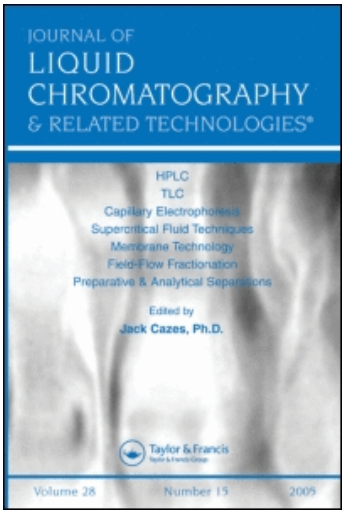

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Analysis of α-Ketocarboxylic Acids by Ion Exchange Hplc With UV and Amperometric Detection

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ANALYSIS OF α-KETOCARBOXYLIC ACIDS BY ION EXCHANGE HPLC WITH UV AND AMPEROMETRIC DETECTION

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ABSTRACT

Cation exchange high performance liquid chromatography with tandem 200nm spectrophotometric and +1.15V amperometric detection was used for the analysis of several α-ketocarboxylic acids. Derivatization of the α-ketocarboxylic acids was not necessary. This technique was operable at 2.0 ug/ml concentrations of α-ketocarboxylic acids. The sensitivity limit was not investigated.

INTRODUCTION

Recent studies have described the separation, identification and quantitation of α-ketocarboxylic acids.[1-5] These procedures required derivatization followed by GC[2], GC/MS[1] or HPLC[3,4,5] analysis. Derivatization of α-ketocarboxylic acids may not be quantitative and may yield diastereomeric compounds. Conversion of the α-keto moiety to the oxime derivative prior to silylation in a GC or GC/MS analysis of organic acids[6], affords two dia-

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stereomeric products, the E and Z oximes[7,8]. Conversion of the α-keto acid into the quinoxalinol derivative[1,2], prior to silylation, eliminates this problem but does not reduce the potential loss of analyte during derivatization.

We have developed a rapid analysis for α-ketocarboxylic acids that does not involve derivatization. The α-ketocarboxylic acids are separated by ion exchange HPLC and detected spectrophotometrically at 200nm and amperometrically at +1.15V.

MATERIALS

A Varian 5000 HPLC equipped with a Bio-Rad Laboratories Aminex HPX-87 Organic Acid column (300 mm x 7.8 mm) was used for the separation of the α-ketocarboxylic acids. The detection system employed was an Hitachi 100-40 Variable Wavelength UV Spectrometer equipped with an Altex 155-00 Flow Cell. The UV detection system was in series with and up stream from a Bioanalytical Systems LC-4 Amperometric Detector (on loan from the Anspec Company, Inc.) with a glassy carbon electrode. All chromatograms were traced on a Linear recorder.

α-ketoglutaric acid, α-phenylpyruvic acid, α-ketobutyric acid and α-ketovaleric acid were purchased from Sigma Chemical Company and were used without further purification. Distilled and deionized water was glass distilled from alkaline permanganate and was passed through a 0.22 um Millipore filter prior to use.

METHOD

To 0.3 ml of water was added 50 ul aliquots of the following aqueous solutions:

1.0 mg/ml α-ketobutyric acid
1.0 mg/ml α-phenylpyruvic acid
4.5 mg/ml α-ketovaleric acid
4.5 mg/ml α-ketoglutaric acid

A 10 ul aliquot of the solution was introduced via a Valco loop injector on to the ion exchange column. The mobile phase, 4.5 m N H_2SO_4, was passed through the column at 0.8 ml/min. The α-ketocarboxylic acids were detected by an UV spectrometer at 200nm and by an oxidizing amperometric detector (glassy carbon electrode), at +1.15V vs an Ag/AgCl reference electrode, that was in series with but downstream from the UV detector. The amperometric detector inlet was directly connected to the UV detector outlet. The UV detector was attenuated at $4x10^{-2}$ AUFS and recorded at 10mV for full scale deflection while the amperometric detector was attenuated at 50nA/V and recorded at 100 mV for full scale deflection. Analysis of a 10ul aliquot of an aqueous solution that contained 2.5 ug/ml of each α-ketocarboxylic acid showed that both the UV detector ($1x10^{-2}$ AUFS on a 10m V recorder) and the amperometric detector (20 n A/V on a 100 mV recorder) were functional at this concentration.

RESULTS

We were able to separate four non-derivatized α-ketocarboxylic acids of various carbon chain length on an anionic ion exchange HPLC column employing 4.5 m N H_2SO_4 at a flow rate of 0.8 ml/min (Figure 1). The α-ketocarboxylic acids were detected spectrophotometrically at 200nm and amperometrically at +1.15V. The attenuation for the amperometric recorder was ten times less sensitive

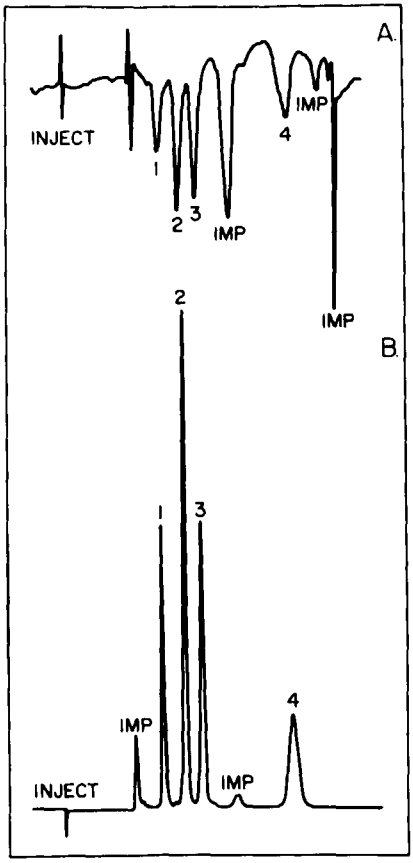

Figure 1: +1.15v Amperometric (A) and 200nm spectrophotometric (B) detection of some α-ketocarboxylic acids: 1) α-ketoglutaric acid (5.6 min, 0. Numole/ml), 2) α-ketobutyric acid (6.9 min, 0.73 umole/ml), 3) α-ketovaleric acid (8.0 min, 0.98 umole/ml), and 4) β-phenylpyruvic acid (13.6 min, 0.67 umole/ml). Separation was effected on a cation exchange column (HPX-87) with 4.5 mN H_2SO_4 as the mobile phase at a 0.8 ml/min flow rate. Amperometric recorder set at 100mV for full scale deflection while the UV recorder was set at 10mV for full scale deflection.

(100 mV full scale) than the attenuation for the spectrophotometric recorder (10 mV full scale). Spectrophotometric and amperometric detection is acceptable when 2 ug/ml concentrations of each α-keto-carboxylic acid is analyzed.

DISCUSSION

HPLC with amperometric detection has been employed in the analyses of phenolic compounds[9], enols such as ascorbic acid[9], oxalic acid[10] and halogenated anilines[11]. These functional groups are characterized by their ease of oxidation when treated with mild oxidizing reagents. Under essentially the same chemical reaction conditions, α-ketocarboxylic acids undergo an oxidative decarboxylation affording carbon dioxide and a carboxylic acid[12]. Thus, it is not surprising that α-ketocarboxylic acids can be amperometrically detected. The +1.15V vs an Ag/AgCl reference electrode that is required to detect the α-ketocarboxylic acids is close to the upper voltage limit of the glassy carbon electrode under these chromatographic conditions. This will affect the sensitivity of the detector. Even so the sensitivity of the amperometric detector toward the α-ketocarboxylic acids studied is such that ng/ml concentrations should be detected.

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