



ELSEVIER

Journal of Chromatography A, 673 (1994) 31–35

JOURNAL OF
CHROMATOGRAPHY A

Quantitative analysis of mixtures of symmetric and mixed anhydrides

Abraham J. Domb[☆]

Department of Pharmaceutical Chemistry, School of Pharmacy, Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem 91120, Israel

(First received December 21th, 1993; revised manuscript received February 24th, 1994)

Abstract

HPLC and GC methods were developed for the determination of symmetrical and mixed anhydrides. Both symmetrical and mixed anhydrides and their respective acids were determined in a single reversed-phase HPLC run using acetonitrile–water as the mobile phase. GC was useful for the quantitative analysis of a mixture of mixed anhydrides symmetric anhydrides. Similar results were obtained from the HPLC and GC analyses of mixtures containing mixed anhydrides of fatty acids with benzoic acid and their corresponding symmetrical anhydrides and acids. These methods are useful in the investigation of anhydride chemistry, the routine analysis of raw materials and the quality control and purity determination of polymers and other compounds containing anhydrides.

1. Introduction

Acid anhydrides are among the most important classes of reagents used in organic synthesis. In the last few years, anhydride materials have been of great interest for use as polymeric drug carriers [1] and as prodrugs [2], to improve administration and bioavailability of drugs. They find widespread use in the formulation of resin systems for advanced composites [3]. Despite their importance, very little has been reported on the determination of anhydrides, particularly mixed anhydrides.

Direct determination of anhydrides is often difficult owing to their susceptibility to hydroly-

ysis. Most published procedures for direct measurements use spectrophotometric techniques, *e.g.*, IR [4] and ¹H NMR spectrometry [5], to monitor functional groups. Indirect techniques such as titration [6], spectrophotometry [7], gas chromatography [8] and liquid chromatography [9] involve hydrolysis and derivatization; these are often multi-step and time-consuming methods. A direct normal-phase HPLC method for the determination of a few commercially important cyclic anhydrides was reported recently [10]. These methods are useful for the determination of total anhydride content, but not for the quantitative analysis of mixtures of symmetrical and mixed anhydrides.

We report on two methods for the quantitative analysis of mixtures of various anhydrides and their corresponding acids in a single step. The methods involve reversed-phase HPLC using

* Corresponding author.

* Affiliated with The David Bloom Center for Pharmacy.

acetonitrile–water as the mobile phase and GC using a methylphenylsilicone column.

2. Experimental

2.1. Chemicals and reagents

Acetic, propionic, butyric, pivalic, hexanoic, octanoic, lauric, palmitic, stearic, benzoic, phthalic, succinic and maleic anhydride, 1,2,5-benzenetricarboxylic anhydride (BTrCA), 1,2,4,5-benzenetetraboxylic dianhydride (BTCA), 1,2,3,4-tetrahydrofuranetetracarboxylic dianhydride (THFTCA) and their respective acids and acid chlorides were purchased from Aldrich (Milwaukee, WI, USA). Mixed anhydrides were prepared by the reaction between acids and acid chlorides (1.1:1 molar ratio) in dichloromethane in the presence of poly(4-vinylpyridine) (Reillex 402; Reilly Industries, NJ, USA) as acid acceptor. After 1 h at 0°C, the solvent was evaporated to dryness and the liquid residue was weighed and analysed for anhydride formation by IR and ^1H NMR spectrometry. HPLC-grade acetonitrile and dichloromethane were purchased from Lab-Scan (Dublin, Ireland). Tritiated water (1 mCi/ml) was purchased from New England Nuclear (Boston, MA, USA) and was diluted to 10 $\mu\text{Ci}/\text{ml}$ using deionized water. Scintillation solution was obtained from Lumax (Landgraaf, Netherlands).

2.2. Apparatus and assay conditions

The HPLC apparatus consisted of an HP 1050 (Hewlett-Packard, Palo Alto, CA, USA) modular system including a diode-array UV detector and an HPCHEM IBM-compatible data system with a ThinkJet printer. A Rheodyne (Cotati, CA, USA) injection valve equipped with a 20- μl loop was used, and the samples (10 μl in acetonitrile) were eluted through a C_8 column (Supelcosil LC-8, 100 Å, 5 μm) (250 \times 4.6 mm I.D.) (Supelco, Bellefonte, PA, USA). Normal-phase analysis was conducted using a silica column (Supelcosil LC-Si, 100 Å 5 μm) (250 \times

4.6 mm I.D.) (Supelco) and hexane–tetrahydrofuran mixtures as mobile phase.

GC analysis was conducted on a HP5890 gas chromatograph (Hewlett-Packard) with an HP 3396A integrator and a flame ionization detector. An HP Ultra 2 (25 m \times 0.2 mm \times 0.11 μm) column packed with methyl–5% phenylsilicone was used. The following conditions were employed: detector and injector temperatures, 260°C; column temperature constant at 150°C; and helium, air and hydrogen flow pressures 40, 60 and 40 p.s.i., respectively (1 p.s.i. = 6894.76 Pa). IR spectrometry was performed on a Perkin-Elmer (Norwalk, CT, USA) Model 1310 spectrophotometer. Samples were cast on to NaCl plates. Acids and solid samples were either pressed into KBr pellets or dispersed in Nujol on to NaCl plates. ^1H NMR spectra were obtained on a Varian (Palo Alto, CA, USA) 300-MHz spectrometer using 1% (w/v) solutions in CDCl_3 containing tetramethylsilane (TMS) as internal reference. Radioactivity counting was conducted on a Model 1211 RackBeta liquid scintillation counter (LKB–Wallac, Finland).

2.3. Anhydride stability in mobile phase

The hydrolytic stability of the anhydride molecules in the HPLC mobile phase mixtures was determined using 1 mg/ml solutions of benzoic and hexanoic anhydride in acetonitrile–water mixtures (50:50 and 70:30, v/v) maintained at 25°C. Samples taken from the solution every 10 min for 60 min were analysed by HPLC (see above). The 60-min aliquots were freeze-dried and the solid residue was dissolved in deuterated chloroform and analysed for anhydride by IR and ^1H NMR spectrometry.

In a second experiment, 1 mg/ml solutions of benzoic and lauric anhydride in a radioactive mixture of acetonitrile–water (7:3, v/v; $^3\text{H}_2\text{O}$ 10 $\mu\text{Ci}/\text{mL}$) were maintained at 25°C for 30 min. The radioactive mixtures were prepared from acetonitrile and 10 $\mu\text{Ci}/\text{ml}$ tritiated water. After 30 min, the solutions were freeze-dried and dissolved in Lumax scintillation solution and the radioactivity was determined.

3. Results and discussion

3.1. Recovery and stability of anhydrides in the HPLC mobile phase

Initial work was directed at determining the stability of the anhydride molecules towards hydrolysis in acetonitrile–water mixtures. Two experiments were conducted. In the first, solutions of benzoic and hexanoic anhydride in acetonitrile–water (50:50 and 70:30, v/v) were followed for 60 min at 25°C. Samples removed from the solutions were freeze-dried and the residue was analysed by HPLC and IR and ¹H NMR spectrometry. HPLC analysis (see above) of the samples removed from the acetonitrile–water mixtures showed the peaks of the anhydrides with no emergence peaks of benzoic or hexanoic acid. IR and ¹H NMR analyses of the residual materials after freeze-drying gave similar spectra to those of the starting anhydride molecules. In the second experiment, the po-

tential formation of acidic degradation products from the reaction with the water component of the mobile phase was investigated using radio-labelled water. Benzoic and lauric anhydride isolated from acetonitrile–³H₂O (7:3, v/v) after 30 min at room temperature did not contain any radioactivity (the dpms were similar to the background). These experiments prove that the anhydride molecules dissolved in the mobile phase do not undergo hydrolysis during the course of analysis.

3.2. Determination of anhydrides by HPLC

Aliphatic and aromatic anhydrides were determined by reversed-phase HPLC (Table 1 and Fig. 1). Short-chain aliphatic and aromatic anhydrides were separated at retention factor (*k'*) of 0.20–0.80, using water–acetonitrile (50:50, v/v) and UV detection at 237 nm. Long-chain anhydrides were eluted at *k'* = 2.70–4.93, using pure acetonitrile. The corresponding mixed an-

Table 1
HPLC of anhydrides

Anhydride (RCOOCOR)	R	<i>k'</i>	Mobile phase (acetonitrile–water)
Acetic	CH ₃	0.18	50:50
Propionic	CH ₃ CH ₂	0.35	50:50
Butyric	CH ₃ (CH ₂) ₂	0.47	50:50
Hexanoic	CH ₃ (CH ₂) ₄	0.70	50:50
Heptanoic	CH ₃ (CH ₂) ₅	0.79	50:50
Decanoic	CH ₃ (CH ₂) ₈	3.74	70:30
Lauric	CH ₃ (CH ₂) ₁₀	4.02	70:30
Palmitic	CH ₃ (CH ₂) ₁₄	4.37	70:30
Stearic	CH ₃ (CH ₂) ₁₆	4.66	70:30
Succinic	–CH ₂ CH ₂ –	2.18	50:50
Maleic	–CH=CH–	0.48	50:50
Benzenetricarboxylic anhydride		0.44	50:50
Phthalic	–C ₆ H ₄ –	1.11	50:50
Benzoic	C ₆ H ₅	0.27	30:70
THF-tetracarboxylic anhydride		0.56	50:50
Benzenetetracarboxylic anhydride		0.63	50:50
Benzoic acid		0.18	30:70
THF-tetracarboxylic acid		0.19	50:50
Benzenetetracarboxylic acid		0.19	50:50
Aliphatic acids	CH ₃ (CH ₂) _{0–10}	0–0.20	30:70

Analysis using a C₈ column, acetonitrile–water as mobile phase at a flow-rate of 1 ml/min and UV detection at 237 nm. The retention factor, *k'*, was calculated from $k' = (t_R - t_0)/t_0$, where $t_0 = 2.70$ min.

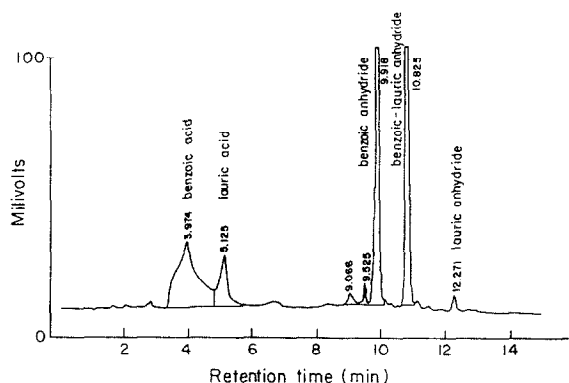


Fig. 1. Chromatogram of the symmetrical and mixed anhydrides of benzoic and lauric acid, obtained by HPLC using a C_8 column with acetonitrile–water (30:70, v/v) as the mobile phase.

hydrides of aliphatic and aromatic acids were eluted at intermediate times. For example, benzoic anhydride, benzoic–lauric mixed anhydride and lauric anhydride were eluted with acetonitrile–water (70:30, v/v) at $k' = 2.67$, 3.00 and 3.54, respectively (Fig. 1). Linear relationships between anhydride concentration and peak area were found with detection limits of 1 and 10 $\mu\text{g}/\text{ml}$ for aromatic and aliphatic anhydrides, respectively.

Several cyclic anhydrides of commercial importance as curing agents and plasticizers in polymer composites and precursors for polyimide formation were studied (Table 1). The cyclic anhydrides were eluted at $k' = 0.48$ –1.96 and their corresponding acids at $k' = 0.20$ –0.48.

For comparison we followed the normal-phase method described by Patterson and Escott [10] using a normal-phase silica column with a mixture of anhydrous hexane and tetrahydrofuran as the mobile phase. Both aromatic and aliphatic anhydrides were eluted at close retention times with $k' < 0.45$. The corresponding acids were eluted at longer retention times, $k' > 0.45$. Attempts to optimize the separation of the various anhydrides by altering the ratio between hexane and tetrahydrofuran and the flow-rate were unsuccessful. Under all conditions, the retention times for the anhydrides were short ($k' = 0.10$ –0.45), which was not useful for the quantitative

analysis of a mixture of anhydride molecules. This method, however, is useful for the determination of the acid content and the total anhydride content in mixtures of acids and anhydrides.

3.3. Determination of anhydrides by GC

Mixtures of various anhydrides and acids were analysed by GC. As shown in Fig. 2, a mixture containing hexanoic acid, benzoic acid, hexanoic anhydride, mixed benzoic–hexanoic anhydride and benzoic anhydride was separated. Aromatic anhydrides were retained longer than the aliphatic anhydrides, while mixed anhydrides were eluted at intermediate retention times.

These HPLC and GC methods were used for the quantitative analysis of reaction mixtures aimed at the formation of mixed anhydrides from acids and acid chlorides. These reaction mixtures contained mixed and symmetrical anhydrides and the corresponding acids. As shown in Table 2, the results obtained by HPLC and GC methods were in good agreement. Both methods showed distinct peaks for the symmetrical and mixed anhydrides and the corresponding acids, thus permitting the direct quantitative analysis of the reaction mixture in a single run.

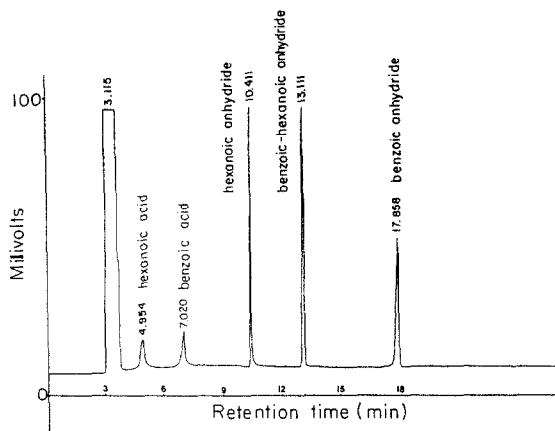


Fig. 2. Chromatogram of the symmetrical and mixed anhydrides of benzoic and lauric acid, obtained by GC using a methyl-5% phenylsilicone column with helium as gas carrier at 150°C and flame ionization detection.

Table 2
HPLC and GC analysis of mixed anhydrides from the reaction between acid and acid chlorides

Acid RCOOH (R)	Acid chloride R'COCl (R')	Total yield (%)	Mixed anhydride (%)	
			HPLC	GC
C ₆ H ₅	CH ₃	89	45	52
C ₆ H ₅	CH ₃ CH ₂	90	50	56
C ₆ H ₅	CH ₃ (CH ₂) ₄	92	65	66
CH ₃ (CH ₂) ₄	C ₆ H ₅	84	74	69
C ₆ H ₅	CH ₃ (CH ₂) ₆	88	70	67
C ₆ H ₅	CH ₃ (CH ₂) ₁₂	89	72	65
C ₆ H ₅	CH ₃ (CH ₂) ₁₆	84	68	65
C ₆ H ₅	4-CH ₃ C ₆ H ₅	80	75	70
CH ₃ (CH ₂) ₁₀	C ₆ H ₅	83	72	67
CH ₃ (CH ₂) ₄	CH ₃ (CH ₂) ₁₆	86	74	65
CH ₃ (CH ₂) ₄	CH ₃ (CH ₂) ₁₂	82	80	68

Analysis of the reaction mixture between the acid and acid chloride (1.1:1 molar ratio) and poly(4-vinylpyridine) (PVP) (2.5 equiv.) as acid acceptor at 0°C for 1 h. Results are for three independent experiments with an error of less than 5%.

Acknowledgements

The author thanks Mishael Haimove and Osnat Shaia for their help. This work was supported by grants from the Eliahu and Tatiana Leszczynski Research Foundation and the United States–Israel Binational Science Foundation, No. 91-0020/1.

References

- [1] A.J. Domb, S. Amselem and M. Maniar, *Adv. Polym. Sci.*, 107 (1993) 93.
- [2] A.J. Domb, presented at ACS Meeting, Division of Organic Chemistry, Washington, DC, August 1992.
- [3] P.M. Hergenrother, in D. Wilson, H.D. Stenzenberger and P.M. Hergenrother (Editors), *Polyimides*, Chapman & Hall, New York, 1990.
- [4] M.M. Koton, V.V. Kurdiavatse and V.M. Svetlichy, *Polyimides—Synthesis, Characterization and Applications*, Vol. 2, Plenum Press, New York, 1984.
- [5] W. Heyde, *Fresenius' Z. Anal. Chem.*, 320 (1985) 667.
- [6] K.K. Verma and P. Tyagi, *Anal. Chem.*, 56 (1984) 2157.
- [7] J. Bartos, *Talanta*, 27 (1980) 583.
- [8] E.P. Usova and G.S. Sirova, *Zh. Anal. Khim.*, 38 (1983) 946.
- [9] R. Geyer and G.A. Saunders, *J. Liq. Chromatogr.*, 9 (1986) 2281.
- [10] S.D. Patterson and R.E.A. Escott, *High Perform. Polym.*, 2 (1990) 197.