Determination of tribromophenol and pentachlorophenol and its metabolite pentachloroanisole in *Asparagus officinalis* by gas chromatography/mass spectrometry*

A GC-MS method was developed and optimized for simultaneous determination of pentachlorophenol (PCP), 2,4,6-tribromophenol (TBP), and pentachloroanisole (PCA) residues in the edible part of *Asparagus officinalis*. For this purpose, two procedures were evaluated: the direct separation of PCP, TBP, and PCA and the separation of acetyl-PCP, acetyl-TBP, and non-acetylated PCA. Better sensitivity and quantitative results, especially for PCP, were obtained after acetylation. The residues of PCP and TBP were extracted as phenolates and acetylated in a carbonate solution. Acetylated compounds were extracted by liquid-liquid extraction with hexane, while PCA was directly leached with this solvent. The proposed method allows the rapid quantification of traces of PCP, TBP, and PCA in a concentration ranging between 1.0 and 8.0 ng mL\(^{-1}\) in solution (corresponding to 0.3 and 8.0 µg kg\(^{-1}\) in asparagus). In this concentration range, typical recoveries for PCA, TBP, and PCP from asparagus samples were 59%, 86%, and 97% respectively (RSDs 3–7%).

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1 Introduction

In recent decades, wood exportation has made a very important contribution to the Chilean economy. In 2000, the forestry industry produced more than 3.0 million m\(^3\) of treated sawn wood in the VIII Region “Bio-Bio” (INFOR, 2000). In this area, sodium pentachlorophenolate (Na-PCP) has been widely used for more than 20 years as a wood preservative [1]. However, due to its hazardous properties, including dermatitis, systemic effect, dyspnea, allergic response, suspected carcinogenicity, and environmental persistence [2–6], the Chilean administration forbade its use in 2000. Nowadays, the wood industry uses 2,4,6-tribromophenol (TBP), which is also considered as hazardous residue by the US Environmental Protection Agency (USEPA) [7].

The persistence and accumulation of PCP residues in soil and the coexistence of forestry and agriculture in the same area lead to the cross contamination of horticultural products with traces of PCP, TBP, and/or their metabolites [8]. Previous reports have shown that in some lots of white musk rose fruit produced in this area, the skin has been contaminated by direct contact with PCP-treated wood in the drier [9].

Other important agricultural exports from this area are berries, apples, and asparagus. The asparagus might be exposed to contamination with PCP or TBP traces through water irrigation, air and soil, or by direct contact with PCP or TBP-treated wood [10]. Pentachloroanisole (PCA) might appear in vegetable samples due to methylation of PCP [11].

Several methods have been developed to determine PCP and other chlorinated phenols in different matrices. HPLC, Gas Chromatography (GC), and Capillary Electrophoresis (CE), combined with different extraction methodologies, such as supercritical fluid extraction (SFE), accelerated solvent extraction, and solid phase microextraction (SPME) have been reported [12, 22]. However, only few methods have been published for the determination of chlorophenol residues in vegetable matrices. A method for the determination of phenols by simultaneous distillation and extraction in alkaline media followed by
acetylation and chromatographic analysis has been presented [23], but sample preparation is time consuming. Diserens [10] recently published a GC-MS methodology, which included in situ extraction and derivatization of PCP and other 18 chlorophenols from wood and fruit samples. In both cases, neither PCA nor TBP were determined. In order to evaluate the possible contamination of asparagus residues of PCP, TBP, and PCA, in the present work the first two are derivatized in situ during extraction and analyzed by GC/MS in SIM mode.

2 Experimental

2.1 Instrumentation

A HP 6890 Series Gas Chromatograph with a split/splitless injector, coupled to a HP 5973 Mass Selective Detector and a HP ChemStation G1701AA Version A.03.00, using a HP-5 MS column of 30 m x 0.25 mm ID x 0.25 μm film thickness (Palo Alto, CA, USA) and electronic helium, Grade 6.0 (99.9999%), supplied by Air Liquide America (La Porte, Texas), was used as mobile phase. Analyte extraction from asparagus was performed with an ultrasonic homogenizer (Cole Palmer Series 4710), liquid-liquid extraction with residues of PCP, TBP, and PCA, in the present work the splitless mode. The working standard solutions were subjected to the same pretreatment procedure.

2.2 Reagents and standards

Chemicals and solvents were residue analysis grade. PCP, TBP, PCA, and 2,2'-difluorobiphenyl (IS) were obtained from Chem. Service, Inc. (West Chester, USA). Hexane and sodium carbonate were provided by Merck (Darmstadt, Germany). Isopropanol was purchased from J.T. Baker (Xalostoc, Mexico) and acetic anhydride was obtained from Sigma (St. Louis, USA). Stock standard solutions of 100 μg mL⁻¹ of each phenol were prepared in isopropanol and stored at 4 °C. The working standard solutions were diluted in sodium carbonate and subjected to extraction with hexane.

2.3 Chromatographic conditions

For direct chromatography of PCP, TBP, and PCA in a poly(5% diphenyl/95% dimethylsiloxane) copolymer bonded column, the initial column temperature was 50 °C for 3 min, followed by heating at 10 K/min until 200 °C (ramp1), and at 15 K/min until 300 °C (ramp2), and a final clean-up at 300 °C for 5 min. For acetyl-PCP, acetyl-TBP, and for PCA, an initial column temperature of 80 °C (for 7 min) was followed by 5 K/min until 150 °C (2.5 min) (ramp1) and 15 K/min until 250 °C (ramp2). The clean-up was carried out at 300 °C for 5 min. In both cases, splitless injection at 250 °C with a carrier flow of 1 mL min⁻¹ was used. The GC-MS transfer line was kept at 280 °C and detection was carried out in scan mode with an electron energy of 70 eV. The ionization source was set at 230 °C and the analyzer temperature at 150 °C. For quantitative analysis, the SIM mode was chosen to enhance the sensitivity and selectivity. The monitored mass fragments were m/z 280, 330, 266, and 190 for PCA, TBP, PCP, and IS, respectively.

2.4 Sampling and sample treatment

The edible parts of asparagus were collected directly from different farms close to sawmills in the Province of Bio-Bio (Chile) in October and November 2001 and they were stored in glass flasks at −20 °C. For analysis, asparagus were cut and chopped in a glass mixer; 5 to 20 g of this were homogenized in 20 mL of sodium carbonate (0.05 M) and subjected to sonication for 30 s in order to extract the phenols from the sample matrix. One mL of acetic anhydride and 5 mL of hexane were added for derivatization/extraction of PCP and TBP, and leaching of PCA. Finally, a liquid-liquid extraction was carried out with stirring for 30 min [10], followed by 5 min centrifugation. One to 3 μL of the hexane layer was manually injected in the splitless mode. The working standard solutions were subjected to the same pretreatment procedure.

3 Results and discussion

To determine PCP, TBP, and PCA in the asparagus samples, two different chromatographic methods were evaluated: the direct separation of PCP, TBP, and PCA and the separation of previously acetylated PCP and TBP and non-acetylated PCA. The acetylation was assayed in order to enhance selectivity and sensitivity [24].

3.1 Separation conditions

PCP, TBP, and PCA can be separated directly in less than 20 min with a resolution of 2.7 between PCP–PCA. However, at low PCP concentrations, a dramatic loss of sensitivity was observed (40%), making this procedure inappropriate for PCP traces analysis, whereas chromatographing PCP as its acetyl-derivative, makes its detection much more favorable. Nevertheless, the retention times increase. The resolution between PCA and acetyl-TBP is only 1.06, but a quantitative determination of both is possible by detection in the SIM mode, using selective fragments for both analytes (Figure 1).

3.2 Extraction and derivatization

PCP and TBP were isolated from the sample matrix by alkaline extraction with sodium carbonate and an ultrasonic homogenizer bar with output control of 80 during 30 s. Better results were not observed when longer time was used. Different pHs were tested in order to obtain the maximal extraction yield of compounds. The optimum value was achieved when 0.05 M of sodium carbonate was
Determination of tribromophenol and pentachlorophenol used (pH = 10). At this pH, the compounds were transferred to alkaline solutions as phenolates. Extraction efficiency did not improve with higher carbonate concentrations.

To test the stability of the acetylated compounds, a mixture of 5.0 ng mL\(^{-1}\) of each analyte was derivatized and injected on the GC periodically. The signal was stable 10 min after derivatization. Acetyl-TBP was stable over 1500 min, whereas acetyl-PCP was stable during 300 min.

### 3.3 Calibration

All quantitative analyses were carried out using the SIM mode in order to enhance the selectivity and sensitivity. Calibration graphs were constructed using a matrix of blank samples spiked with PCP, TBP, and PCA. Six concentration levels were analysed. The individual area-concentration sets were subjected to linear least-squares regression. For non-acetylated PCP and TBP, a linear response was obtained between 1.0 and 6.0 \(\mu\)g mL\(^{-1}\) (\(r = 0.996\) and 0.999, respectively), with a limit of detection (LOD), calculated as three times the blank standard deviation, of 0.6 and 0.3 \(\mu\)g mL\(^{-1}\), respectively.

For acetylated compounds and PCA, the original concentration range studied was between 20 and 100 ng mL\(^{-1}\). The results demonstrated linearity (\(r = 0.995\), 0.989, and 0.966 for PCA, acetyl-TBP, and acetyl-PCP, respectively); however, the concentrations found in samples were less than this range, and a new calibration was carried out between 1 to 8 ng mL\(^{-1}\). In this case, 5.0 ng mL\(^{-1}\) of IS was used to improve the precision of the results. The linear range was between 1.0 to 8.0 ng mL\(^{-1}\) (\(r = 0.98\) for each compound) with a LOD 2 ng mL\(^{-1}\) for acetyl-PCP, acetyl-TBP, and PCA (corresponding to 0.3 \(\mu\)g kg\(^{-1}\) in the plant). The repeatability, obtained by analysis of spiked samples, subjected to the complete method 11 times, including liquid-liquid extraction (or leaching for PCA), derivatization, and chromatographic analysis, ranged between 5% and 8%. This is considered acceptable for trace analysis using manual injection. As demonstrated in this work, acetylation of both compounds is highly recommendable for determining traces of PCP and TBP, since the reaction afforded significant sensitivity enhancement.

### 3.4 Method performance

Figure 1 shows the chromatogram obtained for spiked asparagus samples. The proposed methodology allowed enough selectivity to obtain clean chromatograms with acceptable sensitivity for trace analysis. In order to evaluate the performance of the proposed sample pre-treatment method, asparagus samples spiked with five different concentration levels (injected in triplicate) were subjected to the described procedure. The recoveries were determined as the slopes of the linear regression graphs of added analyte concentration versus measured analyte concentration. In a concentration range between 2.0 and 8.0 \(\mu\)g kg\(^{-1}\) the extraction yield was 59%, 86%, and 97% for PCA, TBP, and PCP, respectively, with a linear relationship between added and found concentration (\(r\) between 0.981 and 0.992). The precision was also acceptable (RSD < 7% in all cases). Recovery of PCA was less than for PCP and TBP, because leaching of PCA from sample matrix into organic phase is more difficult than solubilizing phenolates in alkaline media. However, the presented methodology allows the simultaneous extraction and determination of PCP, TBP, and PCA with adequate precision.

Finally, the methodology was applied to determine the possible contamination of 10 asparagus samples obtained in the Bio-Bio Province. The results showed the low contamination level in the samples obtained in this area. PCP was detected in 9 samples with a concentration range between 0.4 and 4.0 \(\mu\)g kg\(^{-1}\), while 6 samples showed detectable concentrations of TBP (between 0.4 and 1.5 \(\mu\)g kg\(^{-1}\)) and only one sample was positive for PCA with a concentration of 2.0 \(\mu\)g kg\(^{-1}\). In all cases, the detected concentrations were below the maximal allowed concentrations in vegetables (10 \(\mu\)g kg\(^{-1}\)).

### 4 Concluding remarks

GC/MS in combination with the proposed extraction and derivatization system allows the selective determination of PCP, TBP, and PCA at trace levels. Acetylation significantly improved the sensitivity, especially for PCP, whereas PCA was detected directly. Liquid-liquid extraction allowed an acceptable recovery of PCP and TBP (above 86%), while the leaching of PCA gave a lower extraction efficiency (59%). The method allows the simultaneous determination of PCP, TBP, and PCA in the

![Chromatographic separation (SIM) of a spiked asparagus samples subjected to extraction and derivatization (5.0 \(\mu\)g kg\(^{-1}\) of each compound).](image-url)
same sample pretreatment step and with adequate sensitivity for trace analysis.

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