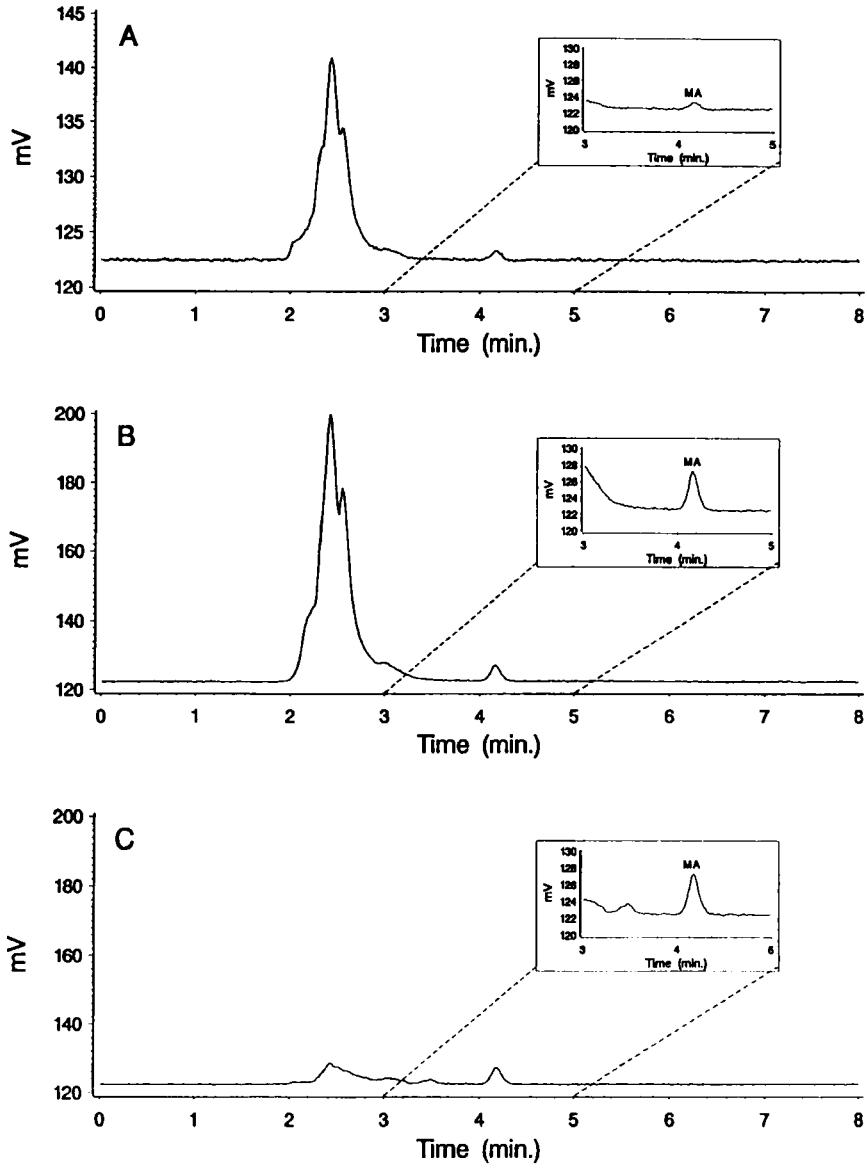

Figure 3. Fluorescence chromatogram of a 0.04 mg/g MA fortified blueberry extract with (A) no clean up or concentration, (B) concentration step and (C) clean up and concentration.

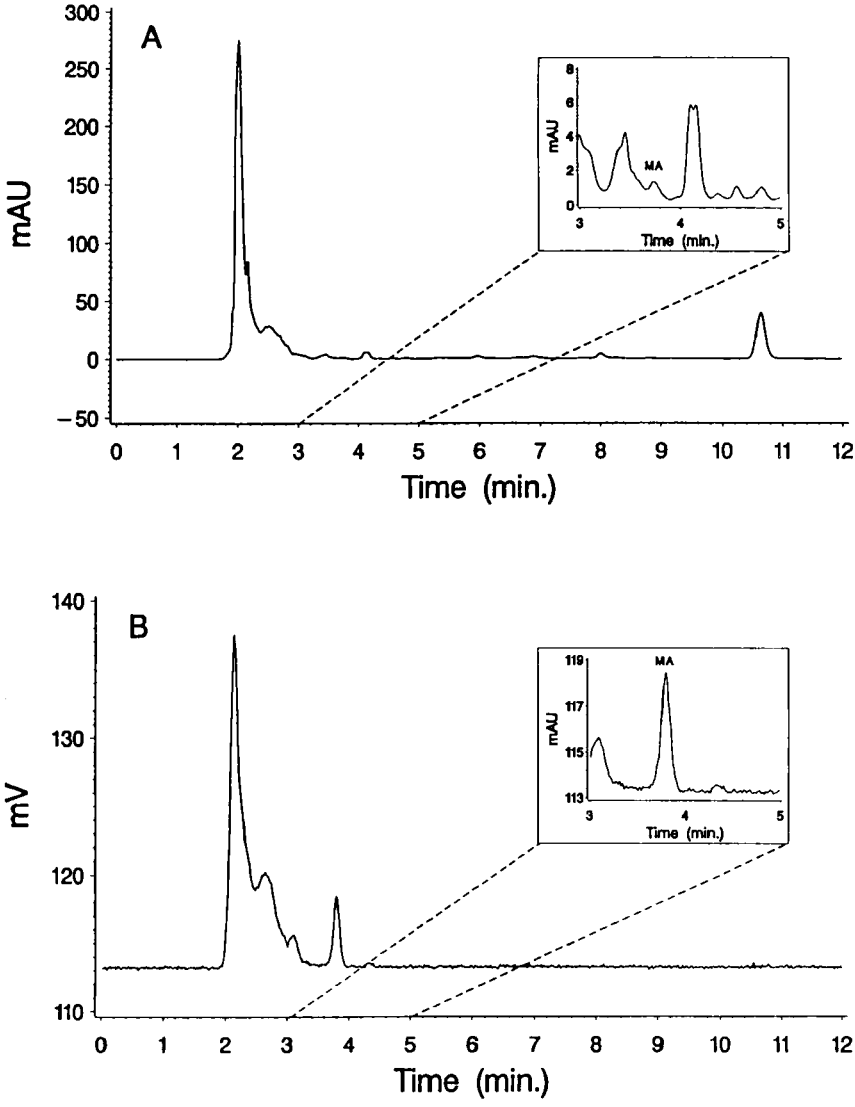

Figure 4. UV (A) and fluorescence (B) chromatograms of extracts from blueberries harvested from control plots.

μg/g but that the uv response for MA in blueberries at 0.15 μg/g is too low to quantify. Comparison of these uv chromatograms with the fluorescence

chromatograms in Figures 1B and 2B illustrate that fluorescence detection provides about a 5 fold increase in sensitivity. Furthermore, the relative response of interfering compounds with respect to the MA is significantly less with fluorescence detection.

The three fluorescence chromatograms presented in Figure 3 illustrate the advantages of the concentration and cleanup sample preparation steps. All three chromatograms are extracts from blueberries spiked with MA at 0.04 μg/g. Chromatogram A resulted from the HPLC analysis of an extract without concentration or cleanup. The addition of the concentration step to the sample preparation resulted in chromatogram B. Comparison of chromatograms A and B illustrate that the concentration step improved the response of MA by a factor of approximately 5. Chromatogram C illustrates the advantages of both the concentration and sample cleanup. The SPE cleanup removed the majority of matrix coextractants without removing MA. With the clean up and concentration step, the method limit of detection for MA in blueberries was determined to be 0.009 μg/g.

The uv and fluorescence chromatograms presented in Figure 4 resulted from the analysis of extracts of blueberries harvested from control plots. Control plots were situated at least 30 meters from the nearest treated plot. Most chromatograms of blueberry extracts from control plots had no detectable MA residues. However, the chromatograms presented in Figure 4B show MA residues of 0.031 μg/g. Minor variations in retention times were due to the use of different HPLC columns. The advantages of selectivity and sensitivity afforded by fluorescence vs uv detection are especially evident in trace level analyses such as these. The low levels of MA detected on these control blueberries by fluorescence detection are probably the result of drift during or shortly after application.

MA residue levels on blueberries were determined for blueberries harvested from 5 different sites. The mean residue level at 6 days after application were approximately 2 μg/g. The mean half-life for MA on blueberries was 3 days. As the method limit of detection for this method was 0.009 μg/g, this method should be able to detect MA residues on blueberries harvested up to 4 weeks post application.

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Mention of commercial products does not constitute endorsement by the

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