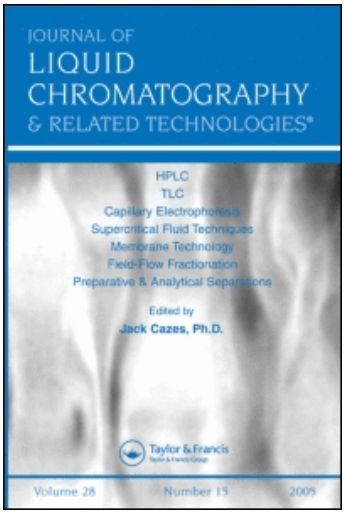

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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R. J. Bushway[a]; J. L. Bureau[a]; Abdulaziz Al-jerayed[a]
[a] Department of Food Science, University of Maine, Orono, Maine

To cite this Article Bushway, R. J. , Bureau, J. L. and Al-jerayed, Abdulaziz(1984) 'High-Performance Liquid Chromatographic Determination of TCNB in Potatoes', Journal of Liquid Chromatography & Related Technologies, 7: 6, 1185 — 1193
To link to this Article: DOI: 10.1080/01483918408074036
URL: http://dx.doi.org/10.1080/01483918408074036

JOURNAL OF LIQUID CHROMATOGRAPHY, 7(6), 1185-1193 (1984)

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF TCNB IN POTATOES

R.J. Bushway, J.L. Bureau and Abdulaziz Al-Jerayed
Department of Food Science
102B Holmes Hall
University of Maine
Orono, Maine 04469

ABSTRACT

A high-performance liquid chromatographic (HPLC) method was developed for the determination of TCNB (tetrachloronitrobenzene), a sprout inhibitor, in potato peels and flesh fortified at levels of 0.16 to 53.5 ppm. TCNB was analyzed on a u Bondapak C_{18} column with UV detection at 210 nm. The mobile phase was acetonitrile-methanol-water (35:35:30) at a flow rate of 1.0 ml/min. Retention time was approximately 10 min. TCNB was extracted by blending for 5 min in acetone. Samples at a level of 1 ppm or higher were directly injected whereas samples below 1 ppm were partitioned into hexane followed by passage through an alumina column. Average recoveries varied from 85.6 to 96.8% with coefficients of variation ranging from 2.18 to 11.68%. A study conducted to test 23 pesticides for possible interferences with TCNB demonstrated that none of them co-chromatographed. The lower limit of detection was 0.08 ppm.

INTRODUCTION

Tetrachloronitrobenzene (TCNB, Fusarex, Tecnazene) is used on potatoes as a sprout inhibitor and as an agent for control of dry rot (Fusarium coeruleum). TCNB has been shown to inhibit sprouting of potatoes up to 11 months. The advantage of TCNB is

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that it does not inhibit wound healing in freshly clamped tubers (1). The recommended application is 1.0 lb of TCNB for every 600 lbs of potatoes (2).

Because of the possibility of sprouting occurring below the application rate and because of the EPA tolerance level of 25 ppm on potatoes, a fast and accurate method for determining TCNB residues is needed. Present methods for the quantification of these residues include polarographic, colorimetric and gas chromatographic. The polarographic method has a very time consuming extraction step whereby the benzene extract must stand for several hours over sodium sulfate (3). There are 3 colorimetric procedures (4-6) in which all are nonspecific. The GC methods (7,8) are good except the extraction and clean-up steps are lengthy.

This paper describes a high-performance liquid chromatographic procedure for determining residue levels of TCNB that overcomes many problems inherent in the other methods.

EXPERIMENTAL

Reagents

All solvents used were HPLC grade obtained from Fisher Scientific Co. Fair Lawn, NJ. The TCNB standard, 99% pure, was obtained from the United States Environmental Protection Agency, Research Triangle Park, NC. The acid alumina, Brockman activity I, 80-200 mesh, was purchased from Fisher Scientific Co. and was used as received.

Liquid Chromatographic System

A model ALC/GPC 244 high-performance liquid chromatograph containing a Model 6000 A pump, a U6K injector and a Model 450 Schoeffel UV detector (Waters Associates, Milford, MA). The detector was set at 210 nm and 0.04 AUFS. A Houston Instruments dual-pen recorder, set at a chart speed of 0.4 in/min, recorded the detector signal.

Column

A Waters Associates 30 cm x 3.9 mm i.d. u Bondapak C_{18} column was used at ambient temperature.

Mobile Phase and Flow Rate

The mobile phase was methanol-acetonitrile-water (35:35:30) with a flow rate of 1.0 ml/min.

Extraction

Fifty g of peel (2-3 mm thick) or flesh were extracted with 100 ml of acetone in a Waring blender, 1 qt jar size, at a high speed for 5 min. The extract was vacuum filtered through Whatman #42 filter paper. The volume was brought to 250 ml using acetone and a 20 ul aliquot was injected into the HPLC. If no TCNB peak was observed, the filtrate was evaporated to approximately 50 ml after which it was tranferred to a liter separatory funnel. One hundred and 30 ml of distilled water was added followed by 1 g of NaCl and 75 ml hexane. The funnel was shaken 2 min and the hexane layer put into another separatory funnel. Fifty ml of hexane was added to the original sample and shaken again for 2 min. The

combined hexane fractions were dried over sodium sulfate. This dried hexane was evaporated to dryness and brought to volume with methanol in a 25 ml volumetric flask.

Sample Clean-up step through alumina. A glass wool plug was placed in the bottom of a 10 ml disposable pipet which had the top 3 cm removed. The column was then dry packed with 4 cm^3 of acid alumina followed by passage of the sample through the column with the 2nd 2ml being collected.

HPLC Analysis

The response curve was determined by taking 0.5, 1.0, 5.0, 10.0 and 20.0 ml aliquots of a TCNB standard (conc 12.8 ppm) and putting each aliquot in a 50 ml volumetric flask and bringing it to volume with methanol. The standards were then passed through an acid alumina column with the 2nd 2 ml being collected. Twenty microliters of each solution were then injected into the HPLC and a curve of detector response vs nanograms of TCNB was plotted. Samples were quantified by comparison of the peak height with that of the standard curve since peak height vs concentration was linear with the range of concentrations used in this study.

Recovery Studies

Both peel and flesh samples (50g) were spiked at levels of 0.16, 0.32, 0.64, 1.07, 6.69 and 53.5 ppm. There were 5 to 6 determinations performed for each spiking level by adding the appropriate concentration of TCNB in liquid form and allowing it to set before extraction. Extractions were performed as described above.

Table 1. Recovery of TCNB Added to Untreated Superior Potatoes

Section of Tuber	No. of Determination	TCNB Added ppm	% Recovery	S.D.
Peel	6	0.16	96.8	4.82
Flesh	6	0.16	93.4	5.82
Peel	5	0.32	89.5	3.00
Flesh	6	0.32	88.4	8.10
Peel	6	0.64	87.7	4.30
Flesh	6	0.64	88.6	4.44
Peel	6	1.07	94.7	7.68
Flesh	6	1.07	89.4	7.77
Peel	6	6.69	89.4	2.96
Flesh	5	6.69	88.3	10.31
Peel	5	53.50	87.3	1.90
Flesh	5	53.50	85.6	1.97

RESULTS AND DISCUSSION

Potatoes were divided into the peel (1st 2-3 mm) and flesh gions. Fifty g portions of each were used in spiking studies and actual samples. Peel and flesh samples were fortified at levels of 0.16, 0.32, 0.64, 1.07, 6.69 and 53.5 ppm. Results of these spiking studies are shown in Table 1. Recoveries were very uniform throughout the different spiking levels with most being 87-90%. Except for the flesh spiked at 6.69 ppm, the coefficients of variation were all below 10%. Most CV% were below 7 % indicating the variation was excellent for a residue method.

Chromatograms of the flesh and peel of fortified potatoes at the 0.32 ppm TCNB level are shown in Figures 1 and Figures 2. TCNB eluted from the column in approximately 10 min with no inter-

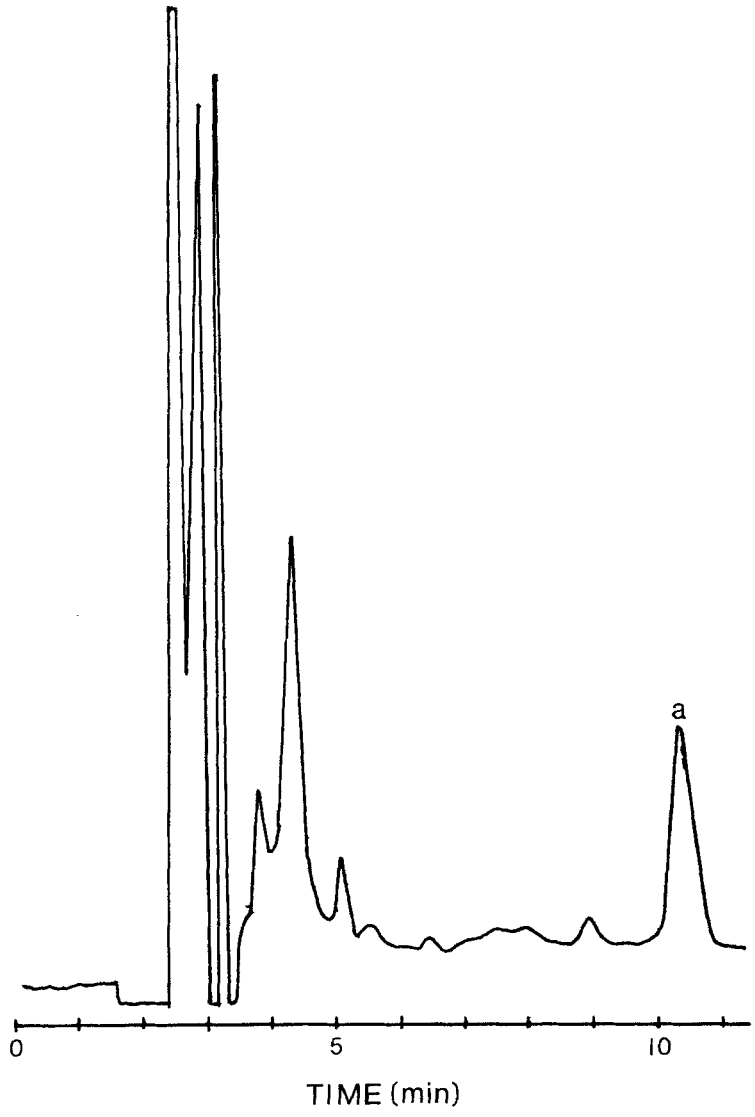

Figure 1. Chromatogram of Superior potato flesh spiked with 0.32 ppm TCNB. Peak (a) TCNB. Chromatographic conditions are given in the text.

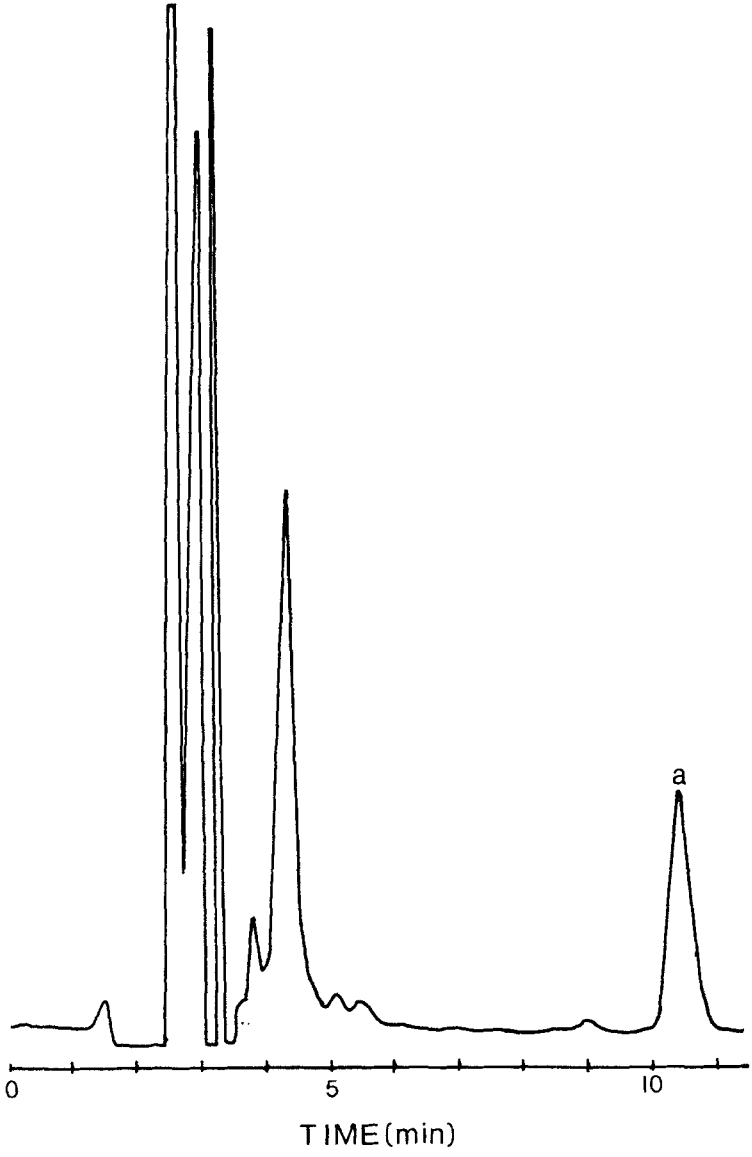

Figure 2. Chromatogram of Superior potato peel spiked with 0.32 ppm TCNB. Peak (a) TCNB. Chromatographic conditions are given in the text.

Table 2. Retention Time of Various Pesticides on C_{18} Relative to TCNB

Pesticide	Relative Retention Time
Guthion	0.49
Carbaryl	0.40
Carbofuran	0.39
PCNB	0.88
Dinoseb	0.19
Dinoseb Acetate	0.68
Maleic Hydrazide	0.21
Chloropropham	0.58
Propham	0.49
PCP	0.21
Amitrole	0.31
Picloram	0.18
Promecarb	0.54
2,4 D Acid	0.18
Monuron	0.39
2,4,5 T	0.18
Dicamba	0.18
Diuron	0.46
Propanize	0.52
Atrazine	0.45
Simazine	0.41
Pirimicarb	0.46
Captan	0.50

ferences. Although the TCNB peak height was low, this added very little to the overall variation of this method which was confirmed by injecting the lowest standard (0.5 ml) 6 consecutive times for which a coefficient of variation of 1.08% was obtained.

The lower limit of detection for this method was determined to be 0.08 ppm. It is doubtful that one could go lower because the blanks begin to show peaks where the TCNB comes off at lower concentrations.

Possible interferences from 23 pesticides were tested by injecting each using the same chromatographic conditions employed for TCNB. As shown in Table 2, none of these pesticides had the same retention time as TCNB.

This HPLC method offers a rapid and precise means of analyzing TCNB in potato flesh and peel, especially at levels equal to or greater than 1 ppm since the samples can be injected directly without cleanup. At concentrations less than 1 ppm a cleanup step must be used like the GC methods (7,8) which then makes the HPLC method valuble as a confirmation procedure.

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