The Determination of Fatty Amides by High Performance Liquid Chromatography

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A high pressure liquid chromatographic technique for separating fatty amides by chain length in the presence of fatty nitriles was developed. The separation used spherical silica with hexane/chloroform/glacial acetic acid (7:2:1, v/v/v) as the mobile phase. The HPLC method can be used to detect trace amounts of fatty amide in the presence of fatty nitrile with a recovery of 99%. Thin layer chromatography was used as a solvent scanning technique. The relationship between k values and R_f values was investigated.

Fatty nitriles are widely used as intermediates in the synthesis of many varieties of fatty amine chemicals. The one-step continuous conversion of fatty acids to fatty nitriles creates fatty amides, which are undesirable side-products (1). The incomplete removal of fatty amide during distillation can result in downstream production difficulties. Assessment of trace concentrations of fatty amide in a fatty nitrile matrix is an important facet of quality control.

Gas chromatography has given excellent separation of the homologs of fatty nitriles (2-3). It has given excellent separation of fatty amides on mixed phase Silar (4-5), but the thermal degradation of amides to nitriles during gas chromatography using a DEGS column has been reported (6). This would preclude the analysis of amides in nitrile using this system, particularly at the low concentrations studied herein. Recently, a gas chromatographic technique successfully separated fatty amides that had been extracted from polyolefin and vinyl resins (7). A variety of derivatization schemes have appeared (8-9) for gas chromatographic analysis of fatty amides. In our laboratories the use of a short (18") Dexsil column was studied (Rath, N.E., private communication), but the complete separation of fatty amide from fatty nitrile was troublesome.

A limited amount of work has been done on fatty amides using HPLC (10-11). The emphasis of that work was on the analysis of fatty amide as slip agents in polymeric films and not as trace contaminants in fatty nitrile production. The work was performed on a reverse phase column. If this system is used for fatty amide analysis in a fatty nitrile matrix, the nitrile would have a very long elution time.

Separation based solely on functionality is possible using TLC. Amides are easily separated from nitriles, but further separation of the chain lengths of the fatty amides has not been achieved and quantitation would be tedious. The use of TLC, however, has enhanced the development of HPLC as a method for amide determination and will be discussed.

Use of HPLC has allowed the observation of chain length distribution within the amide class at low levels in a nitrile matrix. The alteration of amide concentration by thermal degradation is avoided, and no derivatization step is required. Analyses are quickly done and show excellent reproducibility at low levels.

EXPERIMENTAL

Materials. Caprylamide. One hundred and fifty ml of crushed ice and 30 ml of 14 N ammonium hydroxide (reagent grade) were added to a 250-ml Erlenmeyer flask. Fifty four g of capryloyl chloride were added drop by drop with stirring. After standing for one hr, the solution was filtered.

Ten ml of isopropanol and 200 ml of hexane were added to the filtercake. The water fraction was decanted, and hexane was added to the hot solution until the solution became cloudy. The solution was cooled to room temperature and then cooled to 4 C for one hr. The solution was filtered and dried in a vacuum drying oven to a constant weight. Yield, 3.07 g, white platelets.

Capramide. As above, except caproyl chloride was used.

Lauramide. As above, except it was recrystallized twice from hot methanol.

Myristamide. As above.

Palmitamide. As above.

Oleamide. As above.

Stearamide. As above.

Arachidamide. As above, except it was recrystallized from hot ethanol and then further purified by collecting the eluant containing the arachidamide fraction. The HPLC conditions for this purification are specified below. The solvent was removed, and the white solid was dried in a vacuum drying oven.

Solvents. Hexane was ChromAR grade obtained from Mallinckrodt, Paris, Kentucky. Chloroform was Omnisolv grade from MCB, Cincinnati, Ohio. Glacial acetic acid was of reagent grade and came from J.T. Baker, Phillipsburg, New Jersey. All solvents were used as received.

HPLC apparatus and conditions. A Varian 5000 liquid chromatograph (Varian Associates, Sugarland, Texas) equipped with a Varian differential refractive index detector, a Spectra-Physics 4020 Data Interface

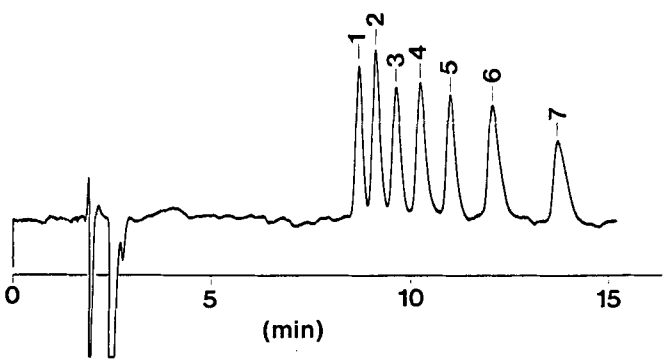

FIG. 1. Separation of the homologs of saturated fatty amides. Peak 1, arachidamide; 2, stearamide; 3, palmitamide; 4, myristamide; 5, lauramide; 6, capramide; 7, caprylamide.

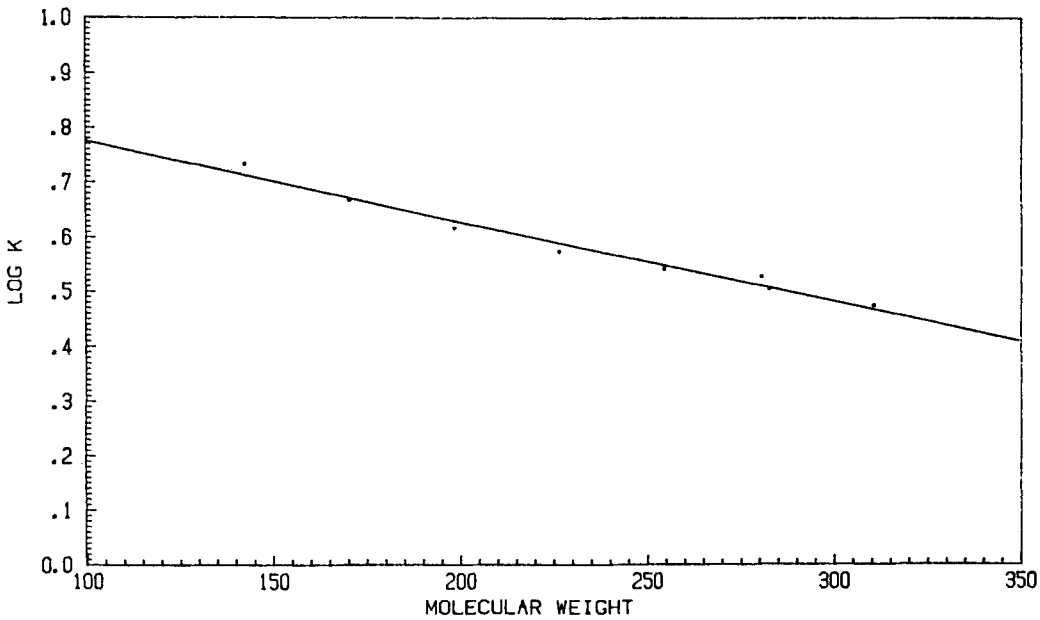

FIG. 2. Relationship between log (K) and molecular weight.

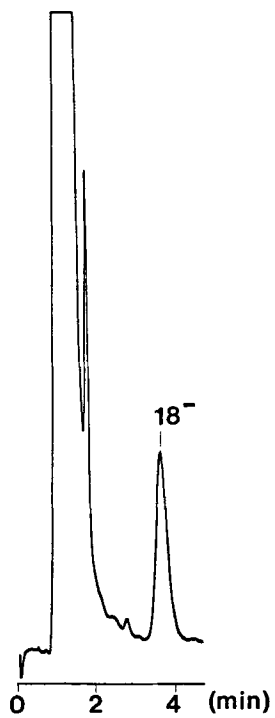

FIG. 3. Oleamide as trace component in soft tallow nitrile. Mobile phase, mixture of 70% hexane, 20% chloroform and 10% acetic acid, by volume.

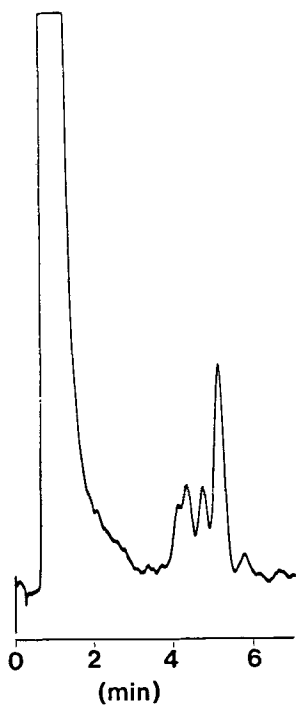

FIG. 4. Mixture of amides present as contaminants in coconitrile. Mobile phase condition is the same as in Figure 3.

TABLE 1

Weight Percent of Amide Added to Fatty Nitrile vs Quantity Measured by HPLC

Amide added	Added (wt %)	Found (wt %)	Percent recovery
Stearamide	0.048%	0.05%	104%
Stearamide	0.11 %	0.10%	91%
Oleamide	0.30 %	0.32%	107%
Oleamide	0.60 %	0.65%	108%
Oleamide	0.90 %	0.85%	94%
Oleamide	1.20 %	1.10%	92%
		Average recovery	99%

TABLE 2

Comparison of K Values Calculated from R and K Values Calculated from Retention Time

Compound	R_f	Calculated K from R_f values[a]	Calculated K from ret. time[b]
Caprylamide	0.14	6.14	5.56
Capramide	0.14	6.14	4.76
Lauramide	0.14	6.14	4.23
Myristamide	0.14	6.14	3.84
Palmitamide	0.15	5.67	3.54
Oleamide	0.17	4.88	3.40
Stearamide	0.17	4.88	3.21
Arachidamide	0.18	4.56	3.02
Stearylnitrile	0.63	0.59	0

[a]Where $K = (1-R_f)/R_f$.
[b]Where $K = (V_r - V_m)/V_m$

and a 4060 Remote Terminal (Spectra-Physics, Piscataway, New Jersey) was used in this study.

The column was 250 mm × 4.6 mm stainless steel packed with five micron Nucleosil 100 (Alltech Associates, Deerfield, Illinois). The flow rate for the optimized conditions was 1.75 ml/min (velocity, 2.14 mm/sec). For routine analyses, the flow rate was 3.25 ml/min (velocity, 3.79 mm/sec). The flow rate was determined by measuring the volume of solvent produced for a given period of time. The solvent was a mixture of 70% hexane, 20% chloroform and 10% acetic acid, by volume. For determining k values of the fatty amides, 0.25% solutions (w/v) were prepared in column solvent. Sample preparation for routine fatty amide analysis was done by mixing one part nitrile with two parts column solvent. All analyses were done at ambient temperature.

Thin layer chromatography (TLC). Silica Gel 60 F-254 TLC plates (EM Science, Cherry Hill, New Jersey) were used as supplied. The thin layer plate dimensions were 5 cm × 10 cm, 0.25 mm thickness.

TLC was carried out in a rectangular glass tank lined with paper saturated with ca. 100 ml of solvent. The solvent was a mixture of 70% hexane, 20% chloroform and 10% acetic acid.

The spots were developed by spraying the plates to saturation with a solution of 10% cupric sulfate in 8% phosphoric acid. The TLC plates were than charred on a hot plate at 190 C for 10 min.

RESULTS AND DISCUSSION

Figure 1 shows the separation of the fatty amides based on chain length using the optimized flow conditions of 1.75 ml/min. It is interesting to note that arachidamide elutes first and caprylamide last. This can be explained by the greater nonpolar character present in the longer chain amides, allowing less interaction on a molar basis with the relatively polar silica resulting in lower k values for the longer chain amides.

Figure 2 shows the relationship between logarithmic k values and the molecular weight of the fatty amides. A linear relationship was found for this class of amides. Base line separation of fatty amides is achieved using this technique.

This method was developed for the purpose of detecting trace quantities of fatty amide in fatty nitrile. Figure 3 is a typical example of oleamide present as an impurity in a soft tallow nitrile. The nitrile elutes with the solvent front and is well separated from the oleamide. Figure 4 is a coconitrile with more than one species of fatty amide present. This separation is not as efficient as the optimized separation in Figure 1, but it is sufficient for quantitation of fatty amide.

The results in Table 1 demonstrate the accuracy of this method. For this study, amide-free nitrile was prepared by collecting the nitrile fraction of the eluant. Purity was verified by reinjecting the nitrile and observing that the area of the chromatogram where fatty

amides elute was free of any peaks. The average recovery of spiked fatty amide was 99%. Various concentration levels of pure fatty amide were then added to the amide-free nitrile on a weight percent basis. The method is accurate to 1000 ppm for all fatty amides. Three-point calibration curves were linear for each fatty amide for one decade over the detection limit. Thus, HPLC is a fast, accurate method for detecting trace quantities of fatty amides in a nitrile matrix.

TLC was used as a rapid scanning technique to assess the effect various solvent combinations have on the separation of fatty amides from each other and from fatty nitrile.

The relationship between K values and R_f for normal phase chromatography can be expressed as $K = (1-R_f)/R_f$ (12). This relationship is generally correct for fatty amides and fatty nitriles (Table 2), but should be used as a guide rather than an exact representation of K values from R_f values.

Trying to predict high performance liquid column K values from thin layer R_f values remains an empirical science. An R_f value of 0.1–0.4 will give reasonable K values for HPLC in normal phase operation with flow rates of 1–3 ml/min. In general, if the R_f of the fatty amide is between 0.1 and 0.4, then the separation can be transferred to the HPLC system. If the R_f is greater than 0.4, then fatty amide will elute with the solvent front. If the R_f is less than 0.1, then the fatty amide will not elute from the column. The TLC system described has enough selectivity to separate the fatty nitriles from the fatty amides, but could not differentiate between the homologs (Table 2). Even so, TLC is a valuable tool for rapid, preliminary investigation of potential solvent systems.

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