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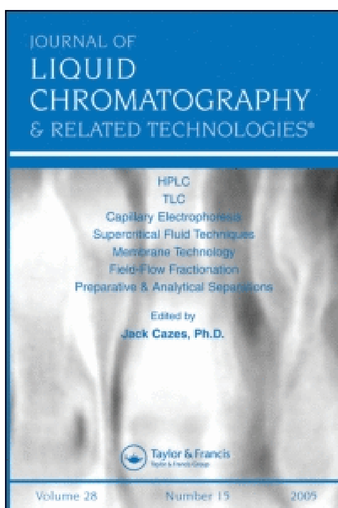

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A Reverse Phase Technique for Separating the Linearc Furano Coumarins in Celery

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A REVERSE PHASE TECHNIQUE FOR SEPARATING THE LINEAR FURANO-COUMARINS IN CELERY

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ABSTRACT

A reverse phase method for separating the four linear furanocoumarins psoralen, xanthotoxin, bergapten, and isopimpinellin in celery is described. The photosensitizing compounds, psoralen, xanthotoxin, and bergapten were also quantified in celery blades and leaves. The method utilizes a phenyl-bonded HPLC column with a methanol-water solvent system. This system allows the use of a detection wavelength (225 nm) more appropriate for these linear furanocoumarins than is currently used with normal phase systems.

INTRODUCTION

Linear furanocoumarins (psoralens) occur widely as constituents of plants from several families (1,2). These potent photosensitizing compounds have been used medicinally for treatment of skin disorders (1,3,4), but much concern has been associated with the medical use of linear furanocoumarins (5). They have been shown to be phototoxic during therapeutic use (1)

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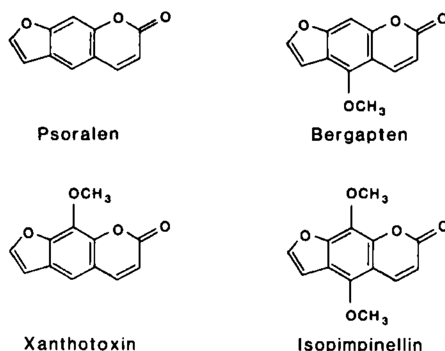

FIGURE 1. Linear furanocoumarins found in celery.

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and are suspected of being photocarcinogenic (6,7). Celery handlers and processors are affected with photodermatitis of the fingers, hands, and forearms which is the result of the linear furanocoumarins in celery (8,9). Recently, photodermatitis among grocery workers possibly caused by the handling of celery was reported by the National Institute for Occupational Safety and Health (10).

Celery has been shown to contain the four linear furanocoumarins shown in Figure 1 (11). During earlier analyses, we used a normal phase HPLC technique; a 25 cm Supelco 5μ Silica Column with a solvent system of ethyl acetate (0.1%) and formic acid (0.1%) in chloroform at 2.5 ml/min and a detection wavelength of 250 nm. This system has been reported as the analysis method in other reports (12-14).

Linear furanocoumarins have been separated by other normal phase systems. Corasil I with a solvent system of chloroform-cyclohexane with UV detection at 254 nm was used to separate furanocoumarins from spring parsley (15). A system using Partisil and a solvent system of methylene chloride-acetonitrile (95:5) at a wavelength of 254 nm was used to analyze xanthotoxin in blood and plasma (16,17). The separation of various furanocoumarins in twelve species of Umbelliferae was accomplished with a solvent

system of cyclohexane-ethyl acetate (3:1) on a microporasil normal phase column at a detection wavelength of 254 nm (18). A system using a MicroPak Si-10 column and a solvent system of chloroform-methanol at a wavelength of 270 nm was used to analyze for linear furanocoumarins in the seed of Casimiroa edulis (19).

While investigating analytical HPLC methodologies for analysis of linear furanocoumarins, a wide range of reverse phase methods have been tried, including C_{18}, NH_2, and CN HPLC columns. We reported a reverse phase C_{18} method for separating eleven of twelve linear furanocoumarins, but bergapten and isopimpinellin elute at the same time with that method as well as with other reverse phase columns (20). The determination of bergapten in perfumes and suntan cosmetics was accomplished with a solvent system of acetonitrile-water (36:65) on a RP-8 (10 μm) column and a fluorescence detector (21). A reverse phase method using UV absorbance would be useful for the separation of the linear furanocoumarins found in celery (Figure 1). Lower wavelengths than 250 nm may then be used for detection which would allow greater sensitivity for xanthotoxin, bergapten, and isopimpinellin.

In this paper it is demonstrated that a reverse phase system will effectively separate the linear furanocoumarins in celery. A reverse phase system of methanol-water (44:56 v/v) on a phenyl HPLC column allows the use of a more appropriate wavelength for these linear furanocoumarins.

CHEMICALS

The linear furanocoumarins used as standards in this study were obtained from the following sources: psoralen (Interchem Corp.), xanthotoxin (Biochemical Laboratories, Redondo Beach, CA), bergapten (Aldrich Chemical Co., Inc.), and isopimpinellin (isolated from Ammi majus as reported by Ivie, 1978). All solvents were Omni-Solv® MCB (22). Water was prepared for sample preparation and HPLC purposes by refluxing reverse-osmosis water (4 liters) with potassium permanganate (2 g) and sodium hydroxide (10

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g) for 2 hr, distilled, and followed by treatment for 2 hr with UV light in a Model 816 H.P.L.C. Reservoir (Photronix Corp.).

MATERIALS AND INSTRUMENTATION

SEP-PAK® cartridges were obtained from Waters Associates, Inc. Beckman Model 112 chromatographic pumps controlled by a Beckman Model 421 controller were used to deliver the solvent system through a Whatman guard column (Pierce Chemical Co.), filled with Pellicular Packing RP ODS (IBM Instruments, Inc.), and then a 4.6 mm ID x 15 cm 5μ phenyl column (IBM Industries, Inc.).

Samples were introduced via a 20 μl loop injector, Model 7125 (Rheodyne, Inc.). The column's effluent was monitored by a Beckman Model 165 detector at 225 nm and recorded with a Hewlett-Packard 3390A integrator.

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METHODS

Extraction and clean-up of celery leaves (2 g) and blades (5 g) with SEP-PAK® cartridges was accomplished as earlier reported (11). The combined eluates from the silica SEP-PAK® were concentrated to dryness by rotary evaporation and taken up to 0.3 ml in methanol/water (44:56 v/v). This solution was directly injected onto a phenyl column being pumped with methanol/water (44:56 v/v) at a flow rate of 1.2 ml/min.

A standard curve was generated by making solutions of the four linear furanocoumarins which contained from 1 ng/μl to 100 ng/μl of each component.

RESULTS AND DISCUSSION

The difference in sensitivities of each linear furanocoumarin as a function of detection wavelength is shown in Figure 2. This is a comparison of the absorbance at 225 nm to the absorbance at 254 nm, the wavelength used in most normal phase situations for linear furanocoumarins. Equal quantities of linear furanocoumarins were separated at each of the two wavelengths 254 nm (A) and 225 nm

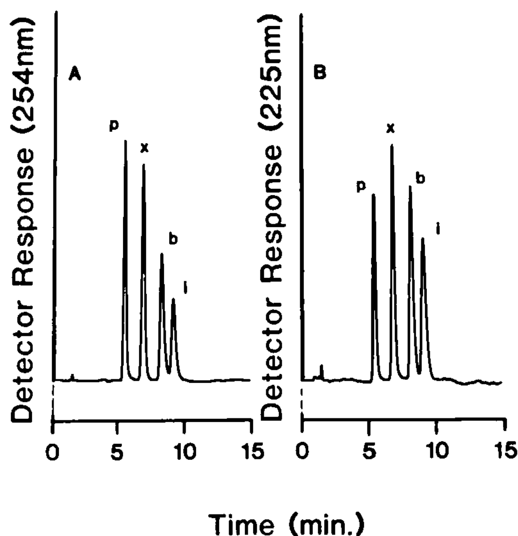

FIGURE 2. HPLC chromatographs of psoralen (p), xanthotoxin (x), bergapten (b), and isopimpinellin (i) at a detection wavelength of 254 nm (A) and 225 nm (B).

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TABLE 1

Quantities of the phototoxic linear furanocoumarins in celery leaves and blades in ppm with four reps per sample.

	Psoralen*	Xanthotoxin	Bergapten
Leaves	0.15 $\pm$ 0.07	3.5 $\pm$ 0.9	1.5 $\pm$ 0.4
Blades	0.11 + 0.03	2.1 + 0.3	0.32 + 0.07

*All values for linear furanocoumarins are in ppm.

(B). This clearly demonstrates that the sensitivities of xanthotoxin, bergapten, and isopimpinellin are nearly equivalent to that of psoralen at a wavelength of 225 nm. At 254 nm however, the sensitivities drop off dramatically from psoralen to isopimpinellin.

The quantitative results of the phototoxic linear furanocoumarins in celery leaves and blades as determined by this method are presented in Table 1. Isopimpinellin was not included because it is not an active photosensitizer (23).
These levels of linear furanocoumarins are about 3 fold higher than what was observed in celery blades grown in different locations of the United States in 1983 (11).

Example tracings of the linear furanocoumarins in the leaves (A), blades (B) and the standards (C) are shown in Figure 3.

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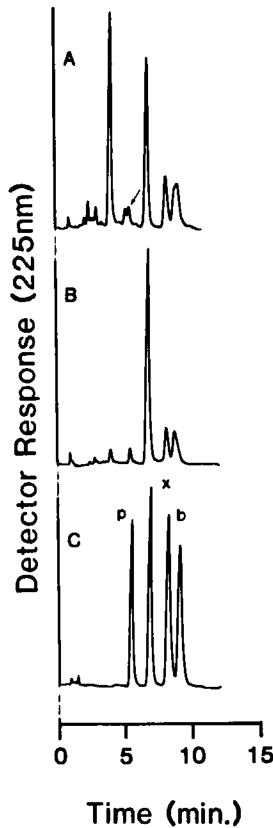

FIGURE 3. HPLC chromatograms of celery leaves (A), celery blades (B), and standards (C) containing psoralen (p), xanthotoxin (x), and bergapten (b).

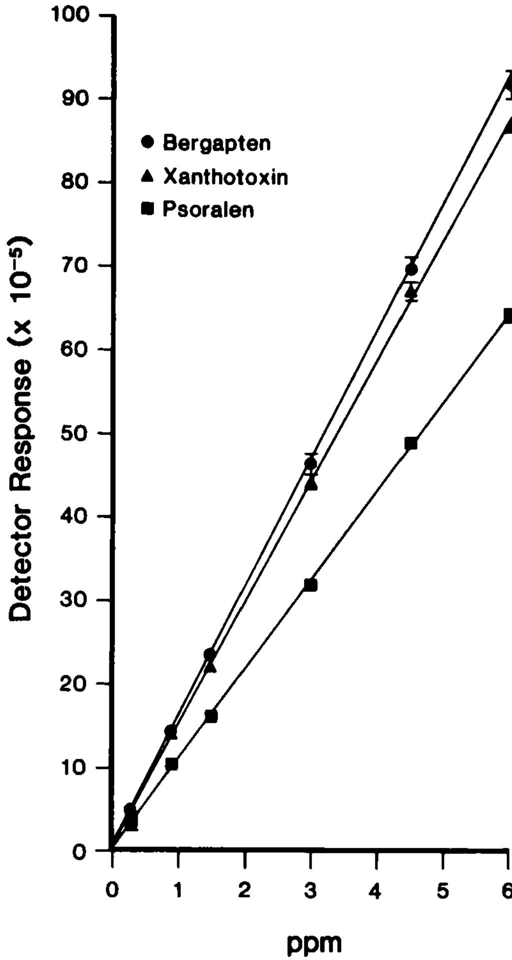

FIGURE 4. Standard curves for bergapten, xanthotoxin, and psoralen. Standard deviations were included when they were larger than the size of the plotted points.

The standard curves used to obtain these results are presented in Figure 4. Standard curves for the three linear furanocoumarins generated over a concentration range of 0.03-6.0 ppm, calculated on the basis of extracted sample weight being 5 g and final sample volume being 300 µl, had correlation coefficients equal to or greater than 0.99967 in each case.

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The reverse-phase HPLC technique reported here extends the HPLC analysis capability for linear furanocoumarins. The analysis of celery with this method can be made with better sensitivity for all phototoxic linear furanocoumarins.

Linear furanocoumarins are biologically active compounds and their potential significance to human health as well as pest control is becoming an active research area. The methods reported here will improve the quantification techniques for linear furanocoumarins in celery and other plants.

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