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Note

High-performance liquid chromatographic analysis of acid chlorides by pre-derivatization*

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The most widely employed methods for the determination of acid chlorides are analyses by titrimetry[1–9]. These non-aqueous titration procedures involve a conversion of the acid chloride to a derivative, such as an amide, and hydrochloric acid. This liberated hydrochloric acid is then titrated with base. However, corrections are required for the amounts of free hydrochloric acid and the free carboxylic acid form of the acid chloride, which may already be present in the acid chloride samples. It may also be necessary to correct for the presence of an amine hydrochloride group, such as that associated with the hydrochloride salts of amino acid chlorides, and of phosphates that may be contaminants in specific acid chlorides as a result of using PCl_3 as a chlorinating agent in the synthetic preparation of the acid chlorides. Hasegawa *et al.*[10] reported a high-performance liquid chromatographic (HPLC) method for the determination of acid chlorides after they were esterified.

This report describes a method that is based on the derivatization of the acid chlorides with 4-chloroaniline and the subsequent separation of the resulting stable amide derivatives from the 4-chloroaniline agent and from the free carboxylic acid form of the acid chlorides on a microparticulate octadecyl column using an eluent consisting of a methanol–ammonium acetate buffer. Optimum reaction parameters to ensure complete derivatization were determined and the applicability of this method to the analyses of several types of acid chlorides was demonstrated. We have also shown the effects of possible interfering compounds on the derivatization.

EXPERIMENTAL

Instrumentation

The liquid chromatograph consists of a Model 6000A pump and a Model U6K septumless injector, both purchased from Waters Assoc. A Spectroflow SF770 ultraviolet detector (Kratos, Schoeffel Instrument Division) was employed for monitoring the column effluent. The column used was a 25 cm × 4.6 mm I.D. 10-μm Chromegabond MC-18 (E.S. Industries).

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Materials

The eluent was prepared by mixing distilled-in-glass methanol and aqueous 0.1 *M* ammonium acetate at a volume ratio of 3:2. Distilled-in-glass acetonitrile was used as the reaction solvent medium. Both organic solvents were purchased from Burdick and Jackson Labs. The derivatizing agent, 4-chloroaniline (99+%), was purchased from Aldrich. α-Aminophenylacetyl chloride hydrochloride, 1-amino-1-cyclohexanecarbonyl chloride hydrochloride, 3-(2-chlorophenyl)-5-methylisoxazole-4-carbonyl chloride and 2-ethoxy-1-naphthoyl chloride and their respective reference amide derivatives were supplied by the Chemical Development Subdivision of Wyeth Labs. The remaining acid chlorides used in this investigation were purchased from Aldrich. The purity (≥99%) and identity of the four reference amide derivatives were established based on the results of titration assays (where applicable), HPLC analyses, melting point determinations, weight loss on drying determinations, elemental analyses, infrared spectra and nuclear magnetic resonance spectra.

Procedure

An accurately weighed amount (approximately 50 mg) of the acid chloride was transferred into a 250-ml volumetric flask. A 25-ml aliquot of 4-chloroaniline in acetonitrile, whose concentration depended on the desired molar ratio of this reagent to the acid chloride, was immediately added, the flask was stoppered and the solution was stirred for a specified time. Upon completion of the reaction, the solution was then diluted to volume with a 50% aqueous methanol solvent. The reference solution was prepared by dissolving an accurately known amount of the specific reference amide derivative in a 50% aqueous methanol solvent. Aliquots (10 μl) of the reaction and reference solutions were injected onto the chromatographic column with the eluent flow-rate at 2.0 ml/min. The detector's wavelength setting was 254 nm and its sensitivity range was 0 to 0.4 a.u.f.s. The amount of acid chloride was calculated by comparing the amide derivative peak height in the reference chromatogram to the peak height of the appropriate component in the sample chromatogram.

RESULTS AND DISCUSSION

Given in Table I are the capacity factors (k') for the three components normally observed in the chromatograms of the solutions prepared by reacting the various acid chlorides with the 4-chloroaniline derivatizing agent. Eluting at or near the solvent front are the carboxylic acid forms which are the most likely contaminants in acid chloride samples. The next component eluted is the excess 4-chloroaniline used in the derivatization step. Finally, the various amide derivatives are observed. Since the elution of the carboxylic acid forms and the 4-chloroaniline occurs so rapidly ($k' < 1$), the ability to separate amide derivatives from them is greatly enhanced which allows for the determination of the wide variety of acid chlorides.

The molar ratio of the 4-chloroaniline to the samples as well as the ammonolysis reaction time were optimized in order to ensure that all of the acid chloride was completely converted to the corresponding amide derivative in a reasonable time. As shown in Table II for four of the acid chlorides, the molar ratio and reaction time needed for complete derivatization depended on the particular acid chloride with 3-(2-chlorophenyl)-5-methylisoxazole-4-carbonyl chloride being the most reactive

TABLE I

HPLC SEPARATION OF COMPONENTS IN DERIVATIZED ACID CHLORIDE SOLUTIONS

Acid chloride	*Capacity factors*		
	Amide derivative of the acid chloride	*4-Chloroaniline derivatizing agent*	*Carboxylic acid form of the acid chloride*
α-Aminophenylacetyl chloride hydrochloride	2.6	0.8	0
1-Amino-1-cyclohexane-carbonyl chloride hydrochloride	2.7	0.8	ND*
Butyryl chloride	1.9	0.8	ND*
Hexanoyl chloride	5.0	0.8	ND*
2-Ethoxy-1-naphthoyl chloride	5.6	0.8	0
3-(2-Chlorophenyl)-5-methylisoxazole-4-carbonyl chloride	4.1	0.8	0
Benzoyl chloride	2.8	0.8	0

* ND = Not detectable at 254 nm.

TABLE II

OPTIMIZATION OF THE MOLAR RATIO OF 4-CHLOROANILINE TO ACID CHLORIDE AND OF THE REACTION TIME AT ROOM TEMPERATURE

A = 3-(2-chlorophenyl)-5-methylisoxazole-4-carbonyl chloride; B = α-aminophenylacetyl chloride hydrochloride; C = 2-ethoxy-1-naphthoyl chloride; D = 1-amino-1-cyclohexanecarbonyl chloride hydrochloride.

Ratio (molar)	*Time (min)*	*% Converted to amide*		*Ratio (molar)*	*Time (min)*	*% Converted to amide*	
		A	*C*			*B*	*D*
15	0.1	100		15	0.1	91	
15	5	101		15	5	99	
				15	10	100	
1.1	30	101					
2.2	30	99		0.9	30	90	
				4.5	30	100	
				4.5	30	100	
50	0.1		68	10	0.1		32
50	10		96	10	5		100
50	15		99	10	10		100
50	30		101				
1.1	30		72	1.8	30		82
5.3	30		91	2.4	30		91
12	30		97	4.0	30		100
25	30		98	6.0	30		101
33	30		100				
44	30		100				

TABLE III

POSSIBLE INTERFERENCES WITH THE DERIVATIZATION OF ACID CHLORIDES

For the names of the acid chlorides A, B, C and D: see Table II.

Interfering compound	*Added (%)**	*% Acid chloride converted to amide*			
		A	*B*	*C*	*D*
Carboxylic form	10	100	100	100	100
Water	0.5	100	100	99	90
	1.0	100	99	86	81
	5.0	100	96	75	62
Diethylamine	0.01	99	12	54	90
	0.10	40	5	48	71

* For the carboxylic form % (w/w) of corresponding acid chloride and for water and diethylamine % (w/w) of the acetonitrile.

and 2-ethoxy-1-naphthoyl chloride being the least reactive. In order to keep the overall analysis time at a minimum, sufficiently high molar ratios were selected for subsequent determinations. Accurate and reproducible results were obtained for the determination of acid chlorides (1) that contain an amine hydrochloride group (α-aminophenylacetyl chloride hydrochloride and 1-amino-1-cyclohexane-carbonyl chloride hydrochloride), (2) that were synthesized with PCl_3 (2-ethoxy-1-naphthoyl chloride) and (3) that do not contain an ultraviolet light-absorbing group (1-amino-1-cyclohexanecarbonyl chloride hydrochloride).

In order to illustrate the specificity of this derivatization procedure, the carboxylic acid forms were taken through the reaction procedure and no detectable derivative was formed. The presence of up to 10% of the corresponding carboxylic acid in the acid chloride sample did not interfere with the ammonolysis reaction, as illustrated in Table III. The effects of two other possible interfering compounds are illustrated in Table III. Water, which may be present in the acetonitrile that is used as the reaction solvent medium, interferes with the reaction. However, the distilled-in-glass acetonitrile used in our studies contained less than 0.01% water. The water content was determined by Karl Fischer titrations. Other interfering substances which may be present in the acetonitrile reaction medium are primary and secondary alkyl amines. To illustrate the alkyl amine interference, diethylamine was added to the acetonitrile at low levels before the derivatization step. For all the acid chlorides studied, there was a significant reduction in the amount of acid chlorides converted to the corresponding chlorophenyl amide derivatives. However, the amine content for the distilled-in-glass acetonitrile we used was less than 0.0005% as determined by titration.

CONCLUSIONS

The method described in this report provides a very useful procedure for the quantitative, reproducible, rapid and easy determination of acid chlorides. The stable amide derivative, whose formation is a result of an ammonolysis reaction, is separated from the 4-chloroaniline derivatizing agent and the carboxylic acid forms on

a microparticulate octadecyl column with adequate resolution in a reasonable elution time.

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