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HPLC Separation of Cotton Bract Extract

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NOTE
HPLC Separation of Cotton Bract Extract

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Abstract

An HPLC method has been developed to separate cotton bract washwater into thirty components using two columns in series.

INTRODUCTION

Dust generated during textile processing contains ingredients other than cotton fibers. One or more of the components of this dust is the causative agent of byssinosis, a disease of the lung (*1*). Physical analysis of this dust indicates that a significant portion is the bract, a leaflike structure found under the cotton boll (*2*). Inhalation of atomized aqueous extracts of cotton bract induces reversible airway narrowing and lung dysfunction similar to those experienced by cotton mill workers with byssinosis (*3*).

Analysis of steam distillates from cotton gin waste, which also contains a substantial amount of bract, was made by Hedin (*4*). The distillate was extracted with ether and methylene chloride, and the organic portion was subjected to gas chromatographic/mass spectrometric (GC/MS) analysis that identified 158 compounds. However, some of the higher boiling compounds were not separated, and interactions of components during analysis are also possible.

Separation of an aqueous extract of cotton bract into specific components using high performance liquid chromatograph (HPLC) with a cyano column was accomplished by Wall et al. (*5*). No attempts were made to propose a general separation technique for the entire spectrum of compounds found in this extract.

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The research presented in this paper offers a method for separation of an aqueous extract of cotton bract or dust into many fractions to facilitate collection of each fraction for further analysis by IR and/or GS/MS methods.

MATERIALS

The HPLC used was a Beckman model 421 with a 165 variable wavelength detector, a Gilson Spectro Glo fluorometer with excitation filters of 330–380 nm and emission filters of 500–600 nm, and a Gilson 201 fraction collector. Columns used were C8, C18, cyano, and amino, and a UV spectrophotometer. Bracts from pima cotton which were grown at Southern Regional Research Center in New Orleans, Louisiana, were used. All water and acetonitrile used was filtered through a 0.45-μm Millipore filter.

METHOD

The pima bract was collected fresh from the cotton plant and ground in a Wiley Mill through a 20-mesh screen. The bract was processed according to a procedure outlined by Buck (*6*) of Yale Medical School. This procedure ensures that the extract still has bioactivity after isolation. Twenty grams of freshly gound cotton bract were soaked in 120 mL of water at 23°C for 2 h, filtered through a Buchner funnel, centrifuged, and then filtered through a 0.45-μm filter. The filtrate was passed through a 1000-dalton Amicon filter, then freeze-dried. Seven-tenths of a gram of the solid was dissolved in 5 mL of water and 35 mL of methyl alcohol. A precipitate that formed was removed by centrifugation. The solution was evaporated to dryness at 40°C with a rotary evaporator, then redissolved in 25 mL water and passed through a diethylaminoethyl cellulose (DEAE) ion-exchange column. The eluent was freeze-dried and dissolved in 3 mL water (for convenience this fraction will be referred to as B6).

An ultraviolet spectrogram was obtained of this extract (B6) from 190 to 350 nm. The spectrogram indicated the most active region was between 192 and 205 nm. Twenty microliters of B6 were injected into the HPLC fitted with a C18 column monitored with a dual UV detector at wavelengths 192 and 205 nm in series with a fluorescent detector. The mobile phase was 50% water and 50% acetonitrile. A typical chromatogram of B6 at 192 nm is seen in Fig. 1. All the peaks are bunched together, and there is no clear separation for collection of specific peaks. Changing the amount of acetonitrile in the mobile phase isocratically or by a gradient, use of methanol as the mobile

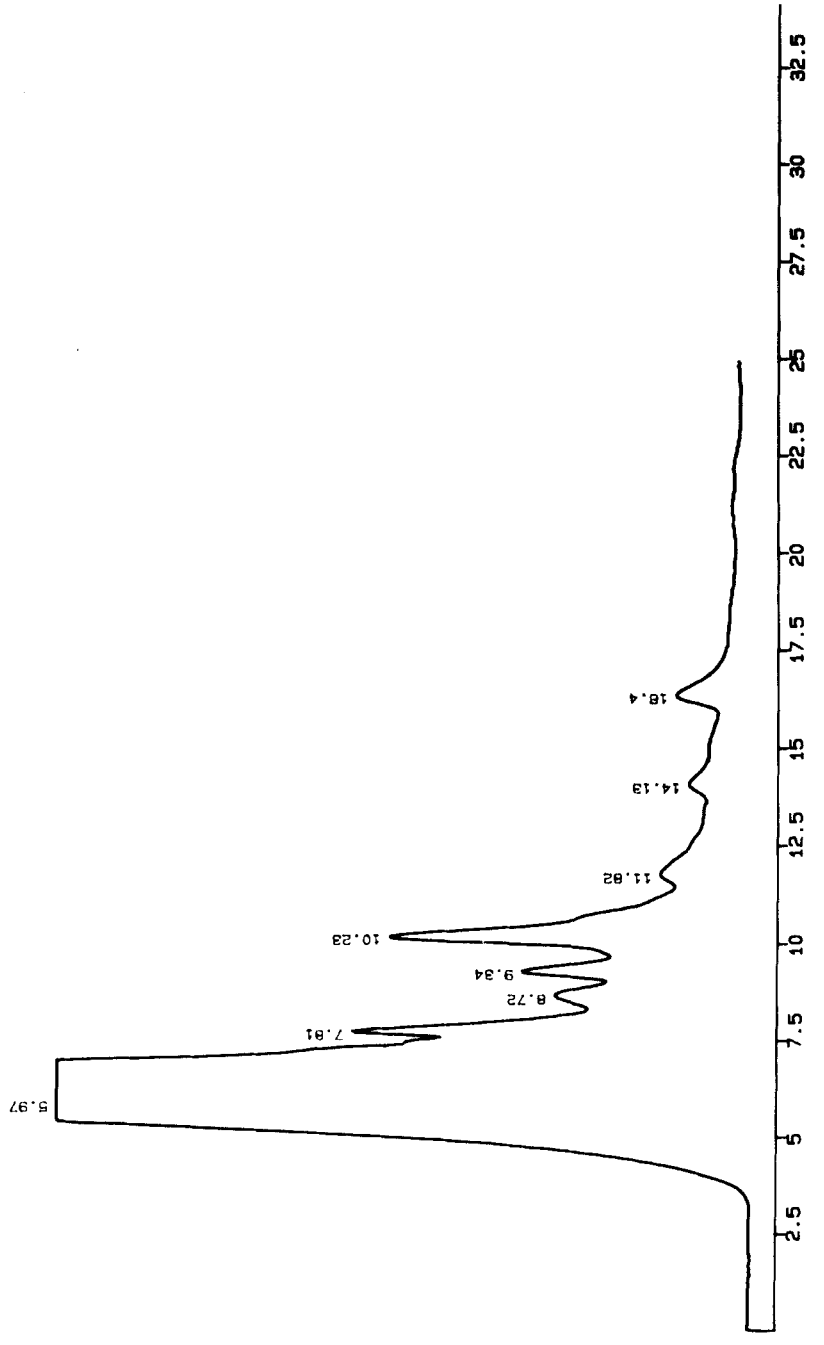

FIG. 1. B6 with C18 column.

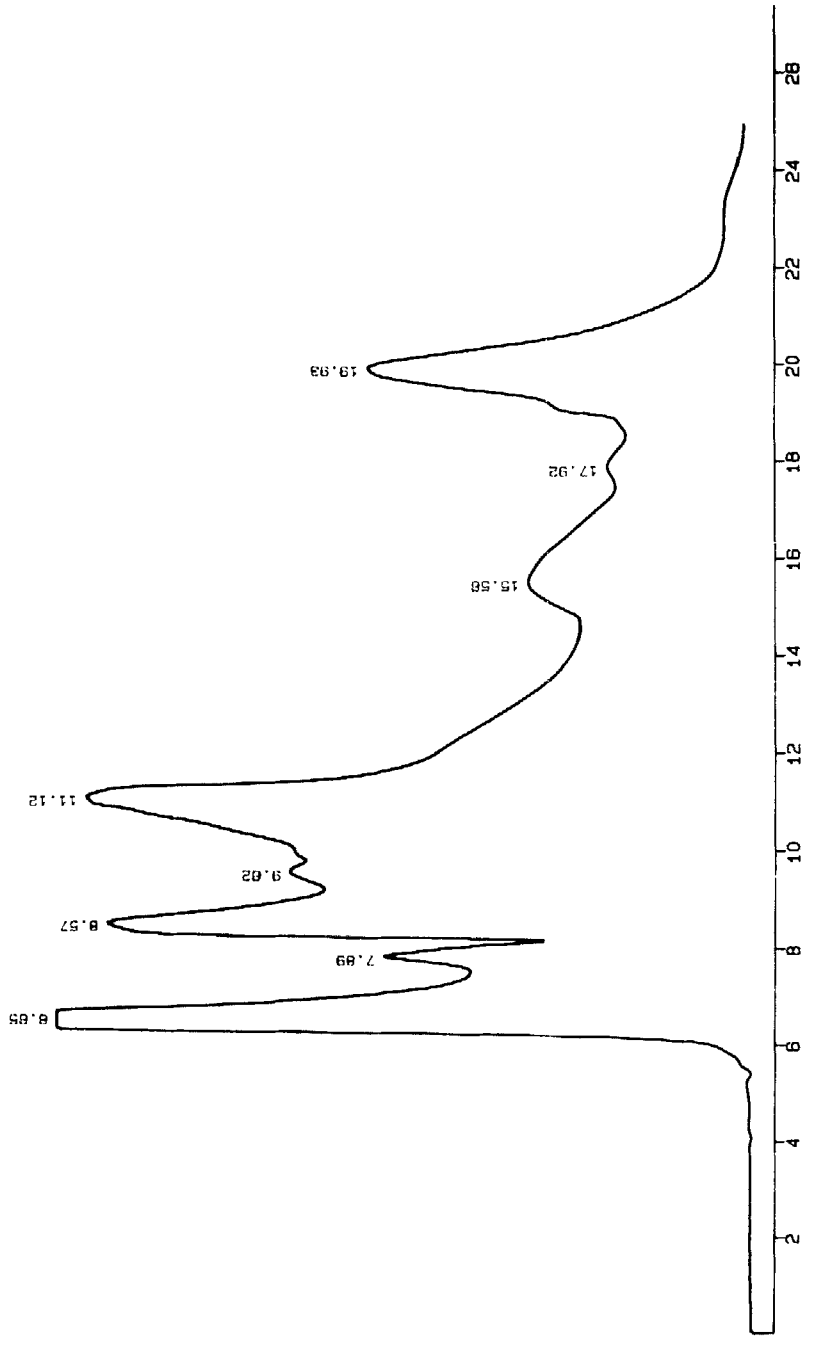

FIG. 2. B6 with C8 and amino column using a UV detector.

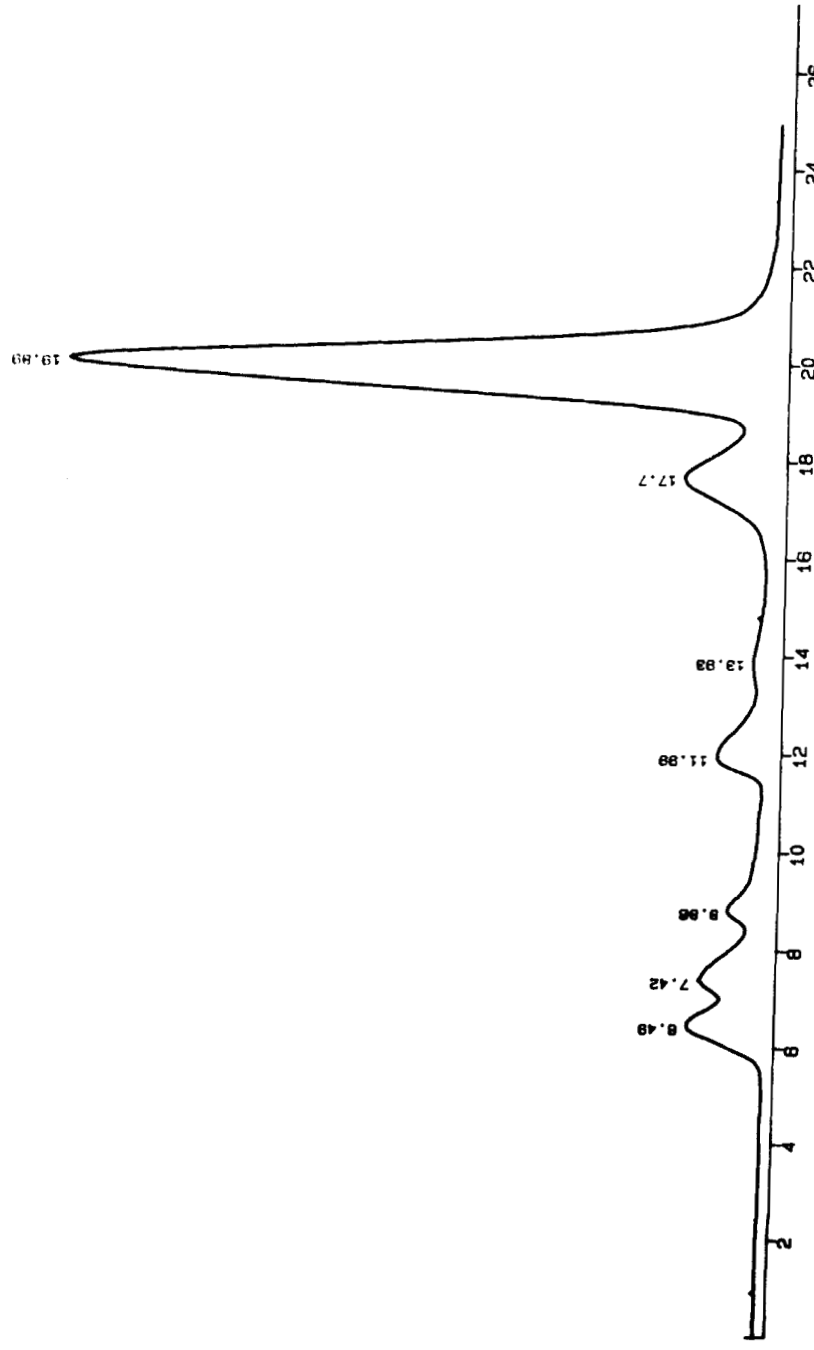

FIG. 3. B6 with C8 and amino column using a fluorescence detector.

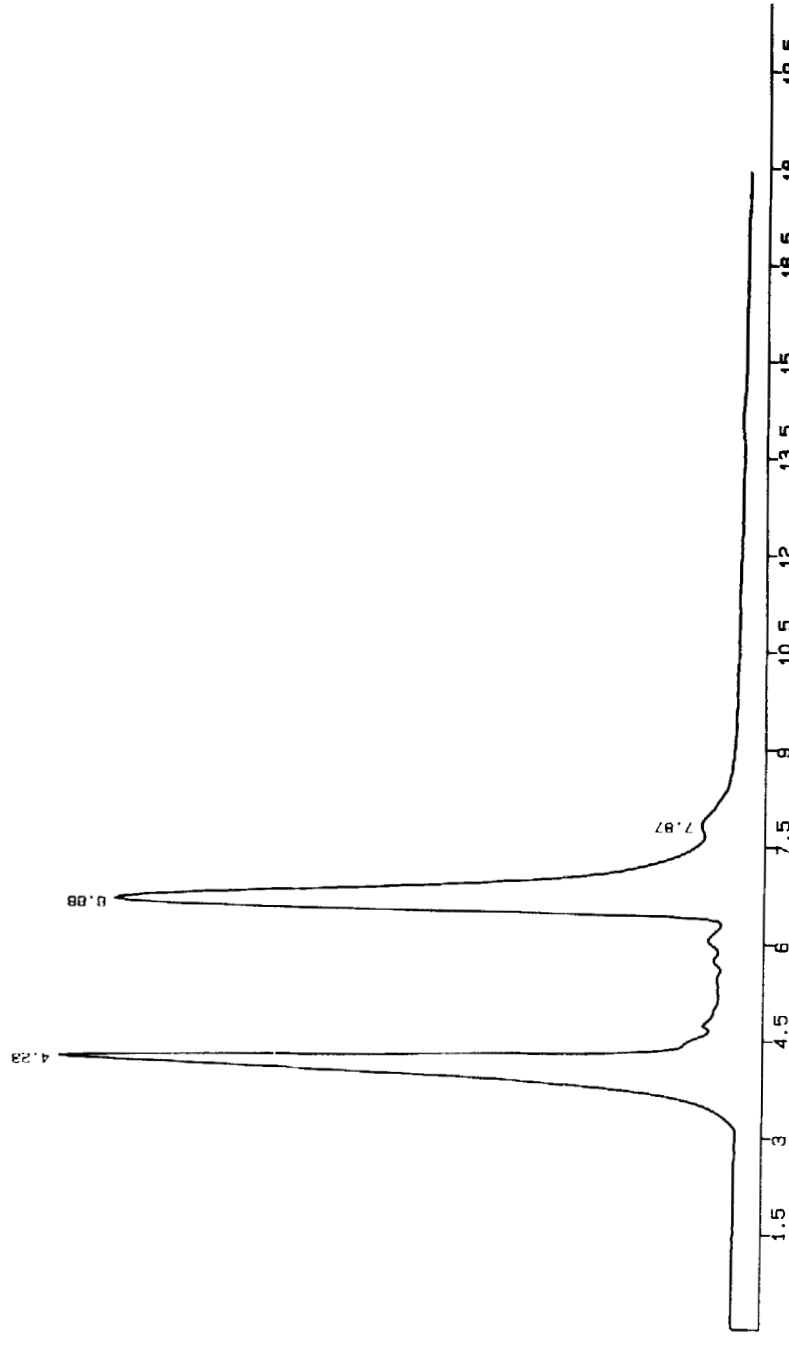

FIG. 4. Peak No. 1 with C18 and cyano column using a UV detector.

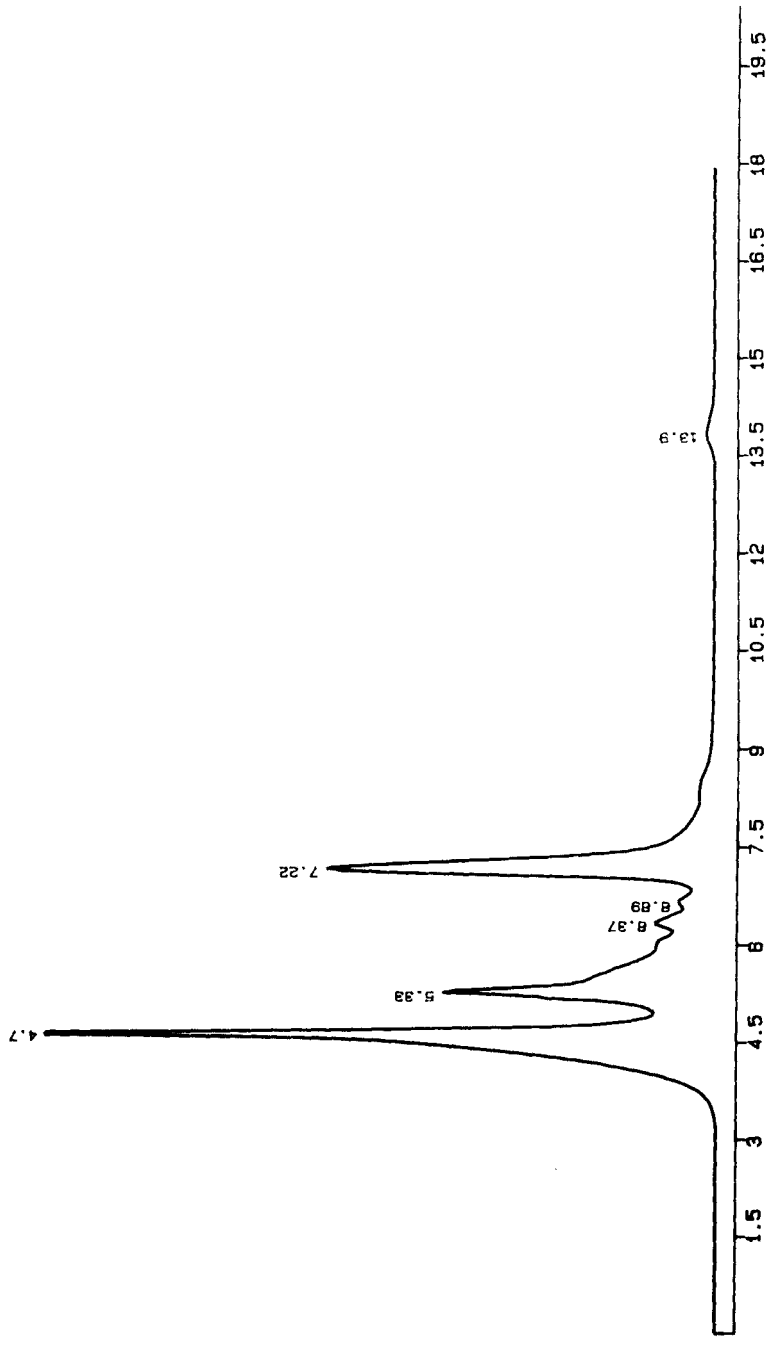

FIG. 5. Peak No. 2 with C18 and cyano column using a UV detector.

phase, and increasing or decreasing the flow rate did not give satisfactory separations. Using a C8, cyano, or amino column did not improve the chromatogram. Although the UV detector at 192 nm did not improve the separation (Fig. 2) when the C8 column was in series with an amino column, the fluoresence detector gave eight clearly separated peaks (Fig. 3) with 50% water and 50% acetonitrile as the mobile phase.

Twenty microliter aliquots of B6 were injected for 12 replicate runs on the C8 and amino columns in series; each of the eight peaks was collected using the peak collector. Each peak, which had a volume about 10 to 12 mL, was freeze-dried and reconstituted to 0.5 mL with water. The eight peak fractions were injected into a C18 column in series with a cyano column, and fractions were detected using the UV detector at 192 nm. Peak No. 1 was separated into two peaks (Fig. 4). After 10 repeated injections, the two peaks were collected for further analysis. Peak No. 2 was separated into five peaks and collected (Fig. 5). The other six original peaks were separated into 23 new peaks, all of which were collected. Thus the original eight peaks were further separated into 30 fractions.

RESULTS

The method described above enables one to separate and collect complex unknown water soluble samples using HPLC as a separation tool. The peak fractions collected in this manner are of a purity suitable for subsequent IR or GC/MS identification of components.

Disclaimer

Names of companies or commercial products are given solely for the purpose of providing specific information; their mention does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned.

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