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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION AND QUANTITATION OF POLYFUNCTIONAL AMINES AS THEIR *m*-TOLUOYL DERIVATIVES

S. L. WELLONS and M. A. CAREY

Union Carbide Corporation, Chemicals and Plastics, P.O. Box 8361, South Charleston, W.Va. 25303 (U.S.A.)

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SUMMARY

We have developed a high-performance liquid chromatographic method to determine aliphatic, polyfunctional amines at low levels in water, ammonia and other amines. This method is potentially valuable for determining trace amine by-products in industrial samples, such as crude and refined piperazines. It is superior to gas chromatography which is plagued by tailing, poor reproducibility and low amine volatility. The lack of a general or specific liquid chromatographic detector for aliphatic amines was circumvented by preparing *m*-toluoyl derivatives measurable with available ultraviolet detectors. These derivatives have desirable solubility characteristics which facilitate isolation by extraction.

INTRODUCTION

Aliphatic polyfunctional amines are valuable chemicals in many areas of industry and medicine. For example, two of the compounds studied, putrescine (1,4-diaminobutane) and cadaverine (1,5-diaminopentane), are present at elevated concentrations in the urine of cancer patients[1]. Other compounds, 1,2-diaminoethane, 1,6-diaminohexane, monoethanolamine and piperazine, are used extensively in agricultural chemicals, polyamide resins (Nylon), ethyleneurea resins, chelating agents, additives for lubricating oils, rubber chemicals[2] and many other areas. As a result, measurement of these compounds at low levels in process streams, urine, end-use products and wastes is important.

Polyfunctional amines have been difficult to impossible to measure by gas chromatography when water, ammonia and/or amine residues are present. Typically polyfunctional amines have low volatility and exhibit tailing and poor quantitative recoveries, because they are so reactive. These characteristics are particularly undesirable when it is important to measure low concentrations of amines in the presence of high concentrations of other amines or water.

High-performance liquid chromatography (HPLC) can overcome these disadvantages of gas chromatography. With HPLC volatility is not a factor. It is obvious

that if ultraviolet (UV)-absorbing derivatives are prepared, the amine derivatives would be detectable at low levels. Preparing such derivatives eliminates the polar amine groups and reduces the likelihood of tailing and poor recoveries. The many solvent–solute and solute–stationary phase interactions that can be manipulated virtually guarantee successful separations.

Satisfactory analyses have been reported for some polyamines such as spermidine, spermine and putrescine as their *p*-toluenesulfonyl derivatives with UV monitoring[3], as their Dns (5-dimethylaminonaphthalene-1-sulfonyl) derivatives with both UV monitoring[4] and fluorescent monitoring[5] and as their fluorescamine derivatives with fluorescent monitoring[6].

EXPERIMENTAL

Materials

The piperazine was obtained from J. T. Baker (Phillipsburgh, N.J., U.S.A.). The 1,5-diaminopentane, 1,4-diaminobutane, 2,5-dimethylpiperazine, 1,2-diaminopropane and *m*-toluoyl chloride were purchased from Aldrich (Milwaukee, Wisc., U.S.A.). The 1,6-hexanediamine, 3,5-dinitrobenzoyl chloride, benzoyl chloride, *m*-nitrobenzoyl chloride and anisoyl chloride were purchased from Matheson, Coleman, & Bell (Norwood, Ohio, U.S.A.). The 1,2-diaminoethane, monoethanolamine, N-methylethanolamine, 2,6- and 2,5-dimethylmorpholine, *p-tert.*-butylbenzoyl chloride and phenoxyacetyl chloride were prepared by Union Carbide (New York, N.Y., U.S.A.). All compounds were of high purity and were used without further purification.

The chromatographic column, 300 × 4 mm I.D., was prepacked with 10-μm average particle size μBondapak C_{18} by Waters Assoc. (Milford, Mass., U.S.A.). The mobile phase was distilled water – acetonitrile (nanograde; Mallinckrodt, St. Louis, Mo., U.S.A.; used without purification) (3:2, v/v). The mixture was filtered prior to use through a 0.2-μm Fluoropore membrane pad (Millipore, Bedford, Mass., U.S.A.).

Apparatus

A Model 820 liquid chromatograph (DuPont, Wilmington, Del., U.S.A.) was used throughout this study. A Chromatronix 200 detector, 254 nm (Chromatronix, Santa Clara, Calif., U.S.A.) was employed. A Valco VSV-6HP 6-port high-pressure valve with a 35-μl external loop (Valco Instruments, Houston, Texas, U.S.A.) was used to inject the analytical samples. The chromatograms were recorded on a Honeywell Electronik 16 strip chart recorder at a 0.254 cm/min (0.1 in./min) chart speed. HPLC conditions: pressure, 750 p.s.i.g. (50 atm); flow-rate, 0.9 ml/min; temperature, ambient.

Procedure

The samples and standards were dissolved 1% (w/w) in pyridine, freshly redistilled from phthalic anhydride, and 1,6-diaminohexane was added as an internal standard. Approximately three times the stoichiometric amount of *m*-toluoyl chloride was added and allowed to react for 5 min at room temperature. 5 *N* Hydrochloric acid was added (to destroy the excess reagent and to prevent the extraction of pyridine)

with stirring and cooling until the pH approximates 2.5. After cooling to room temperature, the derivatives were extracted with Mallinckrodt nanograde dichloromethane. The dichloromethane fraction was washed with 1% Na_2CO_3 in water to remove *m*-toluic acid and other reaction by-products. It was further washed with 0.5 *N* HCl and water as precautionary measures to prevent hydrolysis of the derivatives. The derivatives were found to be stable for at least 6 months in the dichloromethane fraction. Because the dichloromethane concentration in the injected solution cannot exceed 10% without disturbing the HPLC baseline, a final sample concentration of 10 mg/ml was prepared by either diluting the dichloromethane fraction with acetonitrile or evaporating the dichloromethane fraction and redissolving it in acetonitrile.

RESULTS AND DISCUSSION

Previously Carey and Persinger[7] in our laboratory used 3,5-dinitrobenzoyl derivatives very successfully to measure low levels of polyalcohols. When 3,5-dinitrobenzoyl derivatives of several polyfunctional amines were prepared, the piperazine formed a 3,5-dinitrobenzamide that was insoluble in water-immiscible solvents. Extraction into a water-immiscible solvent is a practical means of isolating the derivatives from the spent reagents and reaction matrix, and solubility is required for extraction. Since the ability to measure piperazine was important for our study, seven other acid chlorides were evaluated. Their structures are shown in Fig. 1.

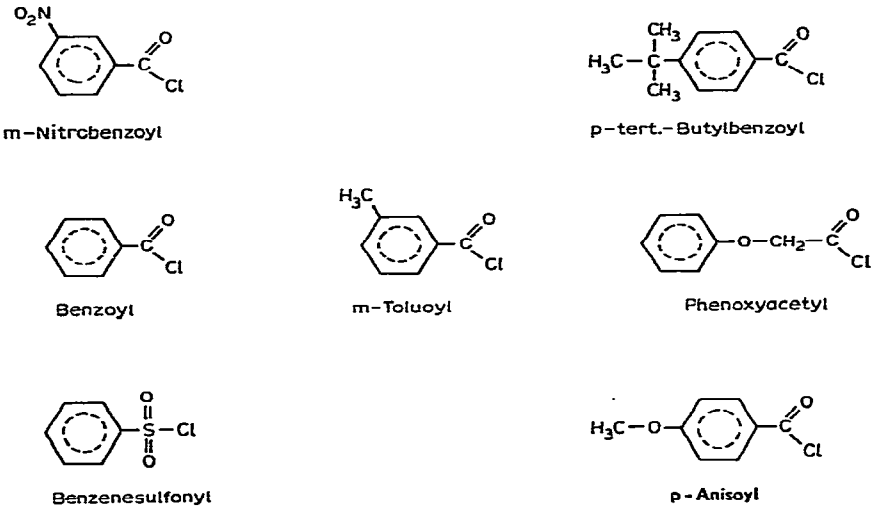
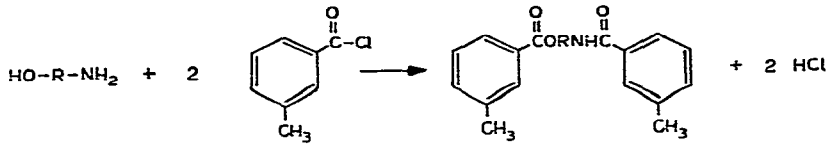


Fig. 1. Acid chlorides evaluated with polyfunctional amines.

m-Nitrobenzoyl and benzenesulfonyl chlorides gave amides of 1,2-diaminoethane and piperazine that were insoluble in water-immiscible solvents. Benzoyl chloride derivatized 1,2-diaminoethane to form an amide that was insoluble in water-immiscible solvents. Phenoxyacetyl chloride attacked the pyridine, and of the other

three reagents, only *m*-toluoyl chloride gave a reaction that was free of by-products. It also provided the derivatives that were most easily separated. The separability of the derivatized components and the absence of side reactions were established by using thin-layer chromatography on silica gel GF plates with a heptane–ethyl acetate (1:1) mobile phase. Furthermore, *m*-toluoyl chloride is easy to handle since it is a liquid and can be dispensed with a syringe or pipette.

The following equation describes the reaction of *m*-toluoyl chloride with polyfunctional amines:



Note that because the analyte is labeled at each active hydrogen, the absorbance for multifunctional compounds is high and the sensitivity is high.

The reaction conditions were optimized with a layered factorial experiment (Table I) in which the reaction time, reaction temperature and extraction conditions were varied. The optimum temperature is room temperature and the optimum time is 5 min or less. 100% Excess of the *m*-toluoyl chloride over the stoichiometric amount required to react with amines, water and ammonia guarantees complete reaction with all of the amines. The amount of an unknown sample required can be calculated easily from the equivalents of water and total amines measured by titration. The HPLC conditions were also optimized and are given in the Experimental section. Fig. 2 shows a model separation of eleven *m*-toluoyl derivatives of polyfunctional amines. The efficiency is about 9000 theoretical plates per meter.

TABLE I

MULTIFACTOR EXPERIMENT USED TO OPTIMIZE DERIVATIVE REACTION

Impurity a, $R_F = 0.1$–0.4 (thought to be *m*-toluic acid); b, $R_F = 0.55$ and c, $R_F = 0.8$. The 1,2-diaminoethane ($R_F = 0.09$), piperazine (0.16), monoethanolamine (0.46) and N-methylethanolamine (0.57) spots were similar in all cases. Later HPLC analyses showed the same amount of derivatives were formed in the A through D cases.

Label	*Temperature (°C)*	*Reaction time (min)*	*Time of vigorous extraction funnel shaking (sec)*	*Impurities detected by TLC*			
				Unwashed	*Na_2CO_3 washed*	*HCl washed*	*H_2O washed*
A	25	5	30	a			
B	25	30	180	a			
C	25	60	30	a			
D	60	5	180	a			
E	60	30	30	a, b	b	b	b
F	60	60	180	a, b	b	b	b
G	98	5	30	a, b	b	b	b
H	98	30	180	a, b, c	b, c	b, c	b, c
I	98	60	30	a, b, c	b, c	b, c	b, c

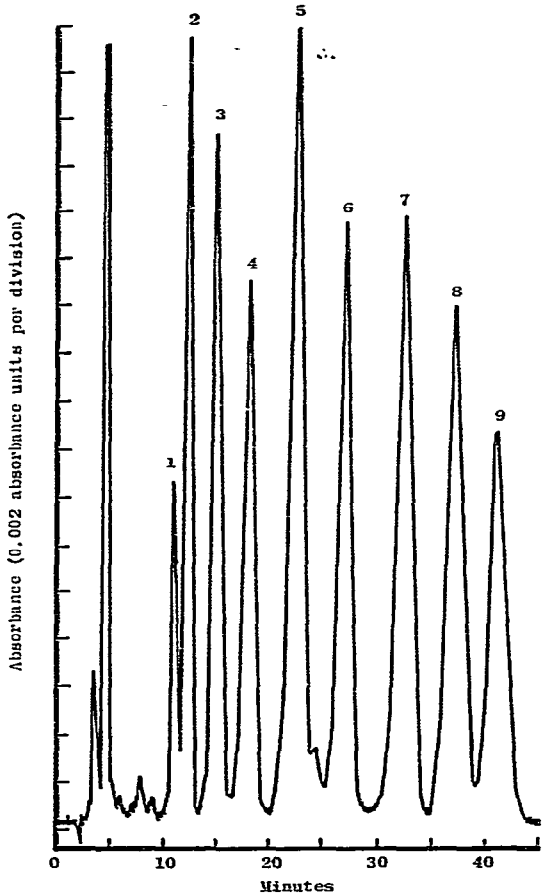


Fig. 2. Model separation of 11 *m*-toluoyl derivatives on μBondapak C_{18} with acetonitrile–water (40:60) as eluent. Peaks: 1 = 2,5-dimethylmorpholine; 2 = 2,6-dimethylmorpholine; 3 = 1,2-diaminoethane; 4 = (1,2-diaminopropane, 1,4-diaminobutane and piperazine); 5 = 1,5-diaminopentane; 6 = 2,5-dimethylpiperazine; 7 = 1,6-diaminohexane; 8 = monoethanolamine; 9 = N-methylethanolamine.

Five synthetic mixtures were analyzed; the recoveries and coefficients of variation are given in Table II. The quantities found were calculated using peak heights. The accuracy and precision were investigated for 1,2-diaminoethane from 8 to 80% (w/w), piperazine from 0.2 to 40% and monoethanolamine from 10 to 90%. The overall pooled coefficient of variation was 4% with 29 degrees of freedom. The percent recoveries are quite good, and the lowest level of 5 ng (weight as injected into to LC) piperazine was accurately measured. 1,6-Diaminohexane was used as an internal standard in calculating the quantitative data. Although quantitative data were collected only on three amines, the data should be typical.

No interference from ammonia or water in the sample was found at the levels of amines investigated. Water and ammonia in samples react with the *m*-toluoyl chloride reagent to form *m*-toluic acid and *m*-toluamide, respectively, which are

TABLE II

QUANTITATIVE DATA FROM ANALYSES OF SYNTHETIC MIXTURES

Water and NH_3 contents of sample 5 equal to 25.7 and 10.3% (w/w), respectively.

Amine	*Mixture number*	*Wt. (mg) amine reacted with 1 ml of m-toluoyl chloride*	*No. of measurements*	*Average recovery (%)*	*Coefficient of variation (%)*
1,2-Diaminoethane	1	45.83	off-scale	not measured	—
	2	7.67	6	103.3	3
	3	46.01	3	94.4	3
	4	73.61	4	95.9	4
	5	15.27	3	102.8	2
Monoethanolamine	1	18.07	1	98.2	—
	2	90.85	3	100.2	1
	3	45.43	3	101.2	4
	4	9.09	4	100.7	2
	5	15.06	2	98.3	6
Piperazine	1	40.68	1	100.3	—
	2	0.1715	4	100.0	20
	3	1.715	3	97.7	7
	4	8.575	4	99.4	5
	5	22.60	2	100.9	1

removed in the Na_2CO_3 wash. Sample 5 in Table II shows that water at the 26% (w/w) and ammonia at the 10% (w/w) levels did not significantly affect the percent recoveries or precision of the method, provided, of course, that sufficient reagent is used to react completely with all the amines, water and ammonia.

CONCLUSIONS

From our findings as well as the work of others, many chromaphoric groups can be added to amines; however, such a derivatizing reagent should meet at least the following criteria:

(1) be stable and safe to handle;
(2) react quantitatively with the amine;
(3) form a derivative that easily separates from the sample matrix of water, ammonia, reagent, etc.;
(4) and, of course, the derivatives should be easily separated by chromatography.

If the chromaphoric group is very large, the differences in properties between the amine derivatives become small and separation may be more difficult. The *m*-toluoyl group is almost the smallest UV-absorbing group that can be added, and it minimizes this problem.

This study established an accurate and precise HPLC method for measuring low levels of polyfunctionzl amines in water, ammonia and other amines. The method is superior to gas chromatography which is plagued by tailing, poor reproducibility

and low amine volatility. This method is potentially valuable for determining trace amine by-products in industrial samples, such as crude and refined piperazines or in biological samples, such as urine.

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