High Performance Liquid Chromatographic Separation of Fatty Imidazolines from Their Diamide Hydrolysis Derivatives

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ABSTRACT

A reverse-phase high performance liquid chromatographic method is described which separates long-chain fatty dialkyl imidazolines from their corresponding diamide hydrolysis derivatives. This permits following the synthesis of these compounds and their storage stability. With a mobile phase of methanol/dilute acetic acid and a differential refractometer, both qualitative and quantitative analysis can be done quickly. Elution order follows chain length with the imidazoline eluting before the diamide. Capacity and selectivity factors are presented for the imidazoline-diamide pairs from pelargonic to lauric acid.

INTRODUCTION

Fatty imidazolines are surface-active materials used in a wide variety of applications, such as detergents (1), waterproofing compounds (2), soil conditioners (3), and as intermediates in the manufacture of textile fabric softeners (4). One type of imidazoline is prepared via the reaction between diethylene triamine with fatty acids, triglycerides, or methyl esters of fatty acids. The reaction proceeds essentially in two steps. First an amide forms according to the scheme:

$$2\ RCOOH + HN(CH_2CH_2NH_2)_2 \rightarrow$$

$$R\ CON(CH_2CH_2NH_2)CH_2CH_2\ NHCOR + 2\ H_2O$$

and secondly, the diamide cyclizes, a reversible reaction, to form an imidazoline ring:

$$RCON\ (CH_2CH_2NH_2)CH_2CH_2NHCOR \rightleftharpoons$$

$$RC = NCH_2CH_2NCH_2CH_2NHCOR + H_2O$$

The imidazoline formation occurs readily at elevated temperatures (150-200 C) and is accelerated under a vacuum which aids in water removal (5). The imidazoline is hydrolyzed quantitatively to the diamide in the presence of water in ca. 4 days at room temperature.

The above reaction and the storage stability of the synthesized imidazolines need to be studied by reliable analytical methods. Conventional titration methods have been used for this purpose. One involves titration of free (i.e., unreacted) fatty acid with standard sodium hydroxide. In another, the total amine content is determined by titration with standard hydrochloric acid. The sum of secondary and tertiary amine content is obtained by first reacting the sample with salicylaldehyde, followed by titration with standard HCl. The tertiary amine (imidazoline) content is determined by first reacting the sample with phenyl isothiocyanate for 1 hr, followed by titration with standard HCl.

The drawbacks to the above methodology are several. The concurrent titrations are time-consuming. The primary amine content is determined indirectly (by difference, total amine—[secondary + tertiary amines]) and cannot be differentiated from the primary amine group of the diamide or of the starting diethylene triamine. The secondary amine content (unreacted diethylene triamine) is likewise determined indirectly (by difference, [secondary + tertiary amine] - tertiary amine).

The imidazoline content can be determined directly by ultraviolet (UV) spectrophotometry at 230 nm where it produces a characteristic absorption peak. However, the amide absorption peak at 202 nm does not permit a direct determination of the diamide content since the amido side chain of the imidazoline also absorbs at 202 nm. The UV spectrum of a mixture of imidazoline and diamide frequently gives a spectrum in which the 202 nm absorbance is merely a shoulder on the 230 nm peak. A chromatographic method which would separate imidazoline from diamide would thus offer a tremendous advantage.

Molina (6) used gas liquid chromatography (GLC) to separate imidazoline salts. Recently, Batukova (7) and his associates used GLC to separate alkylimidazolines from their synthetic starting materials and the amide derivative. More recently, Parris (8) demonstrated that high performance liquid chromatography (HPLC) could separate ionic and amphoteric surfactants. This study shows how HPLC can be used to separate, identify, and quantitate fatty dialkylimidazolines from their corresponding diamides.

EXPERIMENTAL

Materials

The imidazolines and diamides were prepared as previously described (5). Methanol and acetic acid were ACS reagent grade (Baker Company) used without further purification. Singly distilled water was used. All solutions were thoroughly degassed prior to use.

Method

The equipment used included a high-pressure liquid pump (Milton Roy Model Mini Pump), a differential refractive index detector (Waters Model R401), and a spring-loading sample injector (Rheodyne Model 7120). A porous metal filter was fitted on the inlet tubing to the pump. The separation column was a 25 cm reverse-phase type (Whatman Partisil-10,ODS-3) protected by guard column (Whatman Company, Co:Pell ODS).

The 200-μL loop was used on the sample injector. A flow rate of 0.93 mL/min was obtained with a pressure of ca. 700 psi. The signal was sent to a 10-in. recorder (Gould, Inc.) operated at 1 mV sensitivity.

Three thin layer chromatography (TLC) microscope slides (Whatman $MKC_{18}F$) coated with a C_{18} reverse-phase material were used as received from Whatman.

RESULTS AND DISCUSSION

Fatty imidazoline could not be separated from its diamide derivative by HPLC with a solvent system of either methanol/water or methanol/chloroform. However, TLC showed that the diamide from pelargonic acid had an R_f of 0.6 under acid conditions. With this information, solutions of methanol/dilute acetic acid were prepared and used to separate imidazolines and diamides of several chain lengths.

Figure 1 shows the separation of imidazoline from diamide where R = C_9. Resolution is estimated to be 1.25, based on a 1:1 standard curve (9). Note that good separation is obtained in less than 12 min. The imidazoline com-

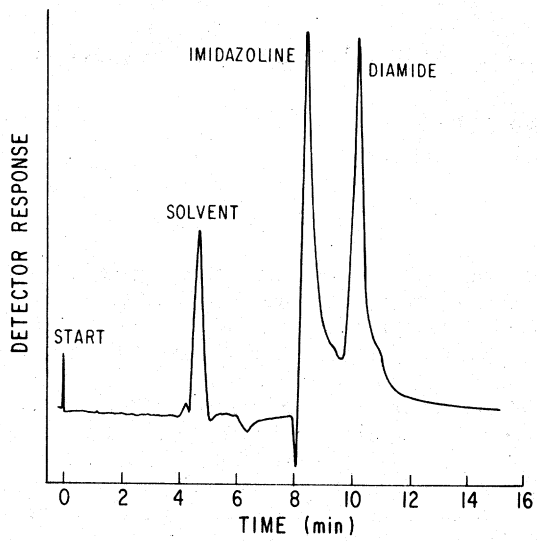

FIG. 1. HPLC separation of the mixture of dialkylimidazoline and diamide derived from decanoic acid. Mobile phase 80:10 methanol/0.1 N acetic acid.

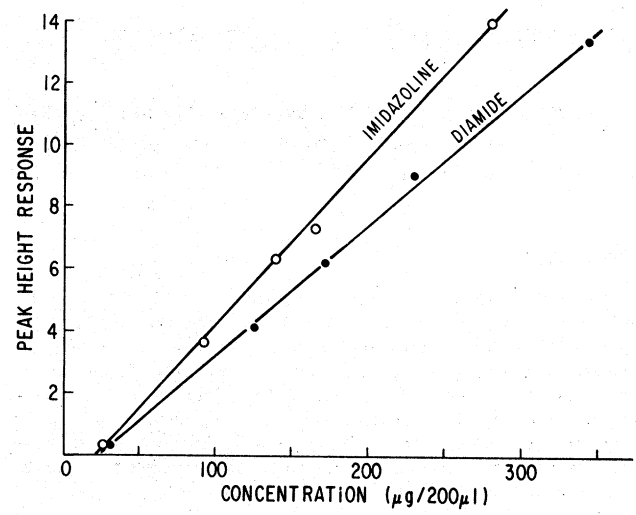

FIG. 2. Peak height response for imidazoline and diamide derived from pelargonic acid in the concentration range 30-340 μg/200 μL. Peak heights are at 8X attenuation, Waters RI detector.

pound is first to elute, followed by the diamide.

It should be noted that imidazolines are hydrolytically unstable. Their solutions, therefore, should be prepared by first dissolving the imidazoline in absolute methanol, preferably with heating. Water and acetic acid should be added to the cold methanol solution, and the resulting solution chromatographed immediately. Imidazoline samples which have been allowed to stand for several hours in solution might show a decreased imidazoline content.

Figure 2 shows the recorder response 8X attenuation as a function of imidazoline and diamide concentration (R = C_8). It can be seen that the peak height response is linear in the range of 30-300 μg/200 μL concentration. Below a concentration of 40μg/200 μL, the compounds are barely detectable. Probably because it elutes first, imidazoline shows a better response than does the diamide.

Table I lists the capacity and selectivity factors for several compounds of different chain length. Each imidazoline or diamide contains two of these R groups as shown in step 2 of the reaction given earlier. Note that the selectivity factors are calculated for the separation of imidazoline from the diamide. The shorter chain material has much more solubility in water than the longer chain material. A solvent system of 70:30 methanol/0.1 N acetic acid provides good k' factors, as well as good selectivity, $1.1 < \alpha < 2$, and thus is a good analytical system for shorter chain material. With increasing methanol, k' decreases rapidly and falls outside the optimum range, $1 < k' < 10$. The lauric acid derivatives are best analyzed by decreasing the water content. A system of 90:10 methanol/0.1 N acetic acid provides adequate capacity factors and selectivity for these compounds. With the 90:10 methanol/0.1 N acetic acid system, the diamide from lauric acid is eluted in 8.4 min, k' is 1.7. Table I shows that the imidazolines, R = C_8-C_{11}, have sufficiently different k' and could be separated in a mixture. This has been done with good results with a solvent system of 80:20 methanol/0.1 N acetic acid. The complete chromatographic run took 20 min. Separation of a diamide mixture has not been tried. It is clear that a mixture of all imidazolines and diamides in the table would not be separated readily. The k' values indicate that there would be some peak overlap, e.g., the imidazoline from decanoic acid and the diamide from pelargonic acid would coelute. However, since these two compounds would not normally be found together, this is not a serious problem. It should be pointed out that the C_{10} group contains one double bond (undecylenic acid derivative) which makes its elution faster than would normally be expected if the compound was saturated.

In conclusion, HPLC has been shown to be useful for the analysis of fatty imidazolines and diamide derivatives. The technique can be used to follow synthesis and storage stability. As Table I shows that good separations are obtained by varying the solvent ratios, and, as mentioned earlier, imidazolines and diamides absorb in the UV region. Therefore, it is expected that gradient elution, increased mobile phase temperature, and UV detection would improve the method even more by optimizing the system selectivity and increasing detector response.

TABLE I

Capacity (k')[a] and Selectivity Factors (α)[b] for Separation of Fatty Imidazolines and Diamides

	Imidazoline k' (α)			Diamide k'		
R	(70:30)[c]	(80:20)	(90:10)	(70:30)	(80:20)	(90:10)
C_8	1.7(1.6)	.56(1.4)	.21(1.5)	2.8	.80	.32
C_9	–	.88(1.4)	.34(1.4)	–	1.20	.46
C_{10}(=)	–	1.10(1.4)	–	–	1.50	–
C_{11}	–	4.80(1.3)	1.20(1.4)	–	6.20	1.70

[a]$k' = (T_R \text{ sample} - T_R \text{ solvent})/T_R \text{ solvent}$, T_R is retention time.
[b]Selectivity factor $\alpha = k'$ (diamide)/k' (imidazoline).
[c]Solvent system is methanol/0. 1 N acetic acid.

ACKNOWLEDGMENT

The authors thank Robert A. Barford for his technical consultation.

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