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Note

Reversed-phase high-performance liquid chromatographic analysis of chloropropanoic acids on a polymer-based column

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The high-performance liquid chromatography (HPLC) of organic acids has been well studied[1]. The easiest way to perform an HPLC analysis of acidic substances is to carry out a reversed-phase separation in the ion suppression mode; a mobile phase with a pH lower than the pK_a of the strongest acid to be analysed is used, which allows an interaction between the undissociated acids and the hydrophobic stationary phase. The technique is limited by the fact that silica gel-based columns lack stability at pH values lower than 2.5.

The availability today of polymer-based columns has lowered this limit to pH 1, thus increasing the range of applications of this technique to moderately strong acids. Using this type of column, an HPLC method has been developed that allows the separation and quantitation of propanoic, 2-chloropropanoic, 3-chloropropanoic, 2,2-dichloropropanoic and 2,3-dichloropropanoic acids. The method has been validated and is suitable for checking the quality of 2-chloropropanoic acid and, after hydrolysis, of 2-chloropropionyl chloride.

EXPERIMENTAL

Reagents

The acids and acyl chlorides used were of the best quality available (E. Merck, Darmstadt, F.R.G., and Fluka, Buchs, Switzerland) and were used without further purification. Chromatographic-grade acetonitrile was obtained from Merck. The water used to prepare the eluents and sample solutions was deionized and passed through a Milli-Q water purification system (Millipore, Bedford, MA, U.S.A.).

HPLC conditions

The HPLC analyses were performed using a Series 4 chromatograph, an LC-75 Autocontrol variable-wavelength UV–VIS detector, an LCI-100 digital integrator and a Model 056 recorder (Perkin-Elmer, Norwalk, CT, U.S.A.). The sample introduction system was a Rheodyne Model 7125S HPLC valve with a 6-μl fixed loop.

The conditions applied for the analysis were as follows: column, Hamilton PRP-1, 10 μm (15 × 0.4 cm I.D.); mobile phase, acetonitrile–water (12:88) (pH 2.0) containing 10.5 ml phosphoric acid (10%, w/v); flow-rate, 1.0 ml/min; wavelength, 210 nm; and temperature, ambient.

Sample preparation

2-Chloropropanoic acid. About 150 mg of the sample, accurately weighed in a 20-ml volumetric flask, are dissolved and diluted to volume with 10% aqueous acetonitrile.

2-Chloropropionyl chloride. About 180 mg of the sample, accurately weighed in a 20-ml volumetric flask, are diluted with 2 ml of acetonitrile; 10 ml of water are slowly added and the solution is set aside for 30 min to ensure complete hydrolysis of the chloride, then the solution is diluted to volume with water.

RESULTS AND DISCUSSION

The analysis of 2-chloropropanoic acid has been performed by gas–liquid chromatography (GLC)[2,3] and by ion chromatography[4]. However, these methods are problematic as GLC requires prior derivatization and ion chromatography requires specialized equipment and columns.

Using the polymer-based column described above, a simple reversed-phase HPLC method able to separate propanoic, 2-chloropropanoic, 3-chloropropanoic, 2,2-dichloropropanoic and 2,3-dichloropropanoic acids was set up. Fig. 1 shows a typical separation obtained with this method.

To check the effect on the separation of the pH of the mobile phase, the capacity factor (k') of each acid in the pH range 1.6–2.8 was determined (Fig. 2). Whereas the effects on propanoic and chloropropanoic acids are negligible, dichloropropanoic acids are strongly affected by changes in pH, and the behaviour of 2,2-dichloropropanoic acid limits the range of pH useful for the separation to 1.9–2.1.

A possible explanation of this effect could be that the pK_a values of the dichloropropanoic acids are similar to the pH of the mobile phase so small changes in the acidity of the eluent have a great effect on the acid–base equilibrium and hence on their k' values. Table I shows the pK_a values of each acid in water and in 12% aqueous acetonitrile determined by the method of Castillo *et al.*[5]. Where possible, the experimental values are compared with literature data. As shown, the pK_a values of 2,2-dichloropropanoic and 2,3-dichloropropanoic acids, in a solvent similar to the mobile phase, lie in the pH range studied for the HPLC separation.

System suitability tests

The following tests were performed to evaluate the performance of the method.

Resolution factors. These were determined for each pair of adjacent peaks and the values found were as follows: propanoic–3-chloropropanoic acid, 6.3; 3-chloropropanoic–2-chloropropanoic acid, 2.4; 2-chloropropanoic–2,2-dichloropropanoic acid, 3.0; and 2,2-dichloropropanoic–2,3-dichloropropanoic acid, 2.7. All the resolution factors are higher than 1.5, a value commmonly used to define the baseline separation of chromatographic peaks. Hence the method can be considered adequate for quantitative purposes.

Linearity of response for 2-chloropropanoic acid. The linearity of the response was evaluated by injection of different amounts of the acid between 20 and 160 mg, corresponding to 6–48 μg injected. The correlation coefficient of the linear regression was 0.9994 and the intercept (area value for zero weight) was zero at the 90% confidence level.

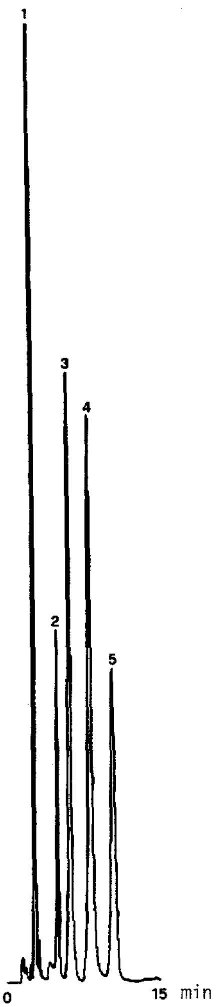

Fig. 1. Typical separation obtained with the method. Acids: 1 = propanoic; 2 = 3-chloropropanoic; 3 = 2-chloropropanoic; 4 = 2,2-dichloropropanoic; 5 = 2,3-dichloropropanoic. For HPLC conditions, see text.

Reproducibility of the assay of 2-chloropropionyl chloride. The reproducibility of the method was tested by analysing separately ten samples of the same batch. The overall standard deviation was 0.40%.

Linearity of response of other acids. The range of amounts in this test was chosen by considering these acids as impurities. The amounts ranged between 0.5 and 4 mg corresponding to 0.3 and 2.5%, respectively, referred to the amount of sample chosen for the method. In this range all the acids showed good linearity (correlation coefficient 0.999) and the minimum concentration that could be determined with good precision was 0.25%.

On the basis of these results, we conclude that this method is sufficiently sen-

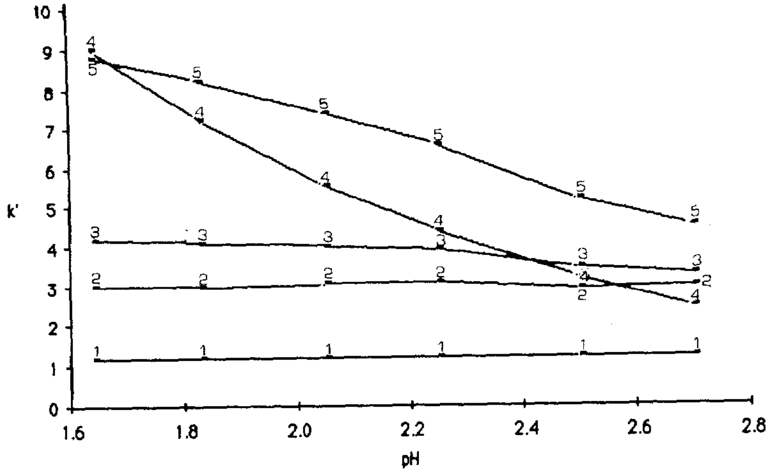

Fig. 2. Plot of k' *vs.* pH of the mobile phase. For identification of substances, see Fig. 1.

TABLE I

pK_a VALUES OF THE ACIDS STUDIED

Acid	*Experimental values*		*Literature value*[6]
	Water	*12% aq. acetonitrile*	
Propanoic	4.79	5.00	4.87
2-Chloropropanoic	2.74	3.12	2.83
3-Chloropropanoic	4.00	4.21	3.98
2,2-Dichloropropanoic	1.82	1.95	–
2,3-Dichloropropanoic	2.07	2.47	–

sitive and reliable and can be used to check the quality of 2-chloropropionyl chloride and 2-chloropropanoic acid.

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