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Quantitative Analysis of Mixtures of Various Linear Anhydrides and Carboxylic Acids

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Abstract

Only a few quantitative methods have been described to analyze mixtures of symmetric anhydrides, mixed anhydrides and their corresponding carboxylic acids. Reversed-phase liquid chromatography using acetonitrile-water as solvent was optimized for the quantitative determination of such mixtures obtained by reacting acetic or propionic anhydrides with various fatty acids (from C3 to C18). Complex mixtures containing up to three mixed anhydrides, four symmetric anhydrides and four carboxylic acids have been quantitatively analyzed successfully in a single run.

Keywords

Column liquid chromatography
Flow rate gradient
Anhydrides, carboxylic and fatty acids

Introduction

Mixed anhydrides, i.e., unsymmetric anhydrides obtained from two different carboxylic acids are molecules finding an increasing interest in the chemical industry due to their high reactivity. They are used for instance in chemical synthesis as co-reagent [1] or in the pharmaceutical chemistry as prodrugs [2]. They are particularly interesting molecules to form ester bonds with

hydroxyl groups and are commonly used in the synthesis of cellulose esters [3] as, for examples, acetoformic anhydride often used as a formylating agent or acetic-propionic and acetic-butyric used in the production of acylated cellulose.

In order to produce mixed anhydrides industrially, three processes are generally employed: the reaction of a carboxylic acid with a ketene, the reaction between an acyl chloride with a carboxylic acid salt and the reaction of a

symmetric anhydride with a carboxylic acid. When synthesizing mixed anhydrides by the latter process, a complex reaction medium is obtained. The mixed anhydride spontaneously reacts again to form the corresponding symmetric anhydrides of the second fatty acid (Fig. 1).

The separation and quantification of such mixtures, containing several symmetric anhydrides, a mixed anhydride, and the corresponding carboxylic acids, is of importance and few analytical methods have been developed to perform direct determination.

Gas chromatography equipped with a methylphenylsilicone stationary phase [2] is an interesting qualitative method to separate these compounds. Nevertheless, it cannot be used quantitatively. The high temperature of the injector can shift the equilibrium of the reaction increasing the symmetric anhydride content in the mixture.

Reversed-phase LC using a mixture of acetonitrile and water as mobile phase [4, 5] has been reported for quantitative determination of symmetric and mixed anhydrides and their corresponding acids. Compounds with short aliphatic chains are separated using eluents with a high content of acidified water, usually 50–90%. Under these conditions, hydrolysis of anhydrides occurs all along

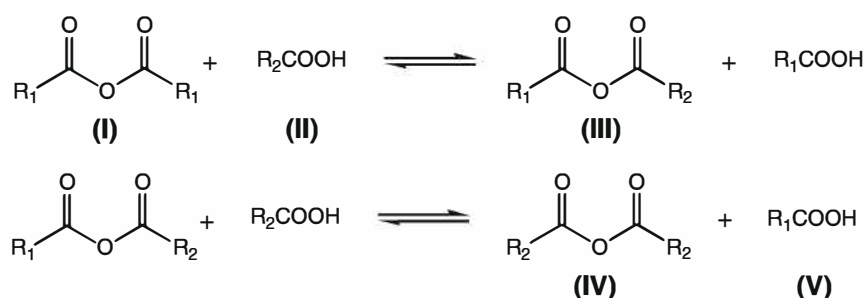


Fig. 1. Consecutive reactions between carboxylic anhydrides and carboxylic acids

the column. The resulting carboxylic acids are liberated continuously and become part of the baseline. It has been demonstrated that the hydrolysis of anhydrides is a reaction with kinetics of the first order [5]. Consequently, as the fraction of anhydride hydrolyzed is proportional to concentration, this phenomenon does not affect the calibration. Moreover, only low molecular weight aliphatic anhydrides, such as acetic anhydride, are significantly concerned by the hydrolysis.

In the case of mixtures containing long aliphatic chains, the polarity of the involved molecules is lower. The LC protocols would require a mobile phase richer in acetonitrile, from 50–100%.

But when it is required to analyze mixtures with compounds containing short and aliphatic chains (as those described in Fig. 1), it is relatively difficult to obtain the separation of all the molecules in a single run, even with a gradient program. In order to make the fatty molecules soluble, it is necessary to prepare eluents with a high concentration of acetonitrile. As a consequence, three problems arise: (a) the resolution of short chain compounds is not achieved, (b) a loss of resolution between the acetic-fatty mixed anhydride and the fatty acid occurs, and (c) the solubility of all the compounds with different polarities in the mobile phase may not be complete.

This paper describes a reversed-phase LC protocol allowing the analysis in a single run of a complex mixture of compounds as described in Fig. 1 with R_1 ranging from C2 to C4 and with R_2 from C6 to C18.

We have investigated different conditions to optimize the said method

by varying the mobile phase gradient, the flow rate gradient and the column specifications.

Experimental

Instrumentation

The liquid chromatography system used for this work consisted of a Dionex modular system including a P680 pump, an ASI100 automated sample injector equipped with a 20 μL loop configured at 10 $\mu\text{L s}^{-1}$ for injection and a UVD340U diode-array UV detector (Dionex, Sunnyvale, CA, USA). The temperature of the column was 23 $^{\circ}\text{C}$ and the room temperature was 20 $^{\circ}\text{C}$.

Samples (10 μL) in acetonitrile were eluted through a C18 column (Varian, Polaris C18-A, 100 A, 5 μm) (250 \times 4.6 mm ID).

Chemicals and Standards

Acetic, propionic, butyric, caproic, caprylic, capric, lauric, myristic, palmitic, oleic, linoleic, and linolenic acids and anhydrides 99% (GC) purity were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Phosphoric acid (85% in water) and technical grade oleic acid 90% were purchased from Acros (Halluin, France). Acetonitrile LC grade was obtained from Scharlau (Barcelona, Spain), and pure water from a Milli-Q water purification system.

Preparation of the Model Mixtures

We prepared various model mixtures by reaction of a short chain symmetric

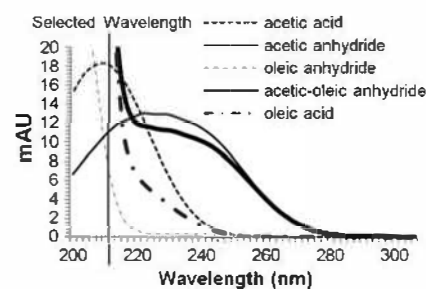


Fig. 2. UV spectra of carboxylic acids and anhydrides with $R_1 = 2$ and $R_2 = \text{C18:1}$

anhydride (acetic or propionic) with a carboxylic acid with R_2 from C3 to C18 without using any catalyst.

Both reagents, with a fatty acid/anhydride molar ratio of 1.5, were introduced in a 100 mL batch reactor equipped with a 350 rpm stirring system and a reflux condenser. Reaction was carried out at 100 $^{\circ}\text{C}$ during 1 h.

Results and Discussion

The choice of a column permitting the analysis of low molecular weight aliphatic anhydrides and their corresponding acids was our first task. We selected standard conditions used in the analysis of fatty compounds, i.e., with a concentration of acetonitrile in the mobile phase higher than 60%. We selected the Varian Polaris C18-A compared to the reversed-phase columns commonly used for this analysis [1, 5] as it resulted to give by far the best resolution factor.

The analytical conditions were optimized with the selected column in order to achieve the separation of a mixed anhydride and its corresponding carboxylic acids.

We first chose to work on a relatively simple mixture obtained at equilibrium by reaction between acetic anhydride with octanoic acid at 100 $^{\circ}\text{C}$. A flow rate of 1.5 mL min^{-1} was selected with an eluent gradient starting at $t = 0$ from 75% acetonitrile/25% water with 0.02 wt% phosphoric acid in water to 100% acetonitrile in 5 min and kept at 100% acetonitrile until analysis. Samples (10 μL) were injected. A wavelength comprised between 205 and 240 nm permitted a quantitative analysis of the

mixture. Nevertheless, 212 nm seemed to be the best compromise between peak shapes and intensity as shown in Fig. 2. Figure 3 shows the chromatogram obtained under the optimized conditions for this mixture. The five compounds described in Fig. 1 were perfectly separated and identified.

Based on these first results, the separation of molecules with a great difference in the length of aliphatic chains was investigated the mixture obtained by reacting acetic anhydride with pure oleic acid was studied. After analyzing this mixture under the previous conditions, a perfect separation of all the five compounds was obtained.

Nevertheless, a relatively important peak asymmetry factor (A_s) value of 3.2 for the oleic anhydride and a high retention factor (k) of 25 did not permit an appropriate quantification of this compound. Asymmetry factor values are considered to be unacceptable when superior to 2 and exact symmetrical peaks have an A_s of 1. We optimized the analytical conditions by applying a flow rate gradient during the analysis as shown on Fig. 4. This permitted to obtain a peak asymmetry factor value of 1.1, which is a satisfactory value, and a retention factor value of 13.5. This permitted the quantitative analysis of all the compounds and diminished considerably the analysis runtime.

More complex mixtures were analyzed and when acetic anhydride reacted over technical oleic acid, which contained small but significant amounts of linoleic and linolenic acids, a mixture with three anhydrides, three carboxylic acids, and three mixed anhydrides was obtained. Several analysis conditions and gradients for both eluents and flow were tested. This succeeded in a perfect separation of all the compounds (Fig. 5). The chromatogram allowed the quantitative analysis of a mixture containing ten compounds of different polarity and even the resolution of various C18 compounds, which are distinguished only by the number of their unsaturations.

It is important to note that these optimized analysis conditions are very sensitive. A change in only one of the parameters (flow, gradient rate or initial

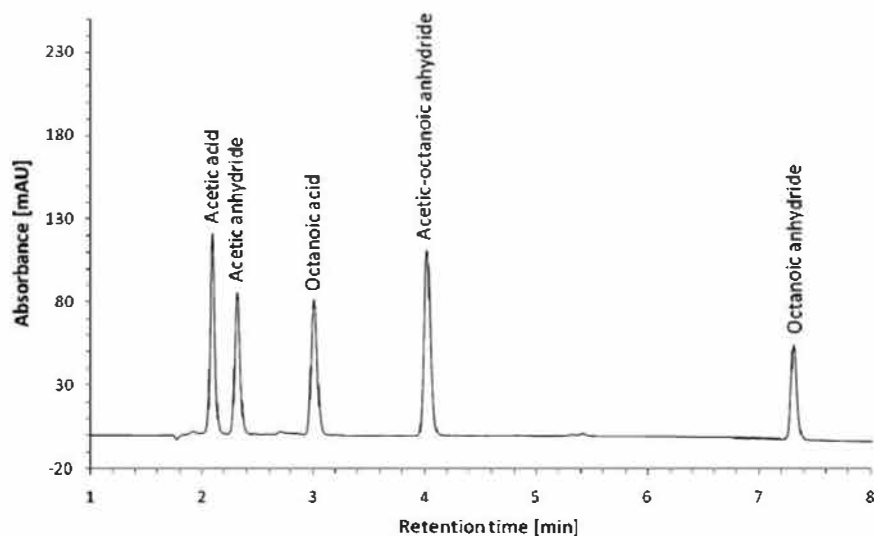


Fig. 3. LC chromatogram of the mixture obtained after reaction between acetic anhydride and octanoic acid. Mobile phase: A = water with 0.2 wt% phosphoric acid, B = acetonitrile. Gradient: 70% A to 100% in 5 min, 1.5 mLmin⁻¹. UV detection at 212 nm

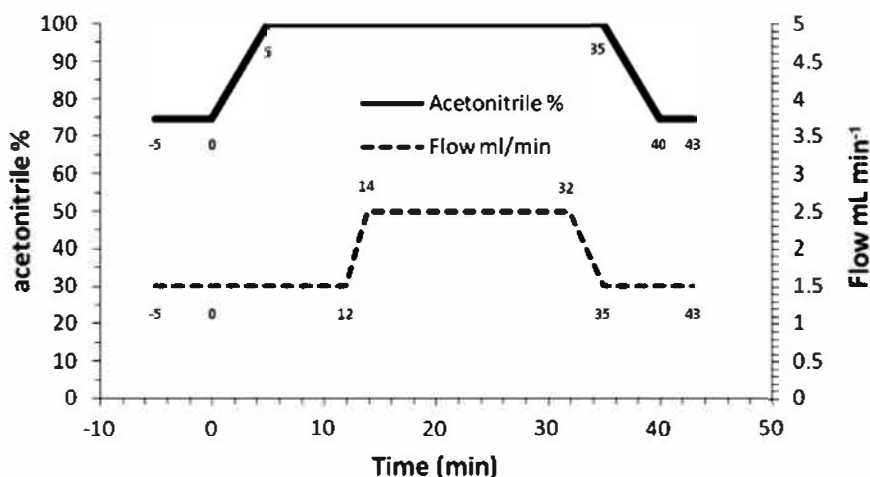


Fig. 4. Optimized conditions retained for eluent concentration and eluent flow

percentage of acetonitrile), prevents the accurate separation of acetic-oleic anhydride and their corresponding acids. For instance, a change of 5% of the initial percentage of acetonitrile (80 or 70%) led to a non-selective separation of linoleic, linolenic, oleic acids and their corresponding acetic-fatty mixed anhydrides.

We finally performed analysis under these conditions of various mixtures synthesized with symmetric anhydrides with R_1 ranging from C2 to C3 and carboxylic acids with R_2 from C3 to C18.

Results of retention factors obtained for each compound are indicated in Table 1. A perfect separation was obtained in every case.

As shown in Fig. 4, we forced the mobile phase to return to 75% acetonitrile and to decrease the flow rate to 1.5 mL min⁻¹ at the end of the analysis. Indeed a period of stabilization of the column under 75% acetonitrile was necessary after or before the analysis in order to obtain a flat baseline for a neat quantitative analysis. The required time of stabilization was 5 min at least.

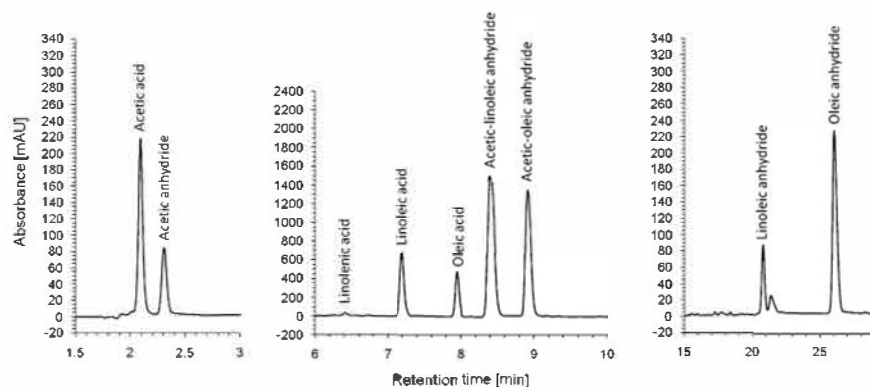


Fig. 5. Separation of the mixture obtained from acetic anhydride and oleic acid (90% technical grade) under the optimized conditions shown in Fig 4

Table 1. Retention factors under optimized conditions

R_1	R_2	k'					
			(I)	(II)	(III)	(IV)	(V)
2	3	0.30	0.22	0.39	0.48	0.18	
2	4	0.30	0.27	0.48	0.78	0.18	
2	6	0.30	0.43	0.80	1.84	0.18	
2	7	0.30	0.52	0.98	2.48	0.18	
2	8	0.30	0.69	1.27	3.12	0.18	
2	10	0.30	1.27	2.01	4.41	0.18	
2	12	0.30	2.06	2.78	6.60	0.18	
2	14	0.30	3.01	3.50	9.75	0.18	
2	16	0.30	4.04	4.23	ins.	0.18	
2	18:1	0.30	3.73	4.02	13.54	0.18	
2	18:2	0.30	3.05	3.48	10.63	0.18	
2	18:3	0.30	2.61	nd	nd	0.18	
3	18:1	0.48	3.73	4.55	13.54	0.22	
3	18:2	0.48	3.05	4.02	10.63	0.22	

(I)–(V) Molecules shown in Fig. 1, nd not determined, ins insoluble in acetonitrile

A dysfunction of the pump valves can occur when increasing the acetonitrile concentration in the eluent. It is therefore necessary, after around 100 injections, to clean the valves in a nitric acid aqueous solution (15% in water) assisted with ultrasonic waves. It is also possible to reduce this problem by using ceramic valves.

Most of the mixed anhydrides are unstable molecules and particularly dif-

icult to isolate. That is why no standards are commercially available. The quantitative analysis of the studied complex mixtures was only possible by applying the method described in this work. Indeed, it permitted to quantify all the other compounds presents in the mixture; it was then possible to determine, by calculation, the concentration of the mixed anhydride. This depended directly on the concentration of all the

other compounds present in the mixture. The only condition to determine the response factor of the mixed anhydride was to know the molar ratio used in the synthesis. Once the response factor was known, the mixture itself could be used as a standard, permitting the analysis of mixtures prepared with other molar ratios. The composition of useful reaction media for many applications can thus be easily determined.

Conclusions

We investigated the analysis of mixtures containing various aliphatic anhydrides, acids and mixed anhydrides and optimized a general reversed-phase LC method to quantitatively determine all the compounds. Controlled eluent flow and eluent concentration gradients were required. By this mean, it was possible to perform the analysis of mixtures containing short and long chains of carboxylic acids and anhydrides in a single run.

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