Journal of Chromatography, 455 (1988) 355–359
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 20 887

Note

Development of an high-performance liquid chromatographic method for the simultaneous analysis of the species involved in diethyl L-tartrate hydrolysis

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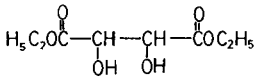
(First received July 13th, 1988; revised manuscript received August 2nd, 1988)

The hydrolysis kinetics of diesters generally follow a two step reaction that involves rate constants, k_1 and k_2, as shown by the following equations.

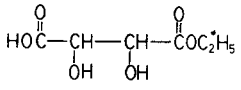
$$\mathrm{Di} + \mathrm{R} \xrightarrow{k_1} \mathrm{Mo} + \mathrm{P} \quad (1)$$

$$\mathrm{Mo} + \mathrm{R} \xrightarrow{k_2} \mathrm{Ac} + \mathrm{P} \quad (2)$$

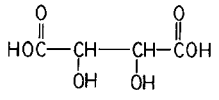
Here, Di, Mo and Ac are starting material, intermediate and final products, respectively. The common co-reactant is R and the common co-product is P. In order to simplify the analysis of data, pseudo-first order conditions, are generally used by maintaining the concentration of the co-reactant, R, at a constant level throughout the course of the reaction. The rate constants can be calculated from the concentrations of



Diethyl Tartrate



Monoethyl Tartrate



Tartaric Acid

Fig. 1. Different species involved in the diester hydrolysis.

0021-9673/88/$03.50

Di, Mo and Ac as a function of time[1]. Under pseudo-first order conditions, diethyl tartrate hydrolysis involves three major species, starting material (diethyl tartrate), intermediate species (monoethyl tartrate), and the end product (tartaric acid). See Fig. 1.

A reversed-phase high-performance liquid chromatographic (HPLC) assay in which the mobile phase is aqueous, would be best suited to analyze these species. This paper describes an HPLC assay that can analyze all the species simultaneously with the added advantage that no pretreatment of samples is required. This can be important as pretreatment could change the kinetics by altering the amounts of one or more of the three species during the pretreatment process.

EXPERIMENTAL

Materials

The following chemicals were used: diethyl L-tartrate and L-tartaric acid (Aldrich, Milwaukee, WI, U.S.A.) and sodium hydroxide (Primary Standard, J. T. Baker, Phillipsburg, NJ, U.S.A.). Monoethyl tartrate was synthesized in the laboratory (see next section). Both methanol (J. T. Baker) and water were distilled and filtered through a 0.2-nm membrane (Millipore, Bedford, MA, U.S.A.). Tetrabutylammonium phosphate (PIC A, Waters Assoc., Milford, MA, U.S.A.) was used as received.

Sample preparation

Stock solutions of the diester, monoester and tartaric acid were prepared by using 243.53 mg of diester, 111.56 mg of monoester and 140.00 mg of tartaric acid. The volume of each solution was adjusted to 100 ml. The samples for HPLC analysis were prepared by mixing 10 μl of each of the stock solutions of diester, monoester and tartaric acid and; diluting the mixture to 10 ml with water. Other samples were prepared similarly by using 20, 30, 40, 50, 100 and 200 μl of each of the stock solutions and diluting the mixture to 10 ml.

Chromatographic equipment and conditions

The HPLC apparatus (Waters Assoc.) was equipped with the following: HPLC pump 6000A, solvent programmer, U6K injector, 730 data module (to record the chromatogram and to calculate the area under the peak), C_{18} column (μBondapak, 10 μm, 30 cm $\times$ 3.9 mm I.D.). In addition an LC-55 variable-wavelength UV detector (Perkin-Elmer, Instrument Division, Norwalk, CT, U.S.A.) was used to monitor the eluent.

The mobile phase consisted of methanol–water (20:80, v/v) with 0.005 *M* PIC A and the pH adjusted to 3.2. Other conditions were: flow-rate, 1.5 ml/min; wavelength, 210 nm; and sample volume, 10 μl.

Preparation of monoester

The following procedure was developed for the preparation of monoester (sodium monoethyl L-tartrate). An amount of 1 g (0.0025 equiv.) of sodium hydroxide pellets was added to 50 ml of chloroform in an erlenmeyer flask. The mixture was placed in a water bath adjusted to 45°C. Over a period of 5 min 5.15 g (0.0025 equiv.) of

diethyl tartrate was slowly added while the mixture was vigorously stirred. A white precipitate began to form immediately. The mixture was stirred for another 30 min and then filtered through Whatmann filter paper. The precipitates were washed at least five times with 20-ml portions of chloroform (dried overnight with anhydrous calcium chloride). The precipitates were air dried overnight and then tested for purity. The product was identified using HPLC assay and confirmed by mass spectroscopy (MS).

RESULTS AND DISCUSSION

During the optimization of HPLC eluents it was found that the retention times of monoethyl tartrate and tartaric acid were extremely sensitive to pH. As shown in Fig. 2, the monoethyl tartrate and tartaric acid peaks were fused at pH 7.0. The order of elution reversed and retention times were slightly reduced when the pH was changed to 4.2. As the pH of the eluent was further decreased to 3.5, the two peaks showed a better resolution. The resolution was further improved when the pH was changed to 1.5 but the first peak eluted with the solvent front. This suggested that an intermediate pH between 3.5 and 1.5 would yield a better separation. In fact, pH 3.2 yielded the optimum separation and was used for all analysis. A typical chromatogram is shown in Fig. 3.

The first and third peaks were confirmed to be tartaric acid and diester, respectively, by using pure samples. Since, monoethyl tartrate was not available commercially and methods in the literature[2] failed to give a pure compound, a synthetic approach was developed to obtain monoethyl tartrate. The method resulted in relatively pure monoethyl tartrate in very high yields (95%). The

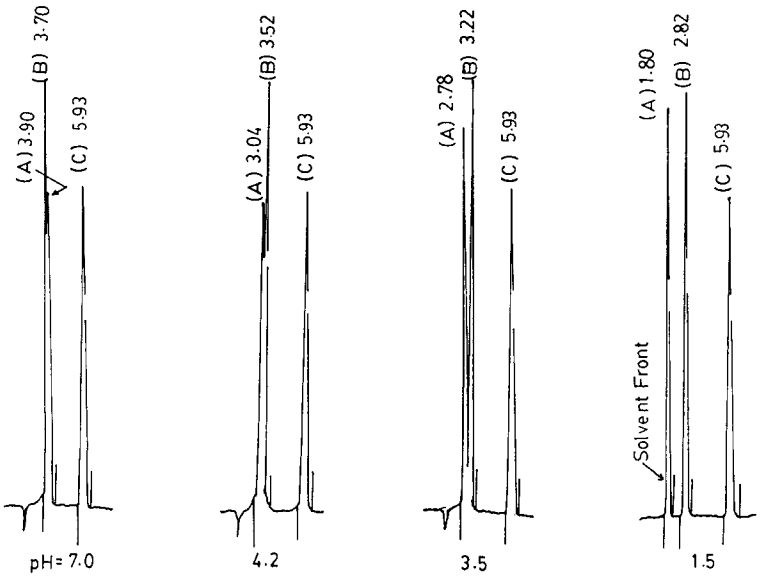
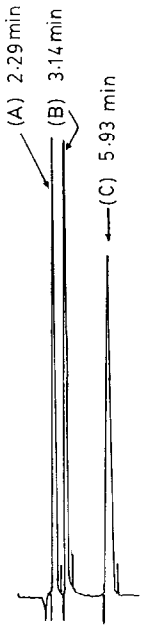


Fig. 2. Effect of the eluent pH on the retention time of diester (C), monoester (B) and tartaric acid (A) using ion-pair chromatography.



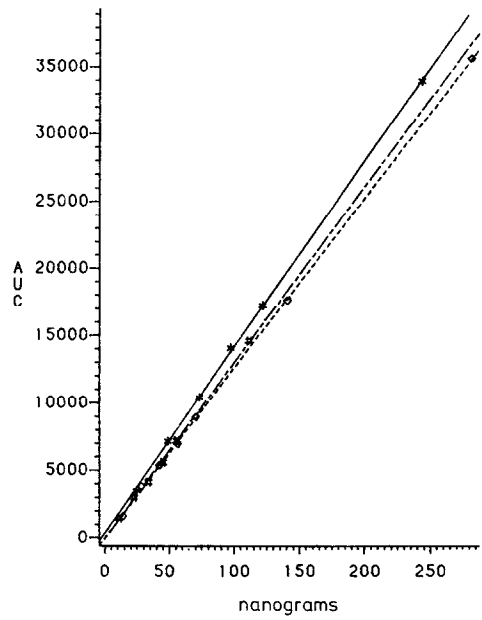


Fig. 3. Separation of diester (C), monoester (B) and tartaric acid (A) using ion-pair chromatography, eluent pH adjusted to 3.2.

Fig. 4. Calibration plot of area under the curve, AUC, *versus* amount injected for all three species. (*) Tartaric acid; (◇) diester; (#) monoester.

synthesized monoester was analyzed by the HPLC procedure described above. The chromatograms of this sample showed only one peak which corresponded to the peak located between the diester and tartaric acid peaks. The eluent corresponding to this peak was collected and analyzed and confirmed by MS.

Fig. 4 shows that a linear relationship was obtained for all three species. The calibration coefficients of the three species were calculated and found to be: diester, 126.4 area units/ng; monoester, 130.7 area units/ng; and tartaric acid, 138.3 area units/ng.

Application to hydrolysis kinetics

The technique was used to follow the hydrolysis kinetics of the diester compound at pH 7.0 and 85°C. Using this assay, the concentrations of all three species as a function of time were determined and the plots of the results are shown in Fig. 5. The figure shows a typical diester to monoester to carboxylic acid plot and indicates that the HPLC assay reported in this communication can differentiate all three species as a function of time and is suitable for kinetic hydrolysis studies.

This assay was used for an extensive investigation of diethyl tartrate hydrolysis kinetics under various conditions. The results of these studies are reported in a subsequent paper[3]. Briefly, the results indicated that only 0.003% diethyl tartrate and 0.0013% monoethyl tartrate will hydrolyze during the chromatographic procedure

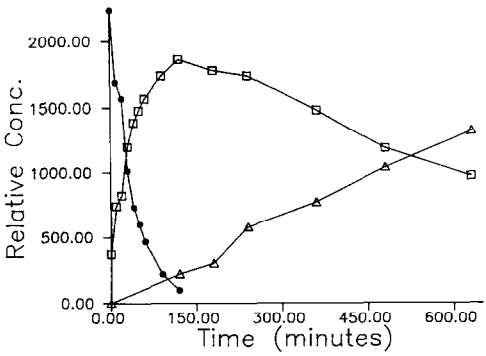


Fig. 5. Experimental kinetic data obtained for diester hydrolysis at pH = 7.0 and temperature = 85°C, (●) Tartaric acid; (□) monoester; (△) diester.

which required 10 min or less. Therefore, for practical purposes, all species were found to be stable during the chromatographic analysis. Further tests also indicated that there were no racemic changes occurring during hydrolysis or analysis. This was expected, since the hydrolysis studies were carried out under acidic and neutral conditions.

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