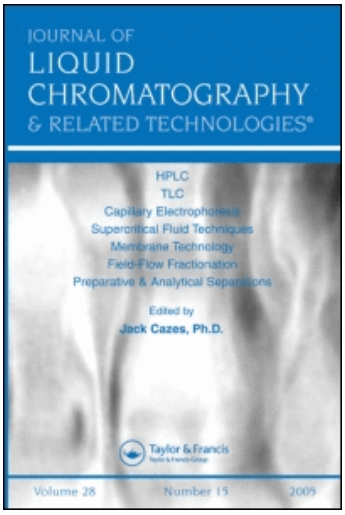


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# Detection of Furfural and 2-Furoic Acid in Bacterial Cultures by High Pressure Liquid Chromatography

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# DETECTION OF FURFURAL AND 2-FUROIC ACID IN BACTERIAL CULTURES BY HIGH PRESSURE LIQUID CHROMATOGRAPHY

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## ABSTRACT

The Furfural and 2-furoic acid present in bacterial cultures were extracted and detected by High pressure Liquid Chromatogrphy (HPLC). Using the methanol/water solvent (70/30), the column, μ Bondapak C18 (Waters Associates) seperated these compounds well. The detection was performed at 254 nm where binary mixtures were absorbed. This method provided a rapid and simultaneous detection for the conversion of furfural into 2-furoic acid followed by the utilization of 2-furoic acid during bacterial growth.

## INTRODUCTION

A number of methods are available for determining furan derivatives. Furfural was quantitatively determined by titration with bromine monochloride (1). Paolo(2) described that the mixtures of furfural and 5-hydroxymethyl furfural (HMF) were seperated by a thin layer chromatography (TLC). The spots were cut and developed with Dische reagent and than estimated by spectrophotometer. Furfuryl alcohol and furoic acid were analysed by Morimoto et al (3), using gas chromatography. Kostenco(4) described a method for determination of furfural and methylfurfural on silica

gel modified by fluorohydrocarbon radicals by High Performance Liquid Chromatography (HPLC).

However, the methods described above were not suitable for the determination of furfural and 2-furoic acid for the following reasons: no selectivity, time-consuming and complicated sample preparation in individual determination of biological mixtures.

This paper describes a new method, using HPLC for the determination of furfural and 2-furoic acid present in biological mixtures.



## MATERIALS AND METHODS

### Bacterial Culture Medium

One litre of culture medium contained: $KH_2PO_4$, 1.0g; $K_2HPO_4$, 2.0g; $KNO_3$, 1.0g; $MgSO_4 7H_2O$, 0.2g; NaCl, 0.2g; $CaCl_2$, 0.01g; furfural, 0.5g; yeast extract, 0.3g. Fifty ml of culture medium was poured into 250 ml Erlenmeyer flasks. After the medium was autoclaved at 121 °C for 15 min and cooled down to room temperature, the test microorganism was inoculated.

### Sample Preparation

Pseudomonas FS1 which was isolated from soil of University Campus was cultivated at 37 °C for three days in G24 Environmental Incubator Shaker (New Brunswick Scientific Co., Inc., Edison N.J. USA).

Five ml samples were withdrawn at appropriate time interval during the culture development and centrifuged at 5,000 rpm for 15 min at 4 °C (Rotor Model RPR20-2, Hitachi Automatic Refrigerated Centrifuge, Hitachi Koki Co., Ltd) to obtain cell-free sample.

This sample was filtered through 0.45 μm porosity milipore filter, 47 mm diameter (Millipore Corp.). The pH of sample was adjusted to 3 with 2N HCl. Two ml of

acidified sample was mixed with 1 ml of diethyl ether in a capped tube and left at cold room (4 °C) until two phases were clearly seperated.

Nonagueous phase was decanted into another capped tube and evaporated at 35 °C. An appropriate amount of this sample was redissolved in the corresponding methanol solvent for HPLC analysis and the other in distilled water for scanning of UV spectrophotometer.

Analysis by UV Spectrophotometer and HPLC

Prepared samples were scanned within the range of 210 nm to 330 nm in wavelength by Perkin-Elmer Model 139 UV-VIS spectrophotometer (Hitachi, Ltd., Tokyo, Japan).

Operational conditions of HPLC (Waters Associates Inc., Milford, Mass. 01757, USA) were as follow: column, μ-Bondapak C18; solvens, methanol/water (100/0, 70/30, 50/50, 10/90); flow rate, 1.0 ml/min; detector, UV Model 440, 254 nm; injection, 10 μl; temperature, room temperature. Methanol solvents (for chromatographic grade, Merck) were degassed and filtered through Millipore filter prior to usage.



## RESULTS AND DISCUSSION

Growth of Pseudomonas FS1 was observed in the culture medium containing yeast extract and furfural as a carbon source. A simple method to determine the quantity of furfural and possible amount of its metabolite(s) in this bacterial culture was needed. Furfural is an aromatic compound and has UV absorbance at 277 nm of wavelength(5). The metabolite(s) would be either furan derivatives such as 2-furoic acid and 2-furfuryl alcohol(3) or glutamate which does not have UV absorbance(6).

If metabolites are furan derivatives, it is certain

that it has UV absorbance. In order to resolve above presumptions UV scanning was carried out and the results are shown in Figure 1.

The peak at 277 nm was the furfural (A,B) and showed the gradual decrease (A,B,C,D). In addition a new peak at 245 nm appeared after 12 hr of growth and thereafter disappeared. A substance which peaks at 245 nm ($E_{245}$) seems to be a metabolite formed extracellularly. The purified $E_{245}$ substance was identified as 2-furoic acid when compared with a standard 2-furoic acid by IR spectrophotometer. 2-Furoic acid was produced due to the oxidation of furfural by an exoenzyme(7).

For simultaneous determination of furfural and



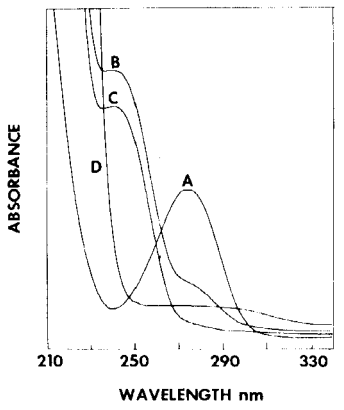


FIGURE 1

UV scanning spectra of culture filtrate during the growth of Pseudomonas FS1. (A) a peak of furfural in culture medium, (B) (C) peaks after 18 hr and 30 hr of growth, respectively, (D) disappearance of two peaks after 55 hr of growth.

2- furoic acid in bacterial cultures, culture filtrate was prepared to be analyzed by HPLC with different mobile phases of methanol as shown in Figure 2.

Furfural and 2-furoic acid could be separated well when solvents other than absolute methanol were used. The optimum condition for retention time of each mobile phase occurred only when 70% methanol was used.

Thus, transformation of furfural into 2-furoic acid and the utilization of 2-furoic acid during the culture development of Pseudomonas FS1 could be detected by HPLC, using 70% methanol solvent.

Pairs of peaks in chromatograms in Figure 3 demonstrated the gradual increase and decrease of 2-furoic



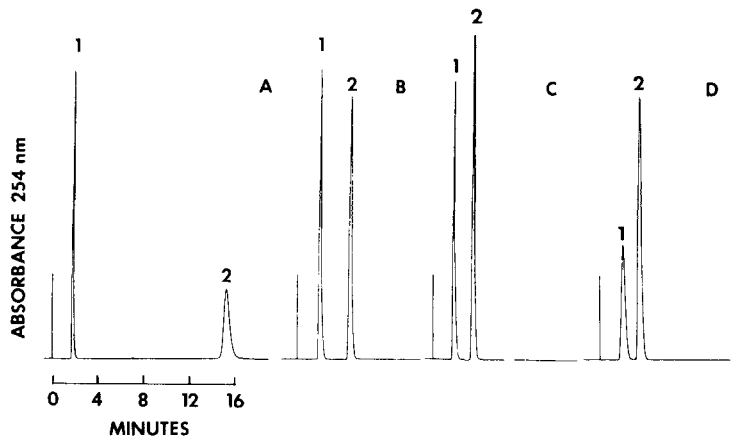


FIGURE 2

Chromatograms of 2-furoic acid(1) and furfural(2) at various concentrations of methanol solvent.
(A) 10% methanol, (B) 50% methanol, (C) 70% methanol, (D) 100% methanol.

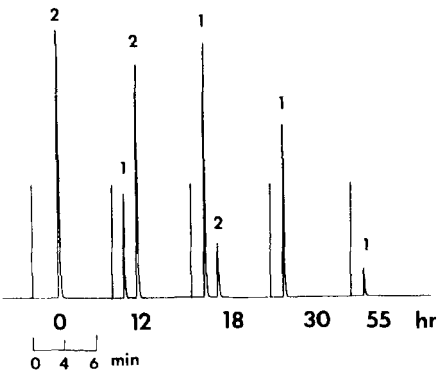


FIGURE 3

HPLC chromatograms of 2-furoic acid(1) and furfural(2) in culture filtrate during the culture development of Pseudomonas FS1. Solvent: 70% methanol. Other conditions are listed in text.



acid and furfural. The peaks that disappeared were completely utilized at the end of growth. As shown in Figure 1, spectrophotometric analysis was not suitable for quantitative determination of each compound in the binary mixtures with high concentration, since absorbances of the two compounds overlapped each other which resulted in error. However, HPLC analysis allowed binary mixtures of furfural and 2-furoic acid to be fractionated and detected rapidly and simultaneously.

Using HPLC method it is posible to estimate the amounts of furfural and 2-furoic acid by measuring peak heights or peak areas with reference to a calibration curve of standard compounds.

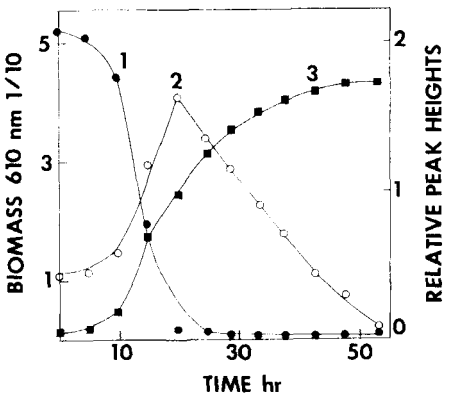


FIGURE 4

Quantitative changes of furfural (1) and 2-furoic acid (2) depending on the increase of cell mass(3).



The quantitative changes of furfural and 2-furoic acid, depending on cell mass expressed as turbidity at the optical density of 610 nm were shown in Figure 4.

Using the HPLC method, we have found unique physiological properties of Pseudomonas FS1 which is of interest in view of elucidating the metabolism of furfural as a toxicant.

2-Furoic acid was not utilized until furfural was completely converted into 2-furoic acid. This indicated that furfural was subjected to oxidation into 2-furoic acid followed by the reutilization of 2-furoic acid as a carbon source. Therefore Pseudomonas FS1 could decompose furan rings by converting furfural into 2-furoic acid.

In summary, the HPLC method described above can be useful for the detection as well as quantitative deter-

mination of furfural and 2-furoic acid present in bacterial cultures.

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