# Analysis of Dimerized Fatty Acids by TLC/FID

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**A rapid method is described for the analysis of the monomer, dimer and trimer components of polymerized fatty acids by thin layer chromatography (TLC) with flame ionization detection (FID) on the Iatroscan. The short- and long-term precisions of the method are discussed, as is the correlation of TLC/FID data with the gas-liquid chromatographic (GLC) data for dimerized fatty acids. The TLC/FID method is shown to be the superior technique for process control applications.**

Dimer acid is the generic name given to the substituted cyclohexenedicarboxylic acid formed from the Diels-Alder type reaction of unsaturated fatty acids such as tall oil fatty acid (TOFA) (1). Its main industrial use is as a coreactant in the manufacture of polymers such as polyamide resins. During the dimerization reaction a series of isomers of the starting material (monomer), the desired dimerized product (dimer), and higher oligomers (trimer, tetramer, etc.) are formed. The quality of the polymers produced in subsequent reactions of this material greatly depends on the amount of each of these species present. A quick, accurate analysis of monomer, dimer and trimer is crucial to the quality control of dimer acid production.

The analysis of fatty acids, and dimer acids in particular, has been investigated for many years. A review article by Firestone in 1963 (2) categorizes the analysis techniques for dimer acids as being molecular distillations or chromatographic methods. Included in this review (3) is a description of a relatively new method of column chromatography which uses silicic acid loaded with methanol to separate the dimer acid components into fractions which are later titrated to determine the acid content. More recently, gel permeation chromatography has been used (4,5) to separate the components for analysis and to obtain standards on a preparative scale. Work involving the use of normal phase HPLC to separate dimer acid components followed by flame ionization detection (FID) is summarized by Veasey (6).

The first methods which described the analysis of dimer acid components by gas-liquid chromatography (GLC) of the ester derivatives (7,8) were limited to direct analysis of only the monomer and dimer components, arriving at the trimer level by difference. In 1975 Nelson and Milun (9) published a GLC procedure from which could be determined trimer as well as monomer and dimer. This procedure separates the dimer acid components by the differences in their boiling points. The boiling point difference between monomer and dimer by the GLC method is great enough that other minor components elute between these two large peaks. These components are collectively defined as "intermediate" and can also be quantitated. This method is a great improvement over previous chromatographic techniques, but still involves a long derivatization procedure and a 45 min chromatographic run for each sample. Also, an extensive column conditioning procedure is required to be able to elute and directly analyze the high-boiling trimer component.

The technique of thin layer chromatography (TLC) has been an attractive separation method in various disciplines for many years. Some of the advantages of TLC over other chromatographic methods include simplicity, economy, versatility and ruggedness. This technique would be appropriate for the analysis of dimer acid because the important properties of dimer acid are by virtue of its functional groups, and silica gel TLC separates on the basis of functional group. The biggest stumbling block to the use of TLC in dimer acid analyses has been the lack of simple and quantitative detection. The development by Padley (10) of ceramic "sticks" which easily could be passed through a flame allowed universal FID for TLC and overcame the shortcomings of earlier attempts of direct FID on the plates themselves (11,12). Padley's concept of TLC/FID was marketed by Iatron Labs in Japan, and many applications from laboratories, mostly in Japan and Canada, appeared in the literature during the years that followed (13,14). Some of the applications have shown the utility of modifying the silica on the quartz rods by impregnation with compounds such as silver nitrate to enhance the separation of unsaturated compound mixtures (15).

The recent popularity of TLC/FID in the lipid field is evidenced by the dedication of an entire journal issue to applications of the technique (16). A recent article by Zeman (17) compared the TLC/FID technique with GLC and column chromatography methods for the analysis of dimer acid components. In his preliminary study, Zeman concluded that the TLC/FID method was the best method for process control purposes because of its inherent speed and simplicity. The present study investigates the application of TLC/FID to the analysis of dimer acid in depth and reports the precision, accuracy and ruggedness of the technique.

## MATERIALS AND METHODS

*Chemicals and equipment.* All solvents were analytical reagent grade (Mallinckrodt, Inc., Pans, Kentucky) and were used without further purification. The standards used in this study were prepared in-house by fractional distillation of the methyl esters of dimerized tall oil fatty acid (TOFA) followed by base hydrolysis to the free acid form. Purities were established on these standards by the gas chromatographic procedure (9) and by acid titration. The three standard materials prepared by this procedure contained 99.4% monomer, 97.5% dimer and 90.3% trimer, respectively.

The Chromarods (SII) were purchased from E.M. Becker Co. (Bala Cynwyd, Pennsylvania) and were prepared for analysis according to the manufacturer's directions. The chromarods were used up to four times before reconditioning in a humidity chamber, and could be used 40-50 times for analysis before loss of resolu-

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tion of the sample components. The manufacturer's recommended cleaning procedures in either sulfuric or chromic acids did not restore the chromatographic performance.

The Iatroscan TH-10 TLC/FID Analyser, Mark III, Iatron Laboratories, Inc., (Newman-Howells Associates Limited, Winchester, England, main distributors) was used for detection. The operating conditions for sample analysis were: scan speed, 3(0.36 cm/sec); hydrogen pressure, 0.9 kg/cm$^2$ (170 ml/min); air flow, two l/min. A Waters 740 Data Module (Millipore, Waters Chromatography Division, Milford, Massachusetts) was used to integrate the resulting chromatographic peaks.

*Procedures.* The standard solution was prepared by weighing monomer, dimer and trimer standards sequentially and exactly (to ±0.1 mg) into a tared, eight-dram vial so that the composition of the standard mixture closely matched the expected composition of the products to be analyzed and the total mass of all three standards was about 600 mg. Then, 25 ml of dichloromethane was pipetted into the vial and the contents shaken to dissolve the standards. The sample solutions were prepared by weighing ca. 125 mg of sample into a two-dram vial, pipetting five ml of dichloromethane into the vial, and shaking to dissolve the sample.

The first step in the analysis procedure was to spot 1.0 µl of the standard solution on each rod by using either a Drummond microdispenser (Drummond Scientific Co., Broomall, Pennsylvania) or a chromatographic syringe. Spotting was difficult to do without touching the surface of the chromarod. Contacting the chromarod with the sharp syringe needle or the microdispenser capillary had to be avoided because the silica easily could be chipped off the quartz rod. To alleviate this problem, a spotting guide, shown in Figure 1, was designed which would accommodate either the microdispenser or a syringe and adjusted so the tip of the spotting device could be reproducibly positioned just above the silica surface without touching it. This spotting guide was built in-house to exact specifications. The Model 3200 Autospotter, available from Ancal Incorporated in Los Osos, California, could be used as an alternate spotter for the chromarods. However, the dispensing tip in this device touches the chromarod during the sample delivery, which may shorten the usable life of the chromarods.

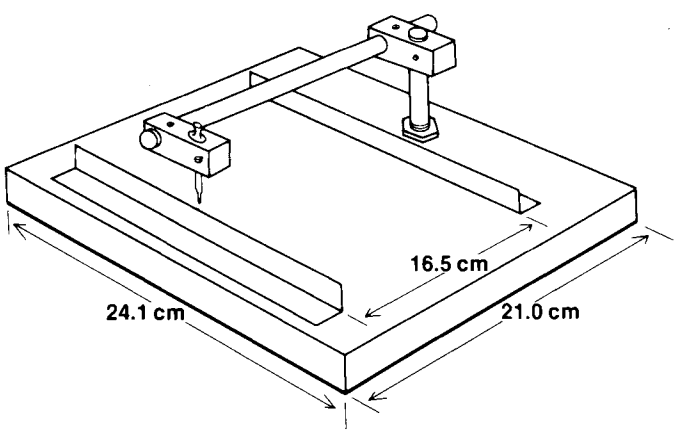



**FIG. 1. Spotting guide for chromarods.**

After spotting the standards, the solvent was allowed to evaporate for 15-20 seconds and then the rack of rods was placed in the chromarod developing tank (supplied by the Iatroscan instrument manufacturer). The eluent for this analysis was 60 ml dichloromethane, 1.0 ml diethyl ether, and 1.0 ml glacial acetic acid. A filter paper saturator soaked with the eluent was required along the inside wall of the tank to obtain short elution times and reproducible chromatography. A flashlight was used to back-illuminate the chromarods to see the solvent front more easily on the chromarods during elution. Elution to the 10-cm mark on the rack took about 23 min, at which time the rack of chromarods was removed from the developing tank and placed in a vacuum oven at 110-115° C. A water aspirator vacuum was applied to the oven, and the vent and vacuum valves adjusted to achieve a small amount of air flow through the oven. The vent line contained glass wool to filter the incoming air. The chromarods were dried for 10 min, removed from the oven, placed in the Iatroscan and scanned through the flame. The scanning process was paused for about 30 seconds between each rod to allow the integrator report to be printed. The time to scan each rod was 0.55 min.

*Calculations.* Before samples were analyzed, the set of rods was calibrated by using the prepared standard solution. The gas chromatographic analysis of monomer, dimer and trimer of each of the standards was used to determine the total weight of each component in the prepared standard mixture. The following calculations were used:

$$W_M = W_{MP} \times (M_{MP}/100) + W_{DP} \times (M_{DP}/100) + W_{TP} \times (M_{TP}/100) \quad [1]$$

$$W_D = W_{MP} \times (D_{MP}/100) + W_{DP} \times (D_{DP}/100) + W_{TP} \times (D_{TP}/100) \quad [2]$$

$$W_T = W_{MP} \times (T_{MP}/100) + W_{MP} \times (T_{DP}/100) + W_{TP} \times (T_{TP}/100) \quad [3]$$

Where W is the weight measured of each standard (MP = monomer primary standard, DP = dimer primary standard, TP = trimer primary standard); M is the percent monomer in the primary standards , D is the percent dimer in the primary standards, and T the percent trimer in the primary standards.

These total weights could then be used to calculate response factors for monomer and trimer relative to dimer from the TLC/FID chromatograms of the standard mixture:

$$RRP_M = (A_D \times W_M)/(A_M \times W_D); \quad RRP_T = (A_D \times W_T)/(A_T \times W_D) \quad [4]$$

Where $A_M$, $A_D$ and $A_T$ are, respectively, the areas of monomer, dimer and trimer peaks in the primary mixture; $W_M$, $W_D$ and $W_T$ are as defined above, and $RRP_M$ and $RRP_T$ are the relative response parameters, normalized to dimer, for monomer and trimer, respectively.

The standard mixture was spotted on each rod within a set, the analysis run, and average RRP values

calculated from all the rods. These average RRP values were then used as calibration for normalized area percent calculations on the samples:

$$\text{Percent monomer} = (A_M \times RRP_M \times 100)/TA \quad [5]$$

$$\text{Percent dimer} = (A_D \times 100)/TA \quad [6]$$

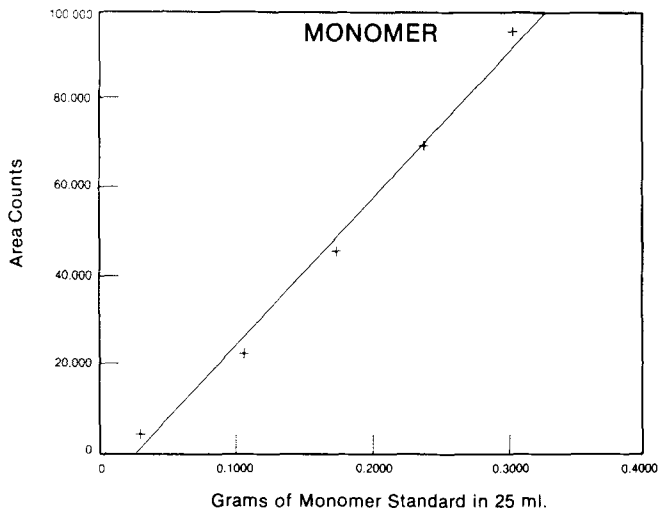
$$\text{Percent trimer} = (A_T \times RRP_T \times 100)/TA \quad [7]$$

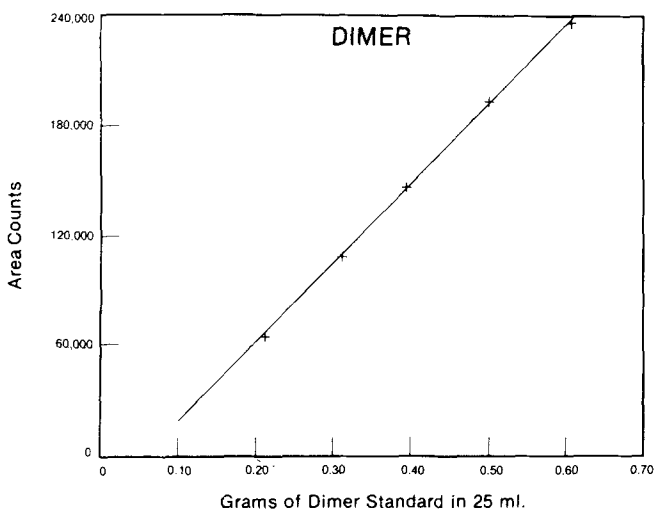
$$\text{Where } TA = (A_M \times RRP_M) + A_D + (A_T \times RRP_T)$$

Results from normalized area percent methods are independent of sample size, thereby eliminating variation due to spotting.

## RESULTS AND DISCUSSION

As Figure 2 shows, the three components are well separated on the chromarods. The separation is good even at very low levels of one or two of the components and a high level of the third component. The effect of varying the eluent components was tested and no differences were found in the separation, even if the amounts of eluent components varied as much as 20% from the recommended levels. The air flow setting on the Iatroscan was not a critical factor in peak sensitivity, but the hydrogen flow greatly affected the peak height and the noise level. Generally, the signal to noise ratio (S/N) improved with higher hydrogen flow rates up to 200 $cm^3$/min. Above this level, the S/N decayed rapidly due to excessive noise. The recommended level is a good compromise between high signal and high noise. A scan rate greater than 3 (0.36 cm/sec) resulted in residual trimer being left on the chromarod and correspondingly lower results for that component. The recommended scan rate allowed complete combustion of all components while still being fast enough to avoid unnecessary thermal damage to the chromarods.



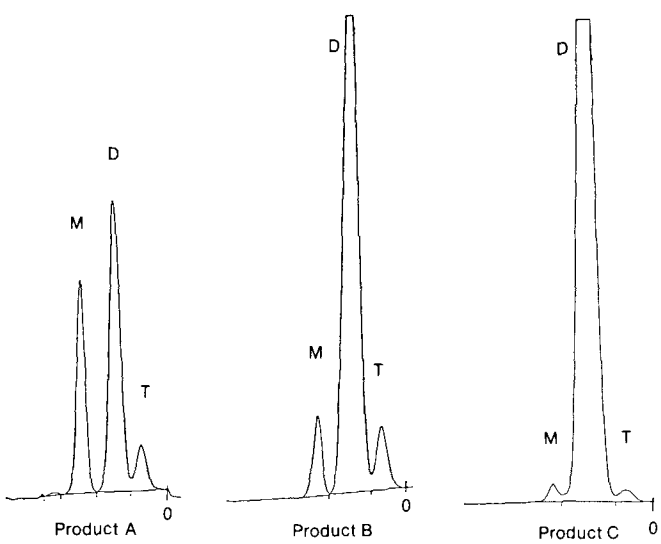




FIG. 2. Representative chromatograms of three different dimer acid products. O represents the spot origin. M is the monomer peak, D is the dimer peak and T is the trimer peak.

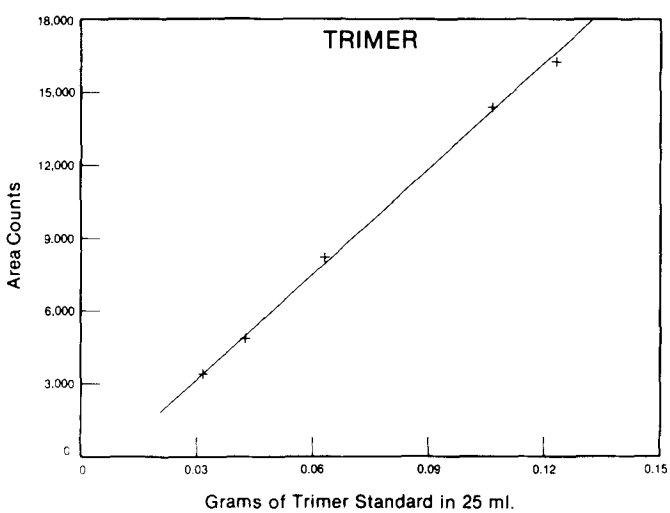



FIG. 3. Linearity of monomer, dimer and trimer detected individually by TLC/FID. Each data point represents the average area count from 4-5 spottings.

TABLE 1

**Short Term Precision for Three Dimer Acid Products**

| Product type | Number of values | %M (±SD) | %D (±SD) | %T (±SD) |
|---|---|---|---|---|
| A | 14 | 39.3 ± 1.0 | 52.3 ± 1.1 | 8.5 ± 0.4 |
| B | 4 | 13.1 ± 0.3 | 74.1 ± 2.2 | 12.8 ± 2.0 |
| C | 4 | 2.6 ± 0.2 | 95.3 ± 0.7 | 2.1 ± 0.4 |

TABLE 2

**Long Term Precision for Two Dimer Acid Products**

| Product type | Number of values | %M (±SD) | %D (±SD) | %T (±SD) |
|---|---|---|---|---|
| A | 414 | 34.7 ± 1.8 | 55.0 ± 1.8 | 10.3 ± 2.4 |
| B | 350 | 7.6 ± 1.5 | 81.1 ± 2.7 | 11.4 ± 2.2 |

As shown in Figure 3, the integrated detector response is linearly related to the amount of each individual standard spotted on the chromarod. This linearity study was done with the standards dissolved individually in 25 ml of dichloromethane from 0.03 g to 0.3 g of monomer, 0.2 g to 0.6 g of dimer, and 0.03 g to 0.12 g of trimer. The coefficients of determination ($r^2$) for the monomer, dimer and trimer plots are 0.9736, 0.9845 and 0.9928, respectively.

Replicate analyses on individual rods within a set, by using the average RRP values from all rods in the set, yielded relative standard deviations ranging from 1.6 to 25%, depending on the size of the peak (the larger the peak, the better the precision). However, if the mean of N multiple runs, on separate rods, is treated as one datum, the precision improves by the square root of N. The values in Table 1 represent the precision obtainable for N=3. This improvement in precision is achieved through a three-fold reduction in sample throughput, but the throughput is still sufficient for production purposes. The data in Table 1 was taken during a one-week period and represents the short-term precision which can be expected from this method. Table 2 represents data taken over a 12-mo period on the same samples run on a daily basis to monitor the integrity of the analysis. This data shows the long-term precision obtainable with this method and illustrates the reliability of this technique for process control.

Because the absolute amounts of monomer, dimer and trimer in any sample cannot be determined exactly, the accuracy of this method was evaluated by comparing the analysis results obtained by this method to the results from the gas chromatographic method. The results for 27 Product A samples, four Product B samples and four Product C samples by both methods are shown in Figure 4. The $r^2$ values for the monomer, dimer and trimer regressions are 0.9918, 0.9880 and 0.8319, respectively. There appears to be good agreement between the two methods. Upon closer examination, there appears to be a bias in the Percent Monomer and Percent Dimer plots toward higher results by

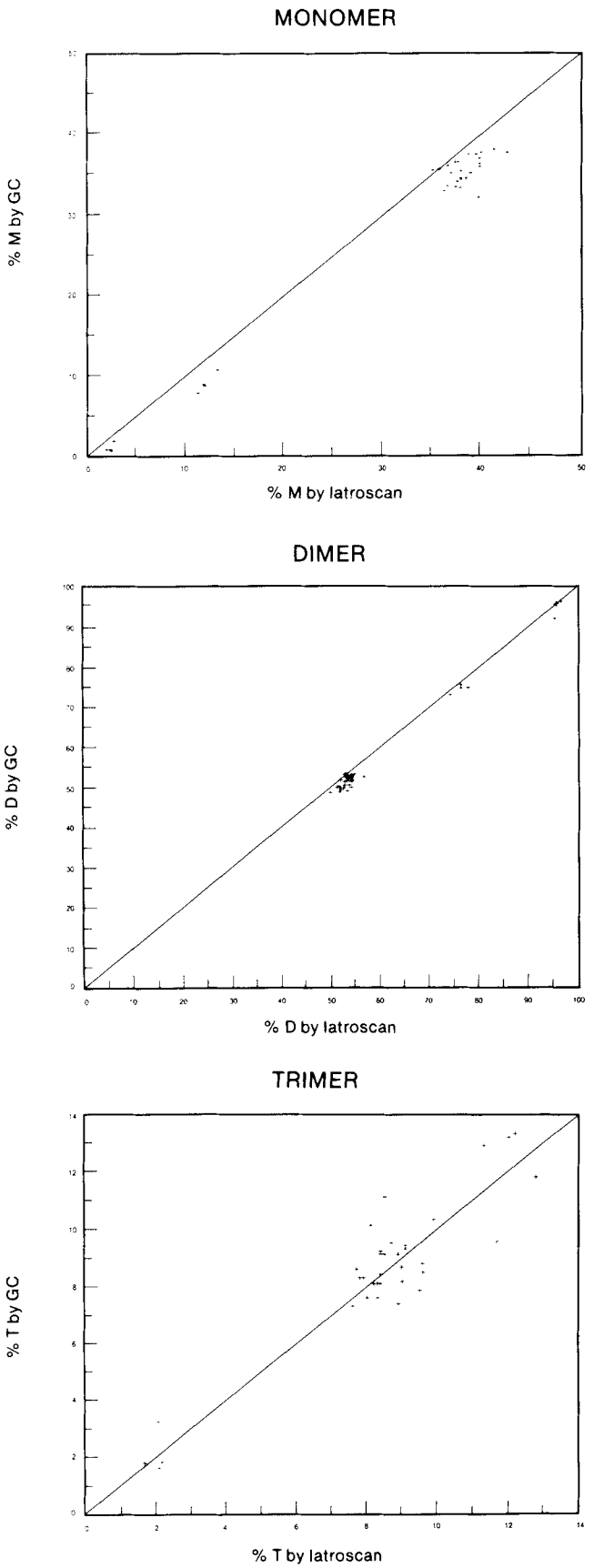



**FIG. 4. Correlation of the GLC method with the TLC/FID method. The one-to-one correspondence lines are shown for comparison of the two methods.**

TABLE 3

**Average Results for Three Dimer Acid Products by GLC and TLC/FID**

| Product type | %M by GC | %M Iatroscan | %D by GC | %D by Iatroscan | %T by GC | %T by Iatroscan | No. of samples |
|---|---|---|---|---|---|---|---|
| A | 35.4 | 38.0 | 51.4 | 53.4 | 8.7 | 8.6 | 27 |
| B | 8.8 | 11.9 | 74.3 | 76.1 | 12.8 | 12.1 | 4 |
| C | 0.8 | 2.2 | 94.7 | 95.8 | 2.1 | 2.0 | 4 |

the Iatroscan method. The results in Table 3 for the average monomer and dimer values from all the samples of each type show that the Iatroscan monomer and dimer values are each about 2% higher than the gas chromatographic values. Interestingly, this total of 4% corresponds to the level of "intermediate" found in these samples by the gas chromatographic method. A sample containing high levels of "intermediate" by the gas chromatographic method was analyzed by the Iatroscan method. Chromatography of this sample on the chromarods yielded two peaks of approximately equal areas at the retention times corresponding to monomer and dimer. This data does not prove that the Iatroscan results are higher because of interference from "intermediate," but the conclusion is consistent with the data. It is indeed surprising that there is as good an agreement between the two methods as there is, considering that the mode of separation is boiling point by the gas chromatographic method and functional group by the Iatroscan method.

Dimer acid analysis by the Iatroscan method has proved to be a much faster, more rugged method than the GC procedure. One operator could easily analyze 30–40 samples in an eight-hr shift by the TLC/FID method as compared to 3–5 samples by the GLC procedure. Results by Iatroscan compare favorably to the results by the GLC method and can be obtained without exhaustive sample pretreatment or column conditioning. Our experience with the use of this method for process analysis leads us to agree with the conclusions of Zeman (17) that the TLC/FID method is the most convenient for dimerization process control.

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