Determination of Dimethylaminopropylamine in Alkylaminoamides by High-Performance Liquid Chromatography

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Abstract

Dimethylaminopropylamine (DMPA), a diamine which, together with salicylaldehyde, forms a Schiff base, is used as a raw material in the synthesis of aminoamides of the fat chain. These aminoamides can in turn be the basis of some compounds from the tensioactive industrial area: betaines, aminic oxides, and quaternary ammonium salts. The derivatization reaction of DMPA is optimized using reversed-phase high-performance liquid chromatography with ultraviolet detection for its determination in samples of aminoamides.

Introduction

Dimethylaminopropylamine (DMPA), which holds great interest for industry, can be directly applied to the field of surfactant agents in the synthesis of alkylaminoamides and precursors of other compounds that are used in commercial formulas. The alkylaminoamides studied, R–CO–NH–$(CH_2)_3$–$N(CH_3)_2$ (where R represents a coconut chain [a mixture of $C_{12}H_{25}$ and $C_{18}H_{37}$] or undecylenic chain [$C_{11}H_{23}$]), can present levels of DMPA waste. Legislation on the handling of hazardous industrial substances (1) established in July of 1980 that products containing between 1 and 10% of polyamines are considered irrritants and those with more than 10% are corrosives.

High-performance liquid chromatography (HPLC) is the technique chosen to determine DMPA. One of the main problems encountered, which is common to all aliphatic amines, is the low ultraviolet (UV) absorptivity of this functional group, which makes their detection difficult. Precolumn or postcolumn derivatization is the most widely employed solution that allows the use of fluorometric and UV–visible (UV–vis) detectors. In a large number of cases, derivatization is carried out before introducing samples in the chromatographic system.

It is desirable to develop an HPLC method for routine analyses using a reagent that offers the advantages of quick, easy derivatization, good stability, and high sensitivity toward the derivative.

Various derivatization agents can be used; most of them have aromatic rings that ensure absorption in the UV region. The reactions tend to be carried out in an organic medium, though they also take place in aqueous media. Acid chlorides make up a large group of potential derivatizing agents that react with primary and secondary amines, but they also may acylate some alcohols and imidazole groups (2–12). Of the acid chlorides, dansyl chloride (one of the most commonly employed agents), benzoyl chloride (a selective agent for amino groups if alcoholic groups are present), and dabsyl chloride require long reaction times (1–16 h), which makes them rather unsuitable for routine analyses. The use of *m*-toluoyl chloride requires the extraction of the derivative before chromatographic analysis, and the 2-naphthalenesulfonyl chloride has been applied in the biomedical field.

Several agents are selective for primary amines (13–16), such as *o*-phthaldialdehyde (which often forms unstable derivatives), acetylacetone (for determination of amine in environmental water samples), and 1-pyrenealdehyde (an aromatic aldehyde with fluorescent characteristics that requires long reaction times).

Another kind of reagents carried out the derivatization in solid phase (17–21). They have been mainly used for the determination of volatile amines in air.

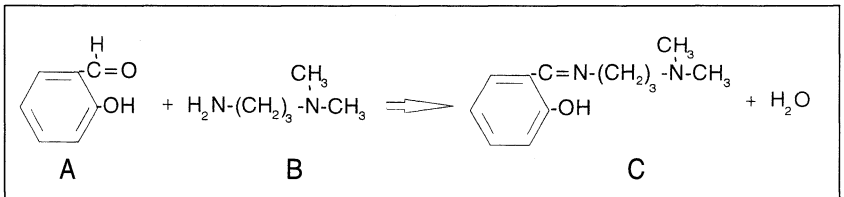

Figure 1. Derivatization reaction.

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A final type of derivatizing agents is characterized by fluorogenic detection (22–28), including halogenonitrobenzofurazans (which causes hypersensitivity; some of its derivatives are known to be very toxic) and luminarins (which, with a highly sensitive derivative, require long reaction times [1 h]). The derivatization time using 9-fluorenylmethylchloroformate is less than 2 min, but the excess reagent forms degradation products that may cause interferences.

There are even some analytical cases without derivatization (29,30) or with ionic reagents (31,32). Finally, another family of chemicals with fluorescent properties and absorption in the visible region (33,34) has application in thin-layer chromatography (TLC) fluorometry and as a spectrophotometric reagent, but without previous application in LC.

In this study, salicylaldehyde (SA) was used as a derivatizing agent of DMPA in aminoamides of the fat chain by reversed-phase chromatography with a cyano column. Using the same reagent, Bauer and Ritcher (35) determined the free amine (DMPA, diethylenetriamine, etc.) of polyaminoamides in epoxy resins by separation with gel permeation chromatography; the retention time was long (16 h), and the columns used were quite expensive. Neither problem occurred with the matrix or with the conditions of chromatographic separation studied here.

SA (Figure 1A) reacts with the primary group of DMPA (Figure 1B) to form the imine derivative (Figure 1C). In our study, the SA was selective with the DMPA because the aminoamide contains only a tertiary group. Additionally, the resultant Schiff base from the reaction absorbs in the UV–vis region, which facilitates detection and quantitation.

Use of this reagent allowed for quantitative derivatization in one step, one phase, and a satisfactory time for a derivatization reaction. The excess SA did not need to be removed because it did not cause solubility problems or reaction with the matrix, and its manipulation held no risk of irritability.

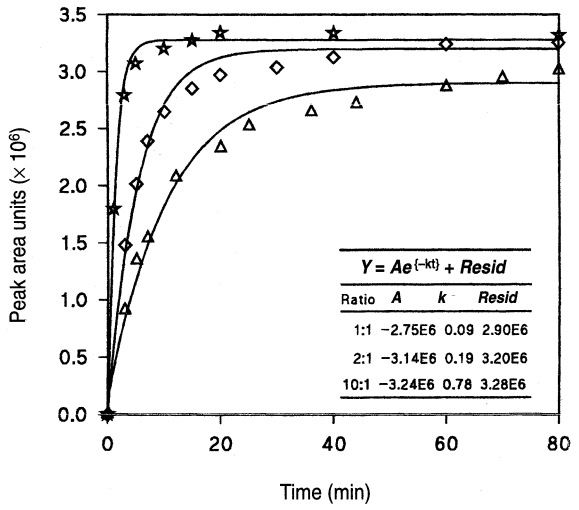

Figure 2. Influence of excess reagent (SA) on DMPA derivatization time at room temperature in acetonitrile (△ = 1:1, ◇ = 2:1, and ★ = 10:1). Kinetic equations are included (see text for details).

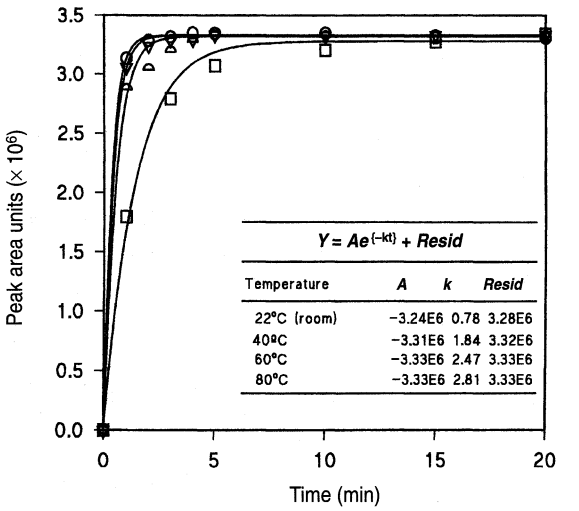

Figure 3. Influence of temperature on DMPA derivatization time for a 10:1 ratio (□ = room temperature, ⌓ = 40°C, ▽ = 60°C, O = 80°C). Kinetic equations are included (see text for details).

Experimental

Reagents and apparatus

The HPLC system consisted of a Hitachi-Merck (Tokyo, Japan) with a gradient control L-500, pump 655A-12, variable wavelength detector 655A, and chromato-integrator D-2000. The analytical column was a LiChrospher CN (125 × 4.0-mm i.d., 5-µm particle size). The solvents and reagents used were acetonitrile (Merck, Darmstadt, Germany), water (Merck), methanol (Merck), SA (Merck), and DMPA (Aldrich Chemical, Steinheim, Germany). An eluent was prepared with 0.1M of CH_3COONa (BDH, Poole, England); acetic acid was used to adjust the pH to 5 and was filtered through 0.2-µm filters (Anotec, Banburry, England).

Separation of excess reagent from the DMPA derivative was carried out in the cyano column using a mobile phase of 80%

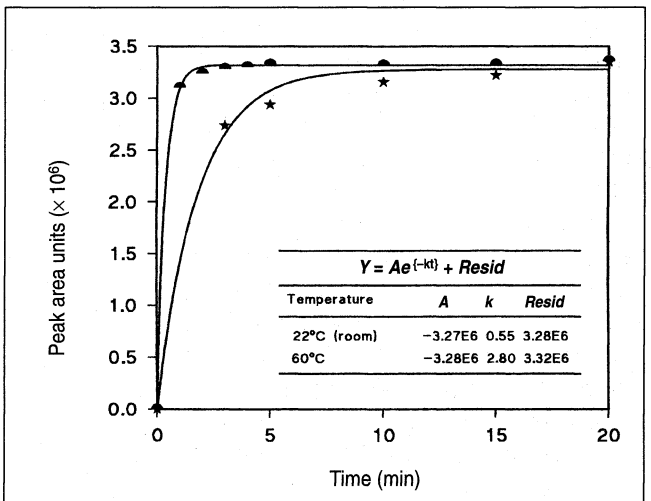

Figure 4. Effect of solvent on the derivatization reaction in methanol for a 10:1 ratio (★ = 22°C, ⌓ = 60°C). Kinetic equations are included (see text for details).

methanol and 20% of the eluent at a flow rate of 0.8 mL/min. The injected volume was 20 µL. Detection was carried out at the maximum absorption of the derivative, 218 nm.

Preparation of standards

Stock solutions of SA (0.02M) and DMPA (0.08M) were prepared in acetonitrile, diluted to 4×10^{-3}M, and used to study different conditions for the derivatization process.

Derivatization procedure

Analyzed samples of coconut and undecylenic aminoamides presented a maximum 1% of DMPA and were derivatized as follows: 1 mL of the product (40 mg/mL acetonitrile) and 2 mL of SA (0.02M) were mixed in a flask in a 10:1 ratio (this corresponds to a minimum of 10-fold molar excess of reagent). This mixture was placed in a 60°C glycerol bath for 4 min. After that, the mixture was diluted in a 10-mL volumetric flask before chromatographic analysis.

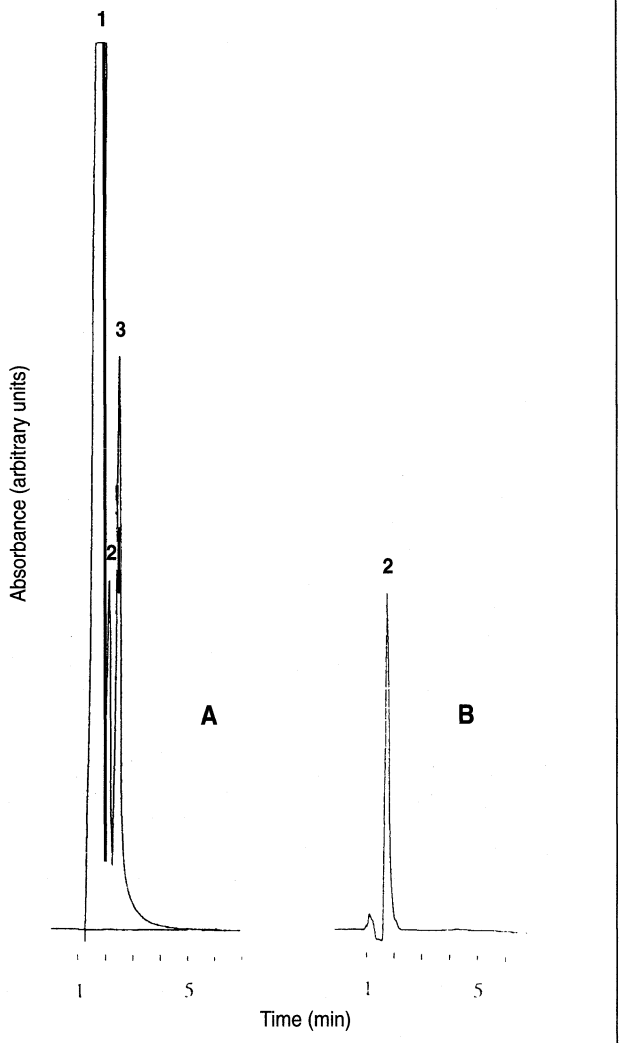

Figure 5. (A) Chromatogram of the coconut aminoamide derivatized in optimized conditions. (B) Chromatogram of the underivatized coconut aminoamide. Column: LiChrospher CN, (125 × 4.0-mm i.d., 5-µm particle size); mobile phase: MeOH–0.1M CH_3COONa at pH 5.0 (80:20); flow rate: 0.8 mL/min; detection: 218 nm. Peaks: 1, excess SA; 2, coconut aminoamide; and 3, DMPA derivative or Schiff base.

Results and Discussion

Derivatization conditions

Derivatization was optimized by selecting the conditions that allowed the total reaction to be achieved as quickly as possible. Various factors were taken into account to get a quantitative reaction: excess reagent, temperature, and solvent. The reaction end point was established by the stabilization of the derivative's peak.

Effect of excess reagent, temperature, and solvent

The effect of excess reagent on the kinetics was determined by using different molar ratios of SA to DMPA (1:1, 2:1; 10:1; 50:1, and 100:1).

A plot of the derivative's peak area versus time (for the different relationships) is shown in Figure 2. An increase in the reaction velocity was achieved with the 10:1 ratio (80 min versus 15 min). Molar ratios of 50:1 and 100:1 were tested, but the peak of excess SA partially overlapped the peak for the derivative; in order to achieve a good separation, it was necessary to increase the percentage of methanol in the mobile phase to 90%. Under such conditions, the peaks were too broad. The 10:1 ratio was chosen, and more variables were optimized.

On the other hand, comparison of the peak areas showed a maximum yield for an excess of derivatizing agent in the ratio of 10:1. The sensitivity improved significantly when the ratio was increased from 1:1 to 10:1. A monoexponential kinetic (first order) decay with residual was confirmed with the experimental values. In the corresponding equation, $Y = Ae^{(-kt)} + Resid$, Y is the derivative's peak area, A is a constant, e is the inverse function of the natural logarithm, k is a first order rate constant, t is the derivatization time, and *Resid* is the peak area at the end of the reaction.

In order to reduce the analysis time, the effect of temperature on reaction velocity was tested (Figure 3). The derivatization was studied at room temperatures (22°C), 40, 60, and 80°C. The higher temperatures allowed the reaction to proceed more rapidly. Increasing the temperature to 60°C led to a stabilization of the chromatographic peak area at 3 min. The time of derivatization was unaffected when the temperature was increased to 80°C; for this reason, 60°C was adopted for the procedure. A certain safety margin is necessary, thus the reaction time of 4 min was used. The peak area did not appear to be markedly increased with respect to that obtained at room temperature. The kinetic equation is the same as shown the one in Figure 2.

The effect of solvent on reaction velocity was evaluated using a methanolic medium at a molar ratio of 10:1. The time of derivatization was 3 min at 60°C and 15 min at room temperature. Results at room temperature and at 60°C (methanol boils at 64.5°C) were analogous to those obtained in acetonitrile (Figure 4). A monoexponential equation for Figure 4 conveys a

Table I. Determination of Free DMPA in Real Samples

Job batch no.	Free DMPA (%)	RSD (%)*
218/92	0.36	1.23
268/92	0.48	0.78
311/92	0.70	0.83
397/92	0.93	0.71
159/93	0.97	0.96

* RSD = relative standard deviation. Six replicates.

Table II. Recovery of DMPA from Alkylaminoamides

Amount (mg/L)			
Added	Found	Recovery (%)	RSD (%)
13.33	13.32	99.90	1.93
23.60	22.90	97.05	1.02
30.83	31.00	100.54	1.12
40.14	40.55	101.03	1.17

kinetic first order relationship, as in Figures 2 and 3.

Methanolic medium could be optimum for other soluble compounds, such as betaines, but due to the good solubility of the matrix studied, acetonitrile was used.

Linearity, detection limit, quantitation limit, and precision

Experimental spectra revealed three absorption bands: 210–230, 250–270, and 300–340 nm. The Schiff base showed a fourth absorbance band around 420 nm, but it was rather less intensive. The 218-nm wavelength was chosen because it presented higher absorption values for the DMPA derivative.

The purity of the peaks was verified in real samples with a diode array detector (Waters, Milford, MA). The linear range was studied by analyzing 10 concentrations from 0.6 mg/L (near the quantitation limit) to 100 mg/L (near the upper levels, the detector signal was saturated), which were referred to the injected solution with 20-µL injections. A correlation coefficent of 0.9991 was obtained (each regression point was the average of two injections for each standard).

Another important analytical parameter for further studies of DMPA traces in formulation is the detection limit (36). A detection limit of 0.13 mg/L and a quantitation limit of 0.4 mg/L were obtained.

Precision for the derivatization procedure included deviations from the sample injection and the reaction of SA with DMPA. For 10 different derivatizations, a relative standard deviation of 1.3% was obtained. It was measured at a concentration of 33.5 mg/L.

Determination of DMPA in real samples

The optimized method was applied for the determination of free DMPA in coconut and undecylenic chain aminoamides. In the volumetric analysis in acetic medium (37), the DMPA values were close to 1%. Five job batches were analyzed (Table I). It was verified that the matrix did not interfere in the analysis because recoveries close to 100% were obtained when different quantities of DMPA were spiked in a coconut aminoamide (Table II). The underivatized coconut aminoamide (Figure 5B) presented a peak at 1.9 min. In the chromatogram of the derivatized sample (Figure 5A), the peak of the DMPA derivative and the reagent excess are also shown.

Conclusion

The use of salicylaldehyde as the derivatizing agent allowed derivatization in a short time and required no extraction. Its derivative was stable. The determination of DMPA in samples of fat aminoamides was achieved under isocratic conditions with short retention times by using solvents that were compatible with the analysis samples (acetonitrile and methanol). The method allowed DMPA quantitation with good linearity and precision. It was possible to detect DMPA down to 0.1 ppm.

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