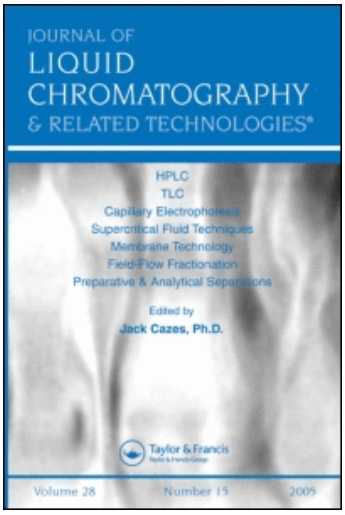

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DETERMINATION OF 2,6-PYRIDINEDICARBOXYLIC ACID IN PEACH JUICE

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ABSTRACT

The quantitation of 2,6-pyridinedicarboxylic acids (2,6-PDA) in fruit juice can be achieved rapidly utilizing High Pressure Liquid Chromatography (HPLC).

The best chromatographic performance was obtained with an aqueous mobile phase containing 15 mM tetrabutylammonium phosphate and 2 mM EDTA; the pH was maintained at 7.2 by 10 mM phosphate buffer.

The influence of the eluent parameters on retention of PDAs has been investigated in order to elucidate the separation mechanisms involved in the ion pair chromatography of these ionizable substances.

INTRODUCTION

In the last years progresses in biochemical and biomedical field have caused a new evaluation about the toxicological effect of the common substances used in industrial preparation, so that a redefinition of the threshold limit below which those substances may be used without risk for the human health is necessary.

Among all pyridinedicarboxilyc acids isomers, 2,6-Pyridinedicarboxylic acid (2,6-PDA) is particularly interesting as a food additive and it represents an example of this situation.

It has been used for water microbiological purification[1] and in fat and oil stabilization at a concentration of 0.005% wt.[2] Moreover, since it is a powerful chelating agent, it has been used, together with hydrogen peroxide, to sterilize food plants at a concentration of 1.2 mg/L[3] and to improve ascorbic acid antioxidant power in foodstuff at a concentration of of 0.005% wt:[4] 2,6-PDA is able to inhibit the oxidative catalysis performed by heavy metals on ascorbic acid, since it can form very stable metal complexes.[5-6]

Since its neurotoxicity is well known,[7] it is necessary to develop an analytical procedure to quantitate 2,6-PDA in real food matrixes. The aim of this work is to optimise the sample preparation in order to minimise matrix effects, and to quantitate 2,6-PDA in peach juice by ion interaction chromatography.

EXPERIMENTAL

Apparatus

A 1090 series II Hewlett Packard (Palo Alto, CA, USA) high pressure liquid chromatograph with factory supplied diode array detector and variable volume 25-μL syringe based auto-injector (Rheodyne sample injection valve Model 7010) was used. The analyses were run at ambient temperature under isocratic elution condition. Solution were centrifuged by a ALC centrifuge (model 4225). pH values were obtained by a Hanna pH meter (model HI842).

Chemicals

2,6-PDA was purchased from Aldrich (Milwaukee, WI, USA); EDTA disodium salt, and tetrabutylammonium phosphate were purchased from Aldrich. Potassium dihydrogen phosphate and disodium monohydrogen phosphate were purchased from Merck (Darmstadt, Germany); NaOH and L(+) Ascorbic acid were Baker Analyzed reagents. $Al_2(SO_4)_3 \cdot 12\ H_2O$ RPE-ACS was obtained from Carlo Erba while Fumaric Acid was purchased from Merk. All chemicals were of the best available quality and used without further purification.

Water was produced by a Milli-Q 185 system (Millipore, Bedford, MA, USA). All analytes were dissolved in the mobile phase and filtered through a 0.2 μm pore size nylon filter (Lida, Kenosha, WI, USA).

The phosphate buffer 81.6 mM, pH 7.2, was obtained by mixing 44.2 mL KH_2PO_4 0.5M and 119.0 mL Na_2HPO_4 0.5M in a 1 L volumetric flask and adding water to a final volume of 1 L. This buffer was then diluted, to optimize the chromatographic performance, to a final concentration of 10 mM.

Chromatographic Conditions

A ODS Hypersil column, 10 cm x 2.1 mm I.D., containing 5 μm particles (Hewlett-Packard) was used. The eluent flow-rate was 1.000 mL/min.

The best chromatographic performance was obtained with a 10 mM phosphate buffer, pH 7.2, containing 15 mM tetrabutylammonium phosphate and 2 mM EDTA. The detector was operated at 270 nm.

The hold-up time (t_O) was determined by injecting 25 μL of water and measuring the time from injection to the first deviation from the baseline.

Prior to use, the reversed phase column was equilibrated with the uncontaminated solvent system to be used for one hour since the dynamic coating of the stationary phase with the ion-interaction reagent is a long lasting process. Equilibration was established by obtaining similar results in duplicate runs at a 15 min interval. All solutions were filtered through a 0.45 μm pore size regenerated cellulose filter (Schleicher&Schuell, Dassel, Germany).

The peak asymmetry factor (AF) was quantitatively expressed as the ratio of the peak half widths at 10% of peak height. Injection volumes were 25 μL Sample size was $2.09 \cdot 10^{-4}$ M in the spiked peach juice.

Sample Preparation

Peach juice was selected as test matrix; it was stored at 4°C in the dark. 10 mL of fruit juice were added to 20 mL distilled water in an Erlenmeyer flask containing a magnetic stirring rod. The mixture was kept under stirring for 15 minutes and then it was transferred into the centrifuge tube. The flask was thoroughly washed with two successive portions of 5 mL H_2O and all washings were added to the centrifuge tube. The sample is centrifuged at 3000 r.p.m. to obtain a clear solution.

The clear solution was filtered through a paper filter. The filtrate was transferred into a 75 mL beaker and 1g $Al_2(SO_4)_3 \cdot 12\ H_2O$ was added to it. NaOH 1.0 M was added until pH=5.1 where $Al(OH)_3$ solubility reaches its minimum. Aluminium hydroxide formation allowed the solution to be cleared up.[8-9]

After centrifugation the supernatant and the washing waters were filtered through a 0.45 μm cellulose nitrate filter into a 100 mL volumetric flask. The pH of the obtained solution was corrected to pH 6.5 with NaOH and then it was diluted to volume with the mobile phase. All samples and solutions were stored at 4°C in the dark.

RESULTS AND DISCUSSION

In spite of the high density of the fruit juice and the presence of solid particles, the clean-up procedure gave a clear solution which was suitable for the chromatographic analysis.

The experimental conditions which were previously optimised in this laboratory[10] for the chromatographic separation of PDAs isomers, were modified to take into account the presence in the real matrix of compounds which could interfere in the quantitation of 2,6-PDA.

When a mobile phase 81.6 mM phosphate buffer, pH 7.2, containing tetrabutylammonium phosphate 15 mM was used the chromatographic peak of 2,6-PDA was not well resolved from the main interferents (L(+) Ascorbic acid and Fumaric Acid). Moreover the chromatographic peak shape was very poor.

According to the physical-chemical retention model proposed by Bidlingmeyer,[11-12] the analyte capacity factor is expected to increase regularly with decreasing buffer concentration. This can be explained by the assumption of a stationary phase increasingly blocked by ion-pairs from tetrabutylammonium and buffer ions. High phosphate concentration limits the formation of ion-pairs between analyte and tetrabutylammonium because buffer ions are involved in a competing for adsorbed lipophilic ions, and this results in a lower retention time.

Hence the buffer concentration was drastically reduced down to 10 mM in order to decrease the mobile phase eluent strength and to obtain enhanced retention for 2,6-PDA which could thereby be separated from the early eluting small interfering organic acids. The addition of 2 mM EDTA in the mobile phase was necessary to prevent analyte complexation with metal impurities in the chromatographic system.

Typical chromatograms of extracts obtained from blank peach juice and 2,6-PDA fortified matrix are shown in Figure 1. The comparison of blank (top panel) and spiked (bottom panel) samples indicates that no endogenous compounds exists at the retention time of 2,6-PDA. Its chromatographic peak was identified by comparison with retention time of 2,6-PDA from a standard solution. The peak shape is good and it allows a quantitative analysis to be performed.

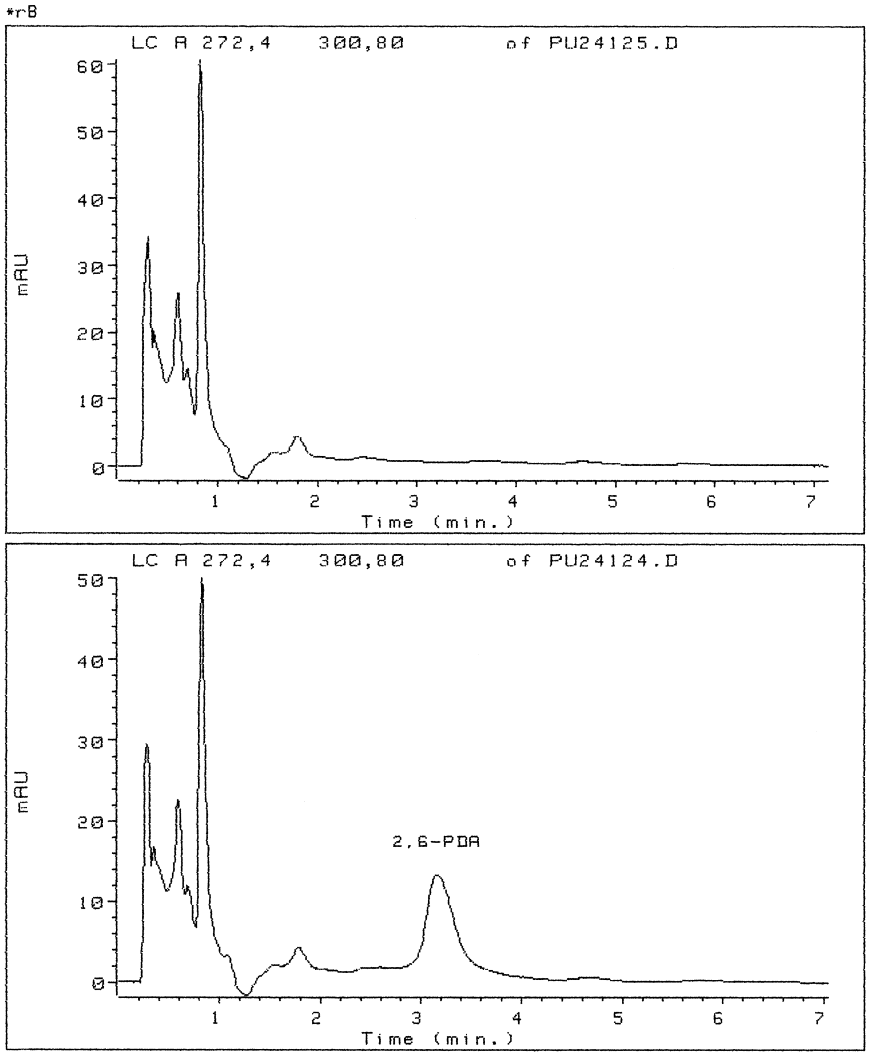

Figure 1. Determination of 2,6-PDA in fruit juice. Conditions: column, ODS Hypersil, 10 cm x 2.1 mm I.D., 5 mm (Hewlett-Packard); flow rate 1.000 ml/min; injections volume: 25 ml; eluent: 10 mM phosphate buffer pH 7.2 containing 15 mM tetrabutylammonium phosphate and 2 mM EDTA. Top panel: blank sample. Bottom panel: 2,6-PDA fortified (2.09·E-04M) sample.

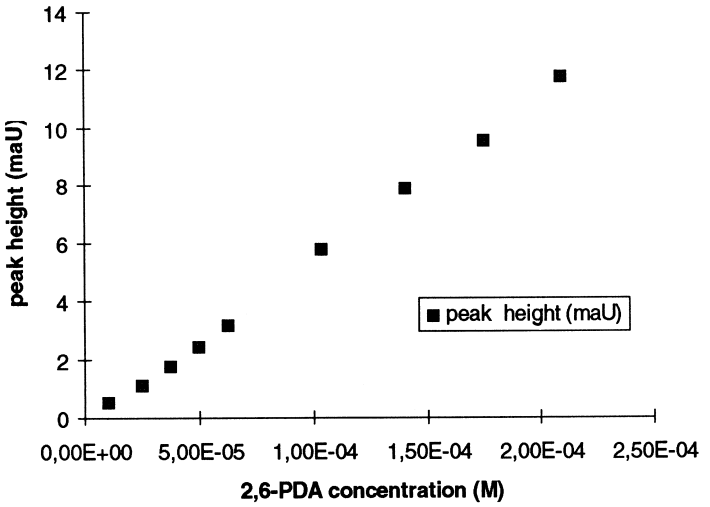

Figure 2. Calibration curve of 2,6-PDA in fruit juice. Conditions: column, ODS Hypersil, 10 cm x 2.1 mm I.D., 5 mm (Hewlett-Packard); flow rate 1.000 ml/min; injections volume: 25 ml; eluent: 10 mM phosphate buffer pH 7.2 containing 15 mM tetrabutylammonium phosphate and 2 mM EDTA. Sample size: see table.

The standard addition calibration curve was obtained by adding successive increasing amounts of 2,6-PDA to the cleared up matrix. The concentration ranged from $1.00 \cdot 10^{-5}$ M up to $2.09 \cdot 10^{-4}$ M. As it is clear from Figure 2 a very good linearity was obtained, the calibration curve ($y = 57218.6 \cdot x - 0.268355$) being characterized by a determination coefficient $R^2 = 0.99864$.

The detection limit was then investigated. The lower limit of detection obtained at a S/N ratio of 2 is $1.8 \cdot 10^{-6}$ M. Therefore, this procedure is very sensitive and suitable for routine analysis, since the recommended concentration of DPA (0.005% wt, e. g. in orange juice and tomato juice)[4] corresponds ca. (is the density is assumed to be 1 mg/mL) $299 \cdot 10^{-6}$ M.

In the analyzed samples 2,6-PDA was not present at a concentration higher than the detection limit.

The recovery of 2,4-PDA was investigated by comparing the concentration found in samples spiked with 2,6-PDA $5 \cdot 10^{-5}$ M and processed according to the described standard operating procedure, to the concentration in the standard solution. Duplicate analyses were performed and average recovery for 2,6-PDA fortified peach juice has been 95%.

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