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Short Communication

Chromatographic determination of citric acid for monitoring the mould process

ZBIGNIEW J. WODECKI
Department of Organic Chemistry, Technical University of Gdańsk, 80-952 Gdańsk (Poland)
BOGUMIŁ TORŁOP
Citric Acid Factory, 81-130 Pelpin (Poland)
and
MAREK ŚLEBIODA*
Department of Organic Chemistry, Technical University of Gdańsk, 80-952 Gdańsk (Poland)
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ABSTRACT

A reliable method for monitoring the mould citric acid production process is presented. The method is based on in-line sample work-up and high-performance liquid chromatographic determination of citric acid with ultraviolet detection. It provides good precision (better than 1% in the range of 0–150 g dm^{-3}) and requires no special equipment.

INTRODUCTION

Citric acid is today the most widely used organic acid in the food and pharmaceutical industries. All the commercial methods of production of citric acid involve the conversion of inexpensive sources of glucose or sucrose, such as treated beet molasses, by selected strings of *Aspergillus niger*. It is important to monitor the mould process by determining the concentration of citric acid in the biosynthetic reaction mixture. From among the several high-performance liquid chromatographic (HPLC) methods of determination of carboxylic acids [1] we chose solvophobic chromatography for its simplicity and for the durability of the octadecyl silica columns. In this method the retention of carboxylic acids is the result of hydrophobic interactions of the hydrocarbon moiety of the solute with the octadecyl chains of the stationary phase, after suppression of ionization of the acidic functional groups by addition of acids or acidic buffers to the mobile phase.

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EXPERIMENTAL

Instrumentation

An HP 1090 Hewlett-Packard liquid chromatograph equipped with a C_{18} guard column (25 × 4.6 mm I.D.), a column-switching valve, a Nucleosil ODS 100-5 analytical column (250 × 4.6 mm I.D.) and a autosampler were used. UV-detection at 210 nm was accomplished with the HP 1040 diode-array detector. The column was operated at temperature of 55°C. The mobile phase flow-rate was maintained at 1.00 $cm^3\ min^{-1}$.

Reagents

Water used as the mobile phase component and as a solvent was freshly redistilled from glass apparatus. Methanol and ethanol (POCH, Poland) p.a. were used as obtained. Orthophosphoric acid (0.1%) (POCH, Poland) p.a. in water was used as a mobile phase for HPLC analysis.

Procedure

Samples taken from the the shell pan citric acid production process were stabilized by the addition of 96% ethanol, in order to obtain a final concentration of 30%, and stored refrigerated. They had a very complex matrix; besides citric acid there were sugars, proteins and enzymes. To avoid the analytical column deterioration caused by strongly retained substances, a sample clean-up step prior to the analysis was necessary. It was done using solid-phase extraction. During off-line as well as on-line work-up procedures the interfering substances were retained on a C_{18} cartridge or guard column, while citric acid was not retained.

Off-line work-up procedure. A solid-phase extraction C_{18} column (SOPHEX, DHN, Poland) was preconditioned by passing through it $2 \cdot 1.0\ cm^3$ of methanol and $3 \cdot 1.0\ cm^3$ of water. The sample (0.10 cm^3) was subsequently introduced and the carboxylic acid fraction was eluted with $3 \cdot 1.0\ cm^3$ of water into a weighed polyethylene vial. The vial was stoppered, weighed again and the content was analysed for citric acid using an HPLC system analogous to that described above but without the guard column in-line.

In-line work-up procedure. The sample (1 mm^3) was injected into the chromatographic system. After the analysis was completed (10 min), the column-switching valve was activated and the guard column was washed with methanol (5 min) to elute substances which previously had been retained and then with the mobile phase (10 min). The column-switching valve was subsequently deactivated and the entire system was washed with the mobile phase (2 min).

Precipitation method (standard industrial method for the determination of citric acid). The sample (10 cm^3) was neutralized with a 1 *M* solution of sodium hydroxide in water and heated on a steam bath. Then a 10% (w/v) solution of calcium chloride (15 cm^3) was added and the precipitate was collected. The filter paper containing the precipitate was ashed in an electric oven (800°C), and the residue was dissolved in 1 *M* hydrochloric acid. The acid was back-titrated with a 1 *M* solution of sodium hydroxide.

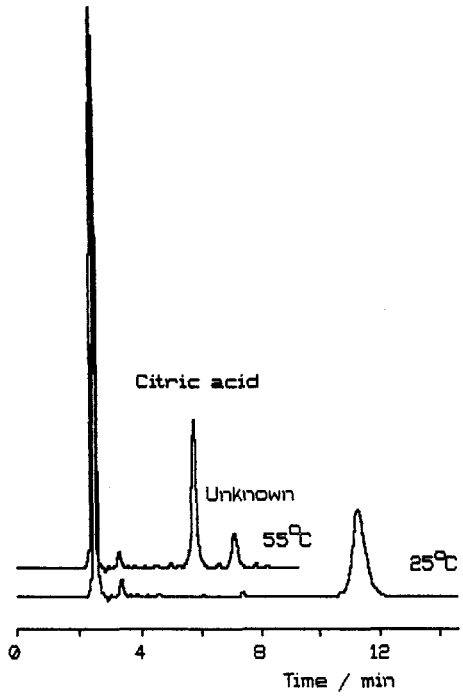

Fig. 1. Effect of temperature on the separation of components of sample taken from the shell pan citric acid production process. For chromatographic conditions see the Experimental section.

RESULTS AND DISCUSSION

The applied chromatographic conditions provided good separation of citric acid from substances eluting close to the void volume, but examination of the UV spectrum of the citric acid peak revealed that an unknown substance present in the sample co-eluted with citric acid. This can be easily avoided by raising the temperature to 55°C (Fig. 1). Because of the very complex matrix, a sample clean-up step prior to the analysis was necessary, otherwise the analytical column deteriorated and interference with the analysis resulted. Usually this is done off-line using solid-phase extraction cartridges. To override this time-consuming and potentially error-causing step, we employed an in-line chromatographic system.

The precision of the in-line HPLC method was estimated to be $\pm$ 1.1 mg cm^{-3} (about $\pm$ 0.9% for the sample containing 100–150 mg cm^{-3} citric acid). The results obtained using both the off-line and in-line work-up procedures were in a good agreement with the results obtained using a standard precipitation method for citric acid determination (within 1.2% at about the 100 mg cm^{-3} level). The method proposed can be a reliable analytical tool for monitoring the biosynthetic production of citric acid.

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REFERENCE

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