ELSEVIER

Journal of Chromatography A, 868 (2000) 41–50

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Determination of aliphatic anhydrides and acids by reversed-phase liquid chromatography

G. William Tindall*, R.L. Perry, A.T. Spaugh

Eastman Chemical Company, P.O. Box 1972, Kingsport, TN 37662, USA

Received 7 June 1999; received in revised form 29 September 1999; accepted 14 October 1999

Abstract

Few chromatography methods have been reported for the determination of anhydrides in mixtures or as mixed anhydrides. The potential reactivity of anhydrides with water and other common eluent components complicates possible schemes for separation and analysis. By optimizing variables that affect hydrolysis, including the stationary phase, conditions can be found to successfully analyze anhydrides as reactive as acetic anhydride. The corresponding acids can be determined at the same time. The effect of the stationary phase on anhydride hydrolysis rates may prove to be a sensitive means of probing stationary phase chemistry. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Aliphatic anhydrides; Aliphatic acids

1. Introduction

Organic anhydrides are used in a variety of high volume industrial processes, for example the manufacture of cellulose esters, pharmaceuticals and plasticizers [1]. In spite of the importance of organic anhydrides, few chromatography methods have been reported for their determination in mixtures or as mixed anhydrides. The reactivity of anhydrides with water, alcohols, organic acids and other anhydrides certainly complicates possible schemes for separation and analysis because most separation possibilities involve both conditions and time for these undesired anhydride reactions to occur. Liu and Lee [2] studied the rates of anhydride hydrolysis under reversed-phase separation conditions. One of the goals of their work was to understand how susceptible various anhydrides are to hydrolysis so that the feasibility of determining them by reversed-phase LC could be assessed. They found that pH and amount of water in the mobile phase, as well as structure of the anhydride, all affect the rate of hydrolysis and, therefore, the feasibility of the separation and determination. For some unsaturated anhydrides, conditions were found where there was insignificant hydrolysis of anhydride during the separation.

Domb [3] reported a reversed-phase separation of aliphatic and aromatic anhydrides, and concluded that anhydride molecules dissolved in the mobile phase do not undergo hydrolysis during the course of analysis. This conclusion was based on a study of hexanoic, benzoic and lauric anhydrides in solutions of 50% or less water. It will be shown that this conclusion cannot be generally extended to lower molecular weight aliphatic anhydrides, unsaturated anhydrides as previously reported [2], nor eluents that are essentially aqueous. Furthermore, Domb's

*Corresponding author. Fax: +1-423-229-4558.
E-mail address: tindall@eastman.com (G.W. Tindall)

0021-9673/00/$ – see front matter
PII: S0021-9673(99)01101-2

conditions result in poor peak shape for aliphatic acids. In many practical applications, it is desirable to know the amounts of corresponding acids in anhydride samples so the ideal separation will be suitable for both classes of compounds. To obtain good peak shapes for aliphatic acids, acidic eluents must be used to suppress ionization of these acids.

To achieve adequate retention of some aliphatic acids, nearly 100% aqueous eluents are essential [4]. Liu and Lee have demonstrated that these conditions are the opposite of what would be desired to lessen the hydrolytic losses of anhydrides. However, we found with an optimization of all the variables that affect anhydride hydrolysis, as well as aliphatic acid peak shape and retention, a successful separation and analysis of even acetic acid and acetic anhydride can be achieved.

The hydrolysis of acetic anhydride has been thoroughly studied in water and partially aqueous solutions. The following conclusions of these investigations provide guidance for minimizing hydrolysis during a reversed-phase separation. First, the hydrolysis of acetic anhydride is not autocatalytic in water [5]. In fact, even strong acids are ''feeble'' catalysts in a largely aqueous environment, however, bases are strong catalysts [6]. Hydrolysis is particularly sensitive to temperature. The rate for acetic anhydride at 0°C is about one tenth that at 25°C [5]. And finally, the rate slows considerably as water is diluted with an unreactive solvent [7]. From this information we predict that hydrolysis will be minimized with a fast separation (high flow-rate) and with as high an organic content and low temperature as practical. The challenge is to take advantage of these factors while maintaining an acceptable analysis of the corresponding acids.

In previously reported work [2,3], the importance of the stationary phase was not investigated. It will be shown that the stationary phase can have a major effect on anhydride hydrolysis during separation, in addition to the variables mentioned above.

2. Experimental

2.1. Materials and instrumentation

A summary of the columns used in this investigation is given in Table 1. Only new columns were used in this investigation. All columns were 4.6×150 mm and nominally 5 μm. Before use the columns were flushed with 50/50 acetonitrile–water for 1 h, or more, while monitoring the eluent absorbance at 205 nm. Flushing was judged complete when the absorbance of the effluent reached the expected value for this eluent, nearly zero. Columns were then conditioned with a 0 to 100% acetonitrile gradient followed by several column volumes at the mobile phase conditions of the experiment. A Waters 510 pump and Model 2487 detector (Waters Corporation, Milford, Connecticut, USA) and Perkin-Elmer Turbochrom data system (Norwalk, Connecticut) were used. Acids and anhydrides were obtained internally. Acetonitrile was obtained from Burdick and Jackson,

Table 1
Columns and their physical characteristics

Column	Manufacturer	Bonded phase	Pore size Å	% Carbon	Surface area, m^2/g	Acetic acid t_r, min	Acetic anhydride t_r, min
PRP-1	Hamilton	None	100	–	–	1.23	5.35
SI-100	ES Industries	None	100	0	300	1.58	1.70
Aquasil	Keystone	Modified C18	100	12	300	1.67	3.72
Zorbax SB-C18	Mac-Mod	C18	90	10	190	1.31	3.17
ACE C18	Mac-Mod	C18	100	15.5	300	1.47	3.34
Hypersil BDS	Keystone	C18	120	12	170	1.41	2.97
ODS-AQ	YMC	Modified C18	120	15	300	1.62	3.04
Inertsil	Keystone	C18	150	18.5	320	1.51	3.58
Spherisorb	Waters	C18	80	12	220	1.41	3.24
Symmetry	Waters	C8	100	11.7	335	1.49	2.87
Aquasep	ES Industries	Modified C8	100	16	450	1.91	4.58
Prism RPN	Keystone	Modified C18	100	11	300	1.54	2.52

Muskegon, IL. Water was obtained from a Milli-Q water system, Millipore, Bedford, MA.

2.2. Procedures

Investigative separations were done at a flow-rate of 1.5 ml/min and 23°C. For quantitative analysis, a flow-rate of 2 ml/min is recommended. The mobile phase was a mixture of acetonitrile and 0.02 wt.% phosphoric acid in water. Most investigative separations were done in 90% aqueous and 10% acetonitrile eluent unless otherwise noted. For practical analysis, this composition can be optimized for the analytes of interest. A sample size of 5 μl was used. Samples were prepared in acetonitrile.

Bulk hydrolysis rate investigations under mobile phase conditions were done in two ways. An amount of acetic anhydride, chosen to give an appropriate sized signal, was dissolved in 1 ml of acetonitrile. This solution was added with vigorous stirring to 9 ml of water or 0.02% phosphoric acid. The disappearance of anhydride was followed spectrophotometrically at 240 nm in a cuvet. Alternatively, samples were taken as a function of time and analyzed by the LC method described above. Either approach yielded equivalent results.

The hydrolysis rate during the separation was determined two ways. In the first method the separation was done at 23 and 0°C. It was assumed (see below) that there was negligible hydrolysis at 0°C. The difference between anhydride peak areas is the amount hydrolyzed. This amount divided by retention time yields an average rate of hydrolysis for the separation. This value is used for comparing columns. This value is not to be confused with a true rate constant. Alternatively, the separation can be done at various flow-rates. The slope of a plot of normalized peak area vs. retention time provides a measure of hydrolysis rate. The measured peak area must be multiplied by flow-rate to get a normalized area that corrects for flow-rate dependence of detector response. Either approach yielded equivalent results for comparing columns.

3. Results and discussion

In a reversed-phase separation of anhydrides, there is justifiable concern that losses of these reactive molecules could occur during the separation and lead to inaccurate results. We found that hydrolysis is the primary cause of anhydride loss during separation and losses by acylation of the stationary phase are undetectable. Conditions must be found to minimize anhydride hydrolysis during the separation while at the same time maintaining adequate retention and peak shape for aliphatic acids. The conditions described in the Experimental section have been optimized to minimize these potential problems for aliphatic anhydrides and their corresponding acids, including acetic anhydride.

Fig. 1 shows the separation of some aliphatic anhydrides of commercial importance. Note that the peak shape for the acids is acceptable and even acetic acid is adequately retained. The Aquasil column, with 0.02% phosphoric acid/acetonitrile mobile phase, has been used in our laboratory for the analysis of 2–12 carbon anhydrides and their corresponding acids. The amount of phosphoric acid modifier required to achieve acceptable aliphatic acid peak shape is somewhat dependent on sample concentration and 0.02% is the lowest amount recommended. Up to 0.2% in the mobile phase will not adversely affect the anhydride analysis (see below). Acetic anhydride is stable in acetonitrile for at least 1 week at room temperature. Acetonitrile dissolves most anhydrides so it is an ideal solvent to use in sample preparation. The absorbance spectra for acetic acid and acetic anhydride is shown in Fig. 2. These curves were recorded within seconds of preparation to minimize the effects of hydrolysis. A wavelength of 210 nm was found suitable for determining both compounds. At 210 nm the molar absorbtivities of acetic acid and acetic anhydride are 31 and 75 l/mole cm, respectively. While these molar absorbtivities are very small, under the conditions described acetic anhydride concentrations as little as 10 ppm can be determined and the calibration curve is linear for at least three orders of magnitude. With a 25 μl sample size we have achieved detection limits of less than 1 ppm for these compounds. For samples where the anhydride concentration is a few percent or greater, the analysis can be cross validated with NMR. Agreement has typically been within the experimental error of the two methods.

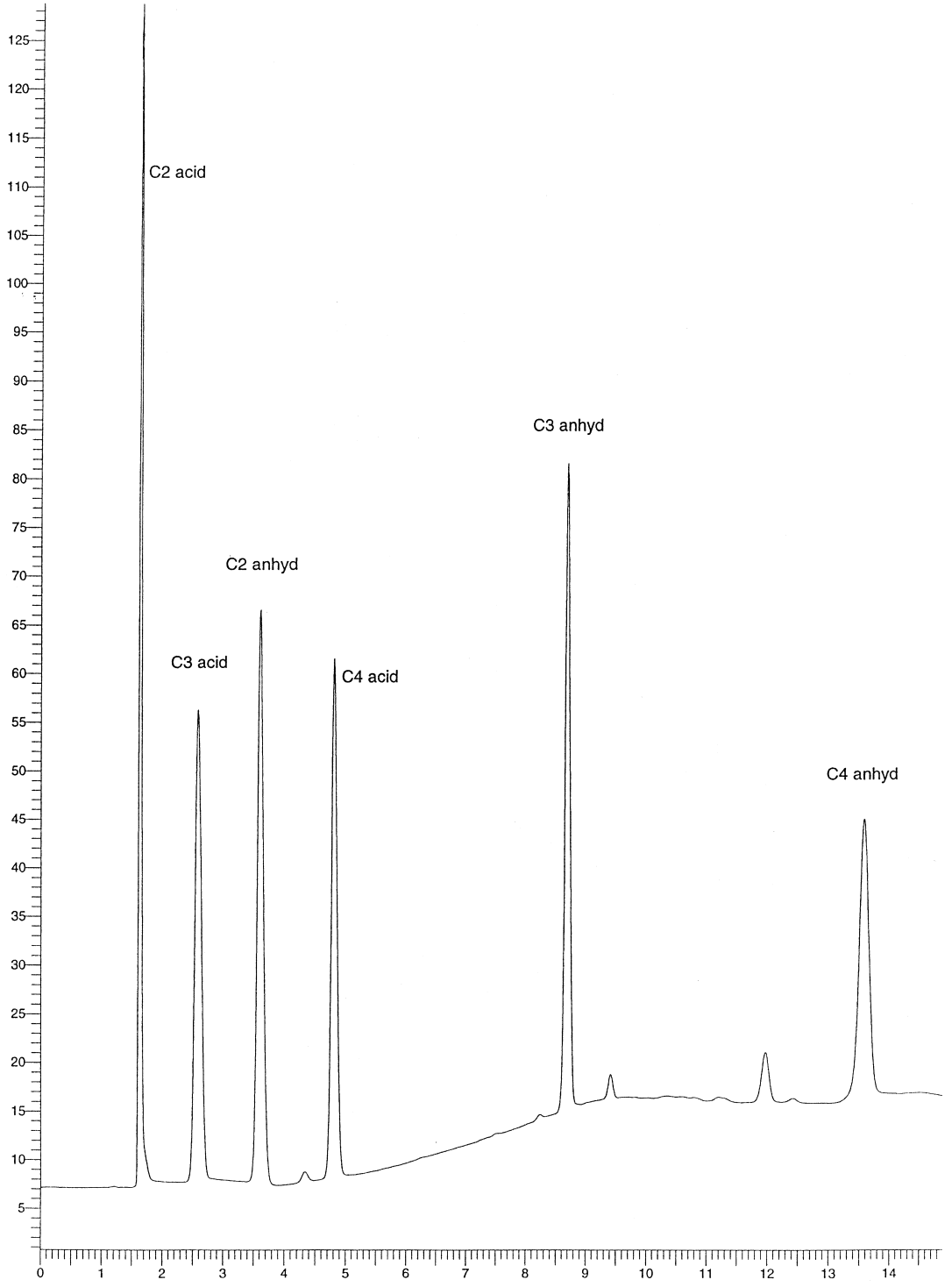

Fig. 1. Separation of aliphatic acids and anhydrides mobile phase: A=90% water with 0.02 wt.% phosphoric acid, B=acetonitrile. Gradient: 10% B for 1 min, 10 to 45% B in 7 min and hold, 1.5 ml/min. Sample concentration 0.25% in acetonitrile, 5 μl samples. Detection at 210 nm.

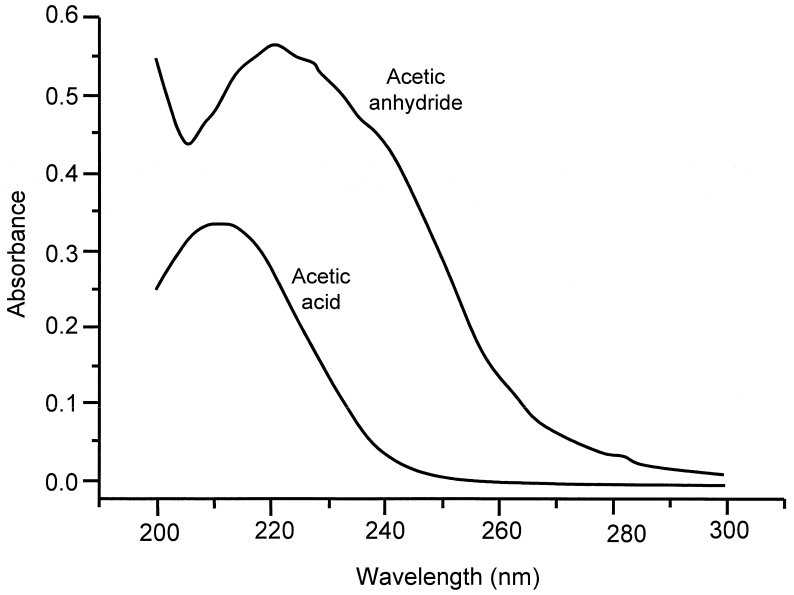

Fig. 2. Absorbance spectra of acetic acid and acetic anhydride solvent: 90% water with 0.02 wt.% phosphoric acid and 10% acetonitrile.

Results from the investigation of factors that affect the separation are discussed in the following sections. Most of the work exploring the feasibility of simultaneously determining anhydrides and acids by reversed-phase LC was done with acetic anhydride and acetic acid because they are most challenging. Of the aliphatic anhydrides, acetic anhydride has the greatest rate constant for hydrolysis in aqueous media [8] and acetic acid is the most difficult aliphatic acid to retain with good peak shape. Any successful analysis for acetic anhydride and acetic acid should be generally applicable to higher molecular weight aliphatic anhydrides and acids, as well as many other anhydrides of commercial importance.

3.1. Column evaluation

While bulk hydrolysis rate experiments can provide information on hydrolysis rates in the mobile phase [2], they do not provide information on reactions in the stationary phase. Losses in the stationary phase could be due to hydrolysis or acylation of the stationary phase. We found that hydrolysis is the main factor affecting anhydride losses in the stationary phase, and this hydrolysis is a very important factor in the analysis of anhydrides by liquid chromatography.

Several columns that we have found to be effective for the separation of low molecular weight, polar molecules in aqueous mobile phases, as well as some columns reported to be made with exceptionally inert silica, were evaluated for anhydride separations. The ideal column should provide adequate retention for aliphatic acids, adequate selectivity for acids and anhydrides and insignificant hydrolysis of anhydrides. We found the most significant difference among columns was their effect on anhydride hydrolysis. The amount of hydrolysis was determined as follows. The same amount of acetic anhydride was injected on each column and the anhydride peak area recorded. To determine the fraction hydrolyzed it was necessary to know the peak area for acetic anhydride under conditions where there was no hydrolysis. This value was determined from a separation done on an Aquasil column at 0°C. At this temperature the amount of acetic anhydride hydrolyzed should be insignificant [5]. By comparing the observed area at 23°C to that found at 0°C the fraction lost can be calculated. The results are summarized in Fig. 3 for the acidic and neutral eluents described. Based on these data the Aquasil

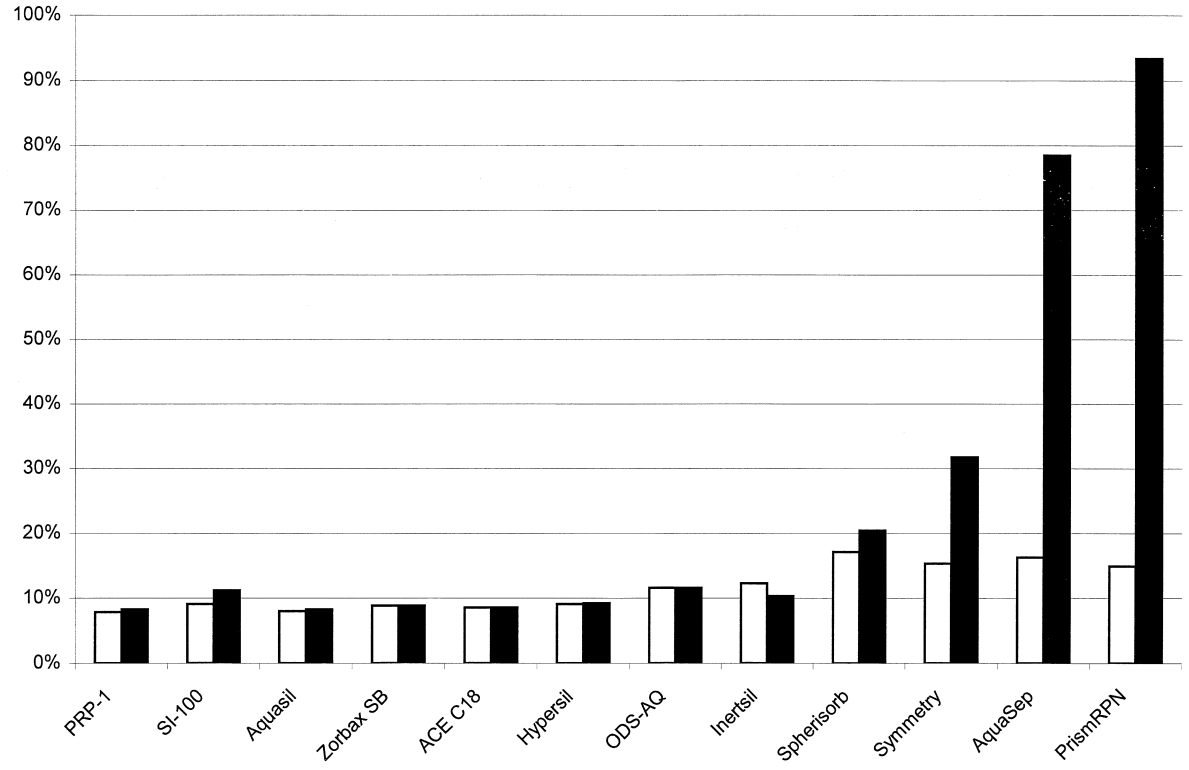

Fig. 3. Percent acetic anhydride hydrolyzed during separation. Separation conditions: 90% water with 0.02 wt.% of phosphoric acid and 10% acetonitrile (clear bars) or 90/10 water acetonitrile (shaded bars), 1.5 ml/min flow-rate, 23°C.

column is recommended for aliphatic anhydride and acid analysis. Anhydride losses are among the lowest on this column, it has superior efficiency to the PRP-1 column and it offers superior retention and selectivity for aliphatic acids.

The results in Fig. 3 show that even on the Aquasil column some acetic anhydride is hydrolyzed during the separation at 23°C. However, this small loss is not necessarily of any significance to the accuracy or reliability of the analysis for acetic anhydride or other anhydrides with similar or smaller rate constants for hydrolysis, because the fraction hydrolyzed is constant for samples and standards and the loss is compensated in the calibration. Anhydride hydrolysis under the conditions of the separation is first order in anhydride so the fraction hydrolyzed is proportional to concentration. This hypothesis is confirmed by the linear calibration curve for acetic anhydride. Therefore, as long as the mobile and stationary phase hydrolysis rate constants remain the same for calibration and analysis, the small loss from hydrolysis is irrelevant. It follows that temperature and mobile phase composition must be the same for calibration and analysis. A possible concern is that some sample component might affect the rate constant. This possibility is unlikely in the mobile phase because acetic anhydride will be rapidly separated from typical sample components during analysis, and the possibility of a sample component with such properties seems remote. Of more practical concern is the long-term accumulation of highly retained components that could affect the stationary phase hydrolysis rate constant. Periodically monitoring the expected peak area of a standard ensures that this potential problem does not affect analyses.

The only problem we have encountered in applying this method to practical samples is the effect the hydrolyzed anhydride has on establishing a proper baseline for integration. Fig. 4 shows an expanded scale chromatogram of an acetic anhydride sepa-

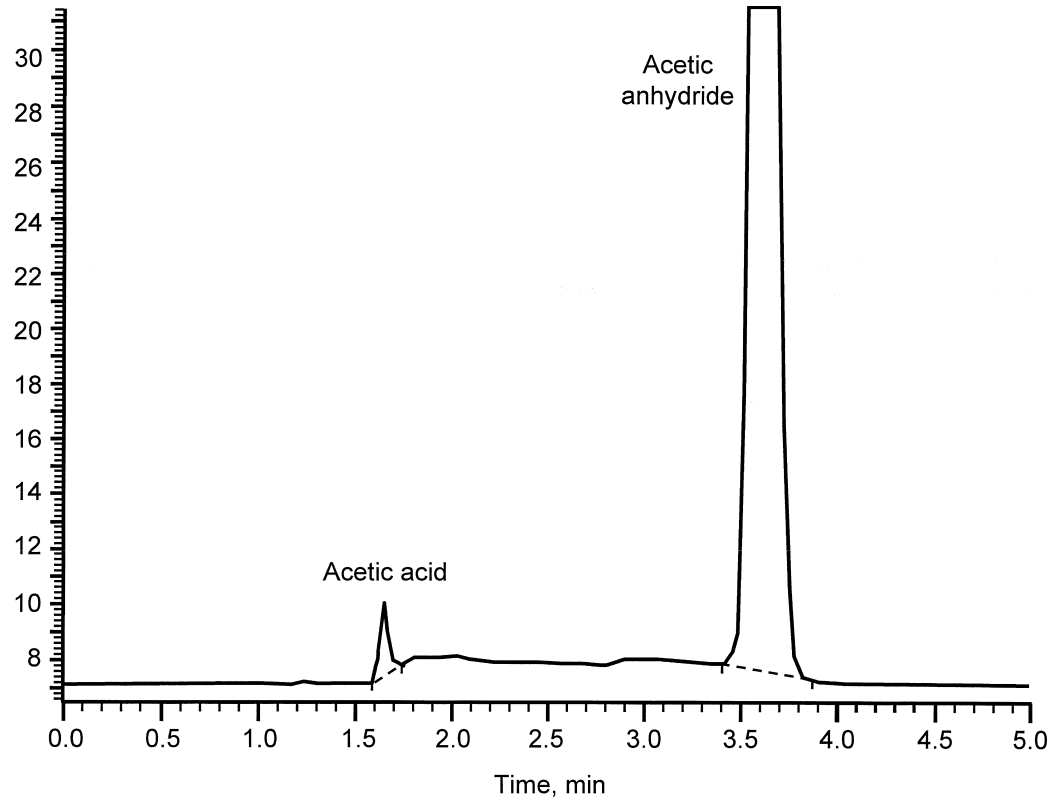

Fig. 4. Separation of acetic anhydride and acetic acid, expanded scale column: aquasil. mobile phase: B 90% water with 0.02 wt.% phosphoric acid and 10% acetonitrile.

ration. This sample contained 0.17% acetic acid as determined by NMR. With detection at 210 nm the anhydride peak will contain some recently formed acetic acid that has not yet separated. The acetic acid peak resulting from acetic acid originally present in the sample will contain some acetic acid formed during the time it takes the anhydride peak to separate from the acid peak after injection, and there will be a continuum of acetic acid formed by hydrolysis between these peaks. The best estimate of baselines for dealing with this problem is shown in Fig. 4, and the data system acquisition parameters need to be set to get this baseline. Alternatively, with multiwavelength acquisition, aliphatic acids can be measured at 210 nm and anhydrides at 240 nm where interference by acids is insignificant. The worst error will occur in the determination of a small amount of acetic acid in nearly pure acetic anhydride, the case shown in Fig. 4. These potential problems are not encountered with propionic and higher molecular weight anhydrides because their hydrolysis rate constants are smaller [8] and no evidence of hydrolysis is detected during a separation.

Although we have not encountered problems using the method described, there could be cases where the small amount of hydrolysis during the separation of acetic anhydride is unacceptable. Or, alternatively, it might be desirable to apply the method to an anhydride less hydrolytically stable than acetic anhydride. There are several changes that can be made to further minimize hydrolysis. The column flow-rate should be high to minimize time spent in the mobile phase, and organic modifier should be high and temperature low to slow the hydrolysis rate. If there are no interfering peaks near the acetic acid peak, the separation can be done at a higher acetonitrile concentration. Response factors are dependent on acetonitrile concentration, so the method must be

calibrated in the mobile phase used for separation. As would be predicted from the effect of temperature on hydrolysis rate [5], at 0°C, there was no evidence for acetic anhydride hydrolysis, i.e. there was no detectable acetic acid eluting between the acid and anhydride peak. These modifications, especially subambient operation, make the method more inconvenient, or less universal, and they should seldom be necessary.

3.2. Investigation of stationary phase effects

The data in Fig. 3 show that the stationary phase can have a significant effect on anhydride losses. During a separation acetic anhydride experiences two environments, the mobile phase and the stationary phase. The substantial differences shown in Fig. 3 result from differences in the stationary phase environment and it is seen that the acetic anhydride hydrolysis rate is a sensitive probe of this environment. During the separation the anhydride is in the mobile phase environment for time t_0, where t_0 is the void time. While the stationary phase has no effect on the hydrolysis rate constant in the mobile phase, it does affect the fraction of anhydride available in the mobile phase at any time during the separation. The amount of anhydride in the mobile phase is only a fraction R of the total, where R is the ratio of t_o and retention time, t_r. Therefore, the observed hydrolysis rate in the mobile phase, based on the total amount of anhydride present, is R times the bulk rate. Assuming equivalent $t_{o,}$ a more retentive column will result in less mobile phase hydrolysis because the fraction exposed to the mobile phase is less. However, a more retentive column will result in more time for hydrolysis to occur in the stationary phase, all things being equal. Therefore, knowledge of hydrolysis in the stationary phase is important for column selection and it may be useful in elucidating stationary phase chemistry.

A measure of hydrolysis rate in the stationary phase can be obtained by subtracting the mobile phase rate from the total rate observed. The mobile phase rate can be calculated from bulk rate experiments. Data for water and 0.20% phosphoric acid are shown in Fig. 5. It can be seen that dilute phosphoric acid has no detectable effect on rate. The rate calculated from these curves multiplied by the fraction of anhydride in the mobile phase, R, yields the mobile phase rate. For purposes of comparing columns only an average hydrolysis rate was calculated for the total rate observed in the separation (see Experimental). Subtraction of the mobile phase rate from the total provides a comparative measure of

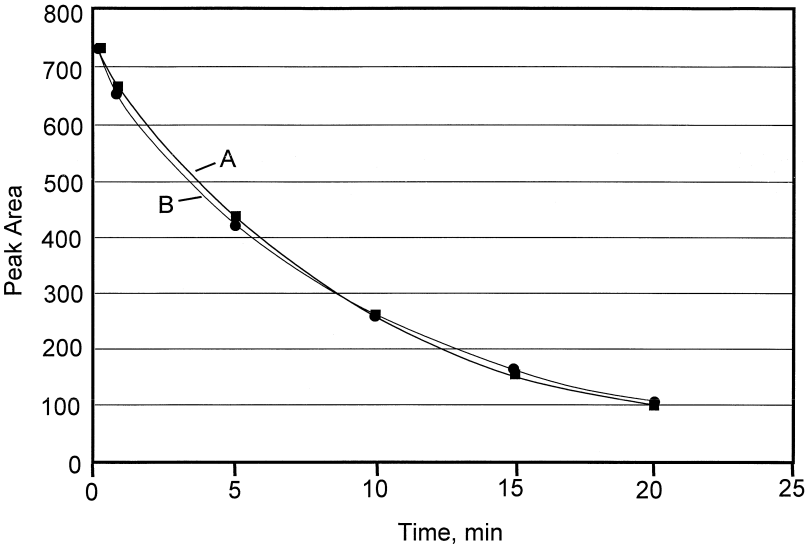

Fig. 5. Hydrolysis rate curve for acetic anhydride. Curve A 90/10 water acetonitrile. Curve B 90% water with 0.20 wt.% phosphoric acid and 10% acetonitrile, 23°C. Samples analyzed by method described in Experimental section.

rate in the stationary phase. These results are shown in Fig. 6.

These rates provide some insight into the chemical environment of the stationary phases. Without detailed knowledge of these proprietary phases it is not possible to confidently rationalize the differences observed, but it is clear the stationary phase has a catalytic effect on rate and it appears that silica is an important contributor. For example, the main reported difference between the AquaSep column and the other pure hydrocarbon phases is the AquaSep column has a very high surface area and modest coverage, which seems to promote hydrolysis. Several columns showed much greater catalytic activity in water than in phosphoric acid. We have found the Prism column contains basic sites that are protonated in the phosphoric acid eluent. In water they are not protonated and in this state they seem to be potent catalysts for hydrolysis. The exceptional inertness of the Aquasil column is more difficult to rationalize. This column is reported to be a C18 column endcapped with hydroxy propyl groups. Perhaps these hydroxyls are hydrogen bonded to the most catalytically active surface silanols. The columns claimed to be made with exceptionally inert silica in general seemed to be more inert with respect to anhydride hydrolysis and most would be acceptable for this application.

None of the columns showed evidence for losses due to acetylation of the stationary phase. This kind of loss could be distinguished from hydrolysis by measuring the amount of acetic acid that appears as a hump between the anhydride and acid peaks. Hydrolysis results in two moles of acid per mole of anhydride, while acetylation yields one mole. A material balance of anhydride lost and acetic acid that appears between the anhydride and acid peaks can be used to distinguish between these losses. It is

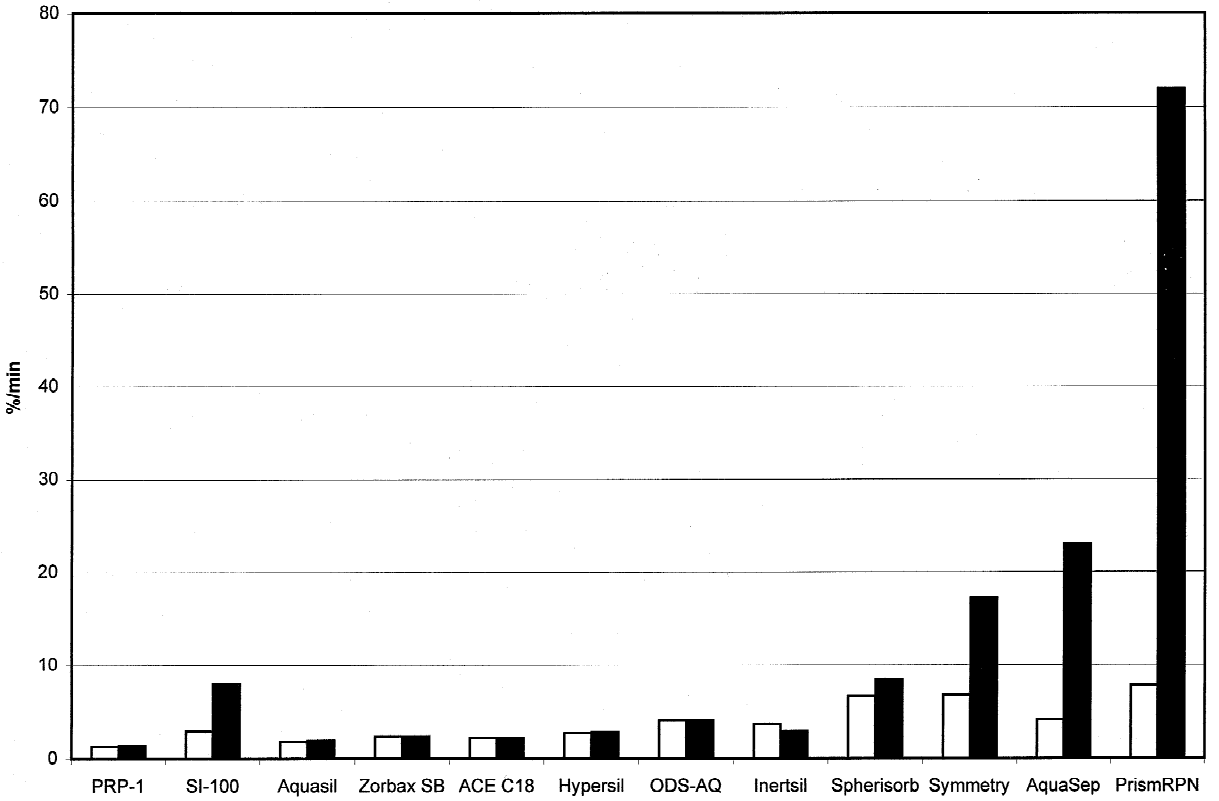

Fig. 6. Rate of hydrolysis of acetic anhydride in stationary phase 90% water with 0.02 wt.% phosphoric acid and 10% acetonitrile clear bars and 90% water and 10% acetonitrile shaded bars, 23°C.

difficult to precisely measure this acetic acid because it appears as a broad hump, but we can confidently conclude that most, if not all, the loss is by hydrolysis for the columns studied.

4. Conclusions

Aliphatic anhydrides and their corresponding acids can be separated without significant hydrolysis losses while maintaining good peak shape and selectivity for the corresponding acids. The stationary phase plays an important role in the potential for anhydride hydrolysis during the separation. Hydrolysis rates in the stationary phase may provide a useful means of probing the chemical environment of the stationary phase.

References

[1] V.H. Agreda, J.R. Zoeller (Eds.), Acetic Acid and Its Derivatives, Marcel Dekker, New York, 1993.
[2] L. Wuping, L. Hian Kee, J. Chromatogr. 805 (1998) 109.
[3] A.J. Domb, J. Chromatogr. 673 (1994) 31.
[4] G.W. Tindall, R.L. Perry, J. Chromatogr. 633 (1993) 227.
[5] A.C.D. Rivett, N.V. Sidgwick, J. Chem. Soc. 97 (1910) 732.
[6] K.J.P. Orton, M. Jones, J. Chem. Soc. 101 (1912) 1708.
[7] V. Gold, J. Chem. Soc. 43 (1947) 506.
[8] W.B.H. Wilsdon, N.V. Sidgwick, J. Chem. Soc. 103 (1913) 1959.