Rapid Analysis of Dimer Acid by HPLC/FID

Robert L. Veazey
Union Camp Technology Center, Methods Development, P.O. Box 3301, Princeton, NJ 08543-3301

A method was developed for the rapid analysis of polymerized fatty acids (dimer acid) using normal phase HPLC with a flame ionization detector (FID). The use of analytical scale HPLC with the FID is a significant improvement over existing methodology for dimer analysis. The HPLC analysis takes only 25 min per sample, with no derivatization required. The FID response is linear for dimer samples from 10% to 90% monomer content. Absolute measurement precision is typically less than 0.5 area percent. Recovery of synthetic dimer blends averaged 102%. Results for the analysis of commercial dimer acid are comparable to those obtained using an HPLC/gravimetric method. The HPLC/FID method is applicable to the analysis of crude dimer as well as the finished dimer product.

The analysis of polymerized fatty acids (also termed dimer acid) for neutrals, monomer, dimer and polymer has been approached by many different techniques, but a truly satisfactory solution has remained elusive until now. Many early analyses were done by distillation of the methyl esters (1), which is tedious and imprecise. Additionally, the integrity of the sample is compromised when subjected to severe distillation conditions. Chromatographic methods have the greatest promise for this analysis, and some success has been realized in applying these techniques. Size exclusion chromatography (SEC) has been used by several workers to separate monomer, dimer and trimer (2,3). This technique separates the oligomers by size, but does not give information about the neutrals content or functionality of the oligomer. The reported SEC separations of dimer are very time-consuming and difficult to quantitate, although the use of new high resolution SEC columns can greatly reduce the separation time.

Paper (4) and thin layer (5) chromatography yield separation of dimer acid, but again quantitation is difficult. Column chromatography of the free acids (6) is slow and imprecise. Gas chromatography of dimer methyl esters at high temperature on a short column also has been reported (7). The separation of trimer from dimer was incomplete in this method, and it is likely that some sample degradation occurs during the analysis because of the high temperatures involved.

More promising approaches have been developed with the application of HPLC to this problem. We have utilized a reversed-phase separation employing infrared detection of the carbonyl species (W.C. Shermer, Union Camp Corporation internal method, unpublished). This separation is similar to the SEC methods, in that monomer, dimer and polymer are eluted in order of size. This method does have the advantage of separating neutral species. There is also some partial separation of the dimer-sized species.

A technique utilizing normal-phase separation was reported which yields good separation and quantitative measurement of the dimer acid species (8, and Emery Industries Method 148.09, unpublished). This method incorporates a preparative separation using a UV detector as an indicator for the elution of neutral, monobasic, dibasic and polybasic fractions. The fractions are collected manually, the solvent is removed and the residue weighed to yield weight percent data. The completeness of the separation was validated by analyzing the purity of the fractions by TLC. Analysis of synthetic dimer acid mixtures yielded recoveries in the range 96 to 110%, with good precision. The normal phase separation employed is advantageous, because in contrast to SEC the separation is on the basis of functionality and not size. Thus, the monobasic fraction contains monomer-sized molecules as well as monodecarboxylated dimer-sized molecules. This is useful information when the dimer is to be used in ploymerization reactions, where the monobasic species act as chain-stoppers.

The accuracy and utility of the normal-phase separation is evident, but the gravimetric method of quantitation has several major drawbacks:

(i) The gravimetric method specifies using preparative chromatographic columns which must be prepared, rather than obtained commercially.

(ii)During the separation, the analyst must observe the UV response for clues to the proper time to make gradient and fraction changes. The judgment of the proper time to make these changes may vary from analysis to analysis and from analyst to analyst.

(iii) The time invested in each analysis is considerable. Typically, only 3-4 samples per day can be analyzed using this technique.

Therefore, we have investigated a novel quantitation alternative utilizing a commercially available analytical-scale HPLC column with a flame ionization detector (FID). Using this detector and a normal-phase HPLC separation, we have obtained quantittative results for analyses of crude dimer and final dimer products.

EXPERIMENTAL

Materials. HPLC grade cyclohexane and isopropyl alcohol (IPA) were used without further purification. Glacial acetic acid was Baker Analyzed.

Samples analyzed were commerical dimer acid products of Union Camp Corporation (Unidyme-18, Unidyme-14) and Emery Industries (Empol 1010 and Empol 1022). Union Camp isostearic acid product Century 1105 also was analyzed, and blended with Unidyme-18 to prepare standard monomer/dimer samples.

Procedure. The samples were separated on a five-μ Supelcosil LC-Si column (Supelco Inc., Bellefonte, Pennsylvania), 25 cm × 4.6 mm, thermostated at 30 C. The column was protected by a Brownlee Labs (Santa Clara, California) 5 cm guard column packed with five-μ silica.

The mobile phases consisted of 99.3% cyclohexane/0.5% IPA/0.2% glacial acetic acid (Solvent A) and 89.8% cyclohexane/10.0% IPA/0.2% glacial acetic acid (solvent B). The flow rate was 1 ml/min. The elution program was a multi-step gradient as follows: 2% B at time zero to

5% B in 6 min, hold 3 min, to 48% B at time 15 min, then to 59% B at time 22 min. Reverse to 2% B in 2 min, and allow baseline to settle before next injection (3 min).

The dimer acid samples were weighed to approximately 0.5 g and dissolved in 10 ml of solvent A. Injection was via an automatic injection valve equipped with a 10-μl loop.

A Tracor model 945 FID (Tracor Instruments, Austin, Texas) was used for detection. This detector uses a continuous quartz braid to transport the column effluent (which is sprayed onto the braid through a 0.1 mm orifice) through a solvent removal zone and into the analytical and cleaning flames. The FID flows were as follows: 140 ml/min H_2 and 400 ml/min air for the analytical flame; 300 ml/min H_2 and 150 ml/min O_2 for the cleaning flame. The attenuator was set to 50, with the filter set on high. The oven temperature control was set to slightly less than mid-range, which yields a block temperature of approximately 140 C. Background subtraction was on.

Integration was done on a Nelson Analytical 4400 series data system, using area percent and normalized area percent methods.

RESULTS AND DISCUSSION

Chromatography. The chromatographic conditions specified produced the chromatogram seen in Figure 1 for Unidyme-18 dimer acid. There are four broad peaks corresponding to neutrals, monobasic, dibasic and polybasic components. Each peak of the chromatogram represents the elution of many isomers. For example, the dibasic peak is composed of linear, cyclic and aromatic dimer, each in various geometric, structural and conformational isomeric forms. The peaks are very broad as a result of the presence of these isomers. Baseline resolution is achieved for all but the dibasic/polybasic pair, for which the resolution is adequate. If required, baseline resolution is achievable for this pair by a slight gradient change. We optimized the gradient to yield a rapid separation of only the four major peaks, but it is possible to modify the conditions to obtain partial separation of isomers of the monobasic and dibasic fractions.

The noise seen in the FID chromatogram (partially obscured by the digitization of the data system) is caused by irregularities in the woven quartz belt of the detector. These irregularities affect the local sample distribution on the belt, and the flame/belt interaction. This is manifested as a repetitive noise signal with a period of 12 sec, corresponding to the rotational speed of the transport system. A background subtraction function is standard on the detector, which can reduce but not completely remove this noise. In our experiments the noise amplitude increased with the solvent B percentage, which indicated that an impurity in the IPA solvent was contributing to the noise. It might have been possible to reduce the solvent-contributed noise by cleaning up the IPA on an ion exchange column, as recommended by Tracor for certain solvents. Increasing the detector block temperature can also affect the solvent related noise. The peaks are wide enough that the presence of the noise did not adversely affect the integration.

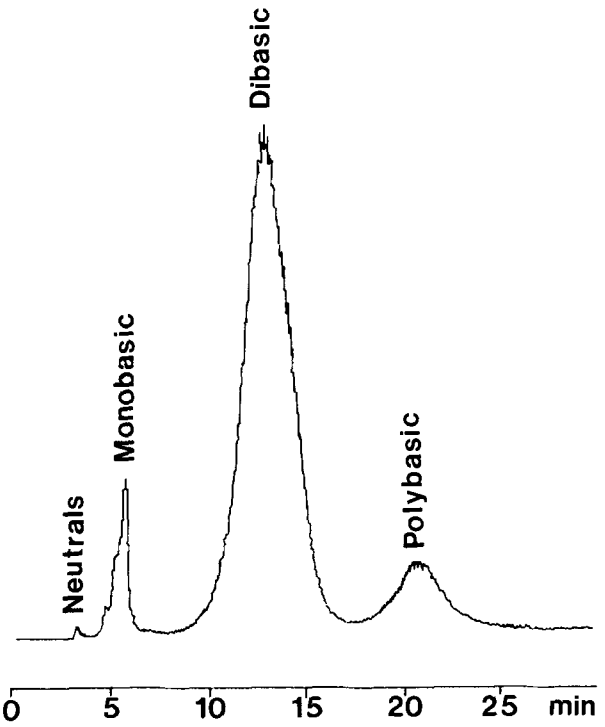

FIG. 1. Chromatogram of Unidyme-18 dimer acid using FID. Chromatographic conditions given in text.

After establishing that the separation and integration were reproducible, experiments were carried out to determine the applicability of the HPLC/FID technique for quantitation of dimer acid samples. Response factors were determined for monomer and dimer and used to obtain recovery data for synthetic dimer acid blends. The FID technique was then applied to the analysis of several commercial dimer acid samples that had been recently analyzed in a collaborative study.

Analysis of blends. A significant difficulty in developing an analysis of dimer acid is that no standards are available for the various components of the mixture. The monomer is available as the distillate from the dimerization, but dimer and polymer can only be obtained by molecular still distillation or by preparative chromatography. Even then, the standards are not pure components but are mixtures of isomers, the compositions of which are dependent on the isolation technique used and on the specific dimer from which they were isolated. For this reason, we chose to create a series of standard blends from Unidyme-18 (Union Camp distilled tall oil fatty acid dimer) spiked with Century 1105 (Union Camp isostearic acid). The isostearic acid (monomer) content was varied from approximately 10% to 80%. These samples were analyzed using the HPLC/FID method, obtaining area percent integration of the components to determine the linearity of the response.

The area percent analysis of the blends showed significant deviation from theoretical recovery. The deviation was more pronounced in the 50/50 monomer/dimer blends than in the 80/10 or 10/80 blends, which indicated that the FID response for monomer-sized components is significantly different from the response

for dimer and larger sized components.

The response of the FID is a complex function of many factors which include the number of carbons in a molecule, its volatility, and the FID operating parameters. We found that the response for dimer is actually greater than that for isostearic acid by a factor of 2.23. The isostearic acid/dimer acid blends were re-integrated using normalized area percent with the relative response factors for monomer and dimer, assuming that the neutral response equals the monobasic response and that the dibasic and polybasic responses are equal. Monobasic dimer present in the Unidyme-18 has the same response as the dibasic dimer, and thus the apparent monomer content must be corrected for this interference. This is done for the comparison of Table 1, which shows an average 102.5% recovery for the blends based on a single analysis of each sample. Although this data may be improved by replication, the worst recovery obtained (128.2%) represents an absolute difference of only 0.25 area percent. The excellent recoveries obtained confirm that the FID response is linear over the range from 10% to 80% monomer. This is greater than the concentration range found in typical crude dimer samples.

Collaborative study. To evaluate the performance of the HPLC/FID method for the quantitation of dimer acid product, four commercial dimer acid samples were obtained. These samples had been analyzed by three laboratories using the preparative HPLC method with gravimetric quantitation in a recent collaborative study sponsored by the AOCS Dimer Acid Subcommittee.

The four dimer acid products are very different from the blends created for the recovery studies. These commercial dimer samples are distilled and contain only traces of monomer-sized components. The monobasic fraction consists of mono-decarboxylated dimer and dimer interesters. Lacking the monomer-sized components, the response of the FID for the remaining components (excluding neutrals) should be essentially the same, and simple area percent integration should yield accurate results. The neutrals content is low enough not to make significant difference in the important monobasic, dibasic and polybasic measurement.

The HPLC/FID analyses in area percent are compared in Table 2 with the weight percent data from the collaborative study. The area percent data will equal the weight percent data only if the FID yields the same response for all components of the samples. The data show this indeed is the case. In almost every category, the FID data fall within the range of the results reported for the collaborative study.

An analysis of variance was performed on the data of

TABLE 1

Percent Recovery of Standard Mixtures

Percent isostearic in dimer	Component			
	Neutrals	Monobasic	Dibasic	Polybasic
10	100.0	114.4	97.0	102.3
20	93.5	108.5	96.9	106.5
40	90.8	100.2	100.2	99.8
60	98.2	98.6	101.6	115.8
80	103.5	101.6	92.7	128.2

$\bar{x}$ = 102.5, RSD = 8.6%

TABLE 2

Comparison of Results Obtained Using HPLC/FID and Gravimetric Procedures

		HPLC/FID[a]		Gravimetric[b]	
Sample	Component	%	s	%	s
Unidyme-14	neutrals	0.16	0.02	0.27	0.12
	monobasic	4.26	0.04	4.33	0.50
	dibasic	89.78	0.18	89.47	1.17
	polybasic	5.79	0.15	5.87	0.61
Unidyme-18	neutrals	0.14	0.01	0.37	0.06
	monobasic	4.56	0.12	4.90	0.26
	dibasic	79.03	0.69	78.47	1.10
	polybasic	16.27	0.68	16.33	1.22
Empol-1010	neutrals	0.12	0.04	0.33	0.12
	monobasic	4.02	0.19	4.20	0.35
	dibasic	93.60	0.09	93.07	0.74
	polybasic	2.27	0.27	2.43	1.06
Empol-1022	neutrals	0.20	0.02	0.23	0.15
	monobasic	6.55	0.11	7.66	0.86
	dibasic	75.64	0.89	75.93	0.94
	polybasic	17.62	0.99	16.23	0.85

[a]Area percent based on triplicate measurements on each sample.

[b]Weight percent data from collaborative study, based on 3 labs' results, each lab reporting duplicate analyses on each sample.

Table 2 using the analytical method as the source of variation. The least significant differences (95% confidence level) of the mean of each component in each sample were compared for the two analytical methods, and found to differ only for the neutrals analysis of Unidyme-18. The results for the neutrals content by the FID method are consistently lower than those obtained by the gravimetric method, but in every case at least one of the collaborating laboratories reported results for neutrals lower than what we obtained using the FID method. It is evident from this comparison that the accuracy of the FID method is comparable to that of the more tedious gravimetric procedure.

Because the neutrals content is a very small percentage of the total sample, the technique of weighing collected fractions can introduce large uncertainties in this measurement. This is evident from the large standard deviation in the collaborators' results (Table 2). The small neutrals peak can be reproducibly integrated, however, yielding a much lower standard deviation for the FID method. In general, the standard deviation obtained with the FID method is lower than that reported for the gravimetric procedure, but it should be kept in mind that the gravimetric data is from an inter-laboratory comparison which will yield greater variance.

The short-term precision shown in Table 2 for the FID analysis is excellent, averaging 0.6% RSD for the dibasic fraction. To determine longer term day-to-day precision, one sample was analyzed on five different days over a two-week period. The results (Table 3) show that the precision over this time period is not significantly different from the within-day precision.

The use of the analytical HPLC column and FID represents a major improvement in the analysis of dimer acid. The method requires no derivatization, is less manpower intensive than existing methodology (requiring only 25 min per analysis), is easily automated, and provides an accurate and quantitative measure of dimer acid composition. Although this work was done using a Supelco LC-Si column, any comparable commercially available silica column should provide adequate separation.

It is evident from our work that the FID has a different response for the monomer sized and dimer sized components. This response may vary with the FID temperature and flow settings. It is possible that the lower response we observed with monomer was due in part to volatilization loss from the heated belt. However, for analysis of final products containing only traces of monomer-sized components, this response difference is not a concern. Simple area percent data yields an accurate and precise measure for the neutral, monobasic, dibasic and polybasic content of dimer product. For analysis of crude dimer which has a significant monomer sized component, normalized area percent analysis using response factors determined for the monomer and dimer will yield data sufficient for estimation of the product makeup. Error will be present in this analysis due to the presence of polymeric monobasic components in the crude dimer, which have a different response from that of the co-eluting monomeric monobasic components. Since for typical crude dimer samples the polymeric monobasic content will be less than 10% of the concentration of the monomer-sized monobasic content, the error will be slight.

TABLE 3

Reproducibility[a] of Dimer Acid Analysis Using HPLC/FID Method

Component	Mean %	Standard Deviation
neutrals	0.17	0.04
monobasic	4.06	0.25
dibasic	93.55	0.51
polybasic	2.29	0.41

[a]Analysis of Empol 1010 over a 5-day period, n=11.

Use of the FID provides sensitive detection of dimer acid components in gradient separations. This should facilitate the investigation of alternative separation conditions which may provide resolution of isomeric dimer species. This could provide additional information concerning the dimer acid composition.

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