# Application of Specific Response Factors in the Gas Chromatographic Analysis of Methyl Esters of Fatty Acids with Flame Ionization Detectors

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## Abstract

The relative responses for flame ionization detectors in the analysis of the longer chain fatty acid methyl esters are shown to correlate with the theoretical responses based on weight percent content of "active" carbon. While particularly affecting estimation of the shorter chain length saturated fatty acids, these corrections have a less marked effect on the estimation of unsaturated fatty acids.

## Introduction

IT HAS BEEN RECENTLY stated (1) that the relative area response for methyl esters of saturated fatty acids in a flame ionization gas chromatography detector may be taken as very close to wt percent composition, and that the difference between saturated and unsaturated fatty acids in this respect is also very small. These views are not impractical, since in systems containing esters of the longer chain saturated acids of only closely adjacent chain lengths, or of related acids with one or two double bonds, the actual difference in response is sufficiently small as to be of the order of, or smaller than, the probable error in peak estimation.

Where a broad range of fatty acids may be present, however, there are correction factors for wt percent composition based on the wt proportion of active carbons in the molecule. This has been recognized in the case of oils themselves (2) and applies to all fatty acid chain lengths and degrees of unsaturation. The present study compares the predicted area response with known data for simple systems of esters of fatty acids to stress the necessity for the use of correction factors where applicable.

## Experimental

*Materials.* Mixtures of the methyl esters of saturated fatty acids were weighed from highly purified materials prepared in this laboratory. In no ester could more than 0.01% impurity be detected by gas chromatography. Methyl esters of linseed oil were freshly prepared by approved procedures and distilled at 1 mm immediately prior to use, a center cut being selected. Spectroscopic examination indicated no significant diene or triene conjugation, and on gas chromatographic examination the materials other than methyl palmitate, stearate, oleate, linoleate and linolenate totalled only 0.3%. Iodine values (I.V.) determined by precision semi-micro hydrogenation (3), and by the Wijs method, were respectively 181.8 and 181.9.

*Gas Chromatography.* Gas chromatography was carried out with a Barber-Colman Model 10, equipped with a Model 5121 flame ionization detector. Hydrogen flow was kept at 40 ml/min and air flow at 400 ml/min. Two glass columns were employed, each 6 ft. x 3 mm bore, packed respectively with 5% SE-30 on 70–80 mesh Anakrom ABS and 10% EGSS-X organosilicone polyester on 100–120 mesh Gas-Chrom P. Operating conditions for these columns were respectively 20 and 10 psi input of argon carrier gas at a column temp of 180C and a flash heater temp of 280C. The electrometer output was recorded by a Minneapolis-Honeywell 5-mv recorder fitted with a Disc Instruments, Inc., integrator counting at 1250 counts/square inch of chart. Injections of material were by Hamilton 7001 N microsyringe, a nominal sample of 0.0001 ml normally given peaks in the range of 300-1000 counts for the mixtures employed.

Individual analyses presented in Table I were selected on the basis of unequivocal return of the integrator pen to a zero level. The rejected analyses were usually satisfactory in interrelating those peaks where a good return to zero was obtained, but tailing, especially of stearate in the saturated ester mixtures, was more noticeable on the SE-30 column.

## Results and Discussion

It has been reported that the carbonyl carbon atom does not give any response in flame ionization detectors (4). The role of the methyl carbon atoms in methyl esters has not been as definitely established, but it appears that primary scission in the flame splits the attached carbon-oxygen bond, permitting this carbon atom to give the full response of an active carbon. There is no evidence that ethylenic carbon atoms do not give a full active carbon response (4,5).

If it is accepted that the methyl carbon gives a full carbon response, and the carboxyl carbon no response, the M responses for the methyl esters of fatty acids with $n$ carbon atoms in the fatty acid chain are then proportional (1) to the active carbon atoms $(n)$. In the longer chain hydrocarbons the addition of methylene groups does not have an appreciable influence on wt percent carbon and detector response is therefore also nearly proportional to weight percent composition (cf. 6,7), but in fatty acids and their methyl esters the presence of the two oxygens results in a more noticeable difference in wt percent carbon and this is reflected in the fact

TABLE I

Comparisons of Area Percent and Corrected Area Percentage with Weight Percentage Composition for Esters of Saturated Fatty Acids

| Methyl ester | Weight | Silicone (SE-30) column | | Polyester (EGSS-X) column | |
|---|---|---|---|---|---|
| | | Area | Corrected area | Area | Corrected area |
| | | % | % | % | % |
| Myristate | 34.8 | 33.7 | 34.3 | 33.9 | 34.6 |
| Palmitate | 29.6 | 30.0 | 30.1 | 29.9 | 29.9 |
| Stearate | 35.6 | 36.3 | 35.6 | 36.2 | 35.5 |
| Laurate | 16.1 | 15.5 | 16.2 | 15.3 | 16.1 |
| Myristate | 19.1 | 19.0 | 19.3 | 18.8 | 19.1 |
| Palmitate | 39.9 | 40.0 | 39.7 | 40.0 | 39.7 |
| Stearate | 24.9 | 25.5 | 24.8 | 25.9 | 25.2 |
| Laurate | 8.4 | 8.1 | 8.5 | 7.9 | 8.4 |
| Myristate | 16.4 | 15.9 | 16.2 | 15.9 | 16.2 |
| Palmitate | 31.1 | 30.7 | 30.7 | 31.0 | 31.1 |
| Stearate | 44.2 | 45.3 | 44.5 | 45.1 | 44.3 |

that detector response for these materials is not proportional to the *number* of active carbon atoms $(n)$, but to the *relative weight percents* of these active carbons in the molecules.

On the basis of detector response proportional to the relative weight percents of $n$ carbon atoms for the methyl esters of saturated fatty acids of chain length $n$, taking stearate as 1.00, the corrections to wt percent composition for methyl laurate, myristate, and palmitate are respectively 1.077, 1.044 and 1.019. The application of these factors, rounded off to 1.08, 1.04 and 1.02, (Table I) gives a significant improvement in correlation of wt percent composition and corrected area response over that for area response alone. Although not statistically significant, the average correction factors calculated from the experimental data are respectively 1.077, 1.049 and 1.022 for laurate, myristate and palmitate relative to stearate as 1.00. The average deviations, respectively of 0.013, 0.007 and 0.011, illustrate however that the application of these correction factors to single analyses where the experimental errors are of the same order of magnitude may be of limited value.

The influence of unsaturation is less marked, and correction factors have only a small effect on calculated iodine values. Appropriate correction factors in the $C_{18}$ chain lengths, relative to stearate as 1.00, are respectively 0.993, 0.987 and 0.980 for methyl oleate, linoleate and linolenate. In analyses of linseed oil methyl esters the application of correction factors to all significant components reduced the calculated I.V. to 182.3 from the value of 183.3 calculated from the uncorrected areas (I.V. Found, 181.9). Similarly in an analysis of a complex methyl ester

mixture from a marine lipid, with acids ranging from myristic to docosahexaenoic, the reduction in calculated I.V. with correction factors was only from 244–238 (I.V. Found, 240). Although in the latter analyses agreement is seldom as close, the correction is normally an improvement.

The theoretical correction factors should be independent of most normal operating variables (cf. 8) excepting detector overload. In practice, in addition to recorder-integrator errors (9) in determining peak areas, numerous other sources of error may occur, such as transesterification with polyester phases (10) or temporary adsorption in the column (11), or systematic errors arising from injection procedure, tailing or partial overlap of certain peaks etc. The application of the theoretical response factors is necessary only when it may be shown that these other sources of error are small. In some cases they could be incorporated into general correction factors including other systematic errors, and in routine analyses of typical samples could be applied arithmetically to the percent area responses.

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# Glass Paper Chromatography and Elatography of Triglycerides[1,2]

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## Abstract

A degree of reverse phase separation of triglycerides can be obtained with ascending chromatography on uncoated glass paper using a pyridine: water solvent system. The same $R_f$ values result when silica gel coated paper is used. To extend the usefulness of this separation, the glyceryl esters are converted to methyl esters in situ using sodium methoxide. This permits, on silica gel coated paper, glyceryl ester separation in one dimension, elatography to the methyl esters, and methyl ester separation in the second dimension. In mixtures of lipids such as serum extracts, the lipid groups may be separated in one dimension and the fatty acid methyl esters in the second dimension.

## Introduction

THE COMPLEX METHODS of separation of triglycerides have made analysis of these compounds difficult. This paper describes a chromatographic micro-method of partial separation of triglycerides and a method of producing methyl esters of fatty acids from triglycerides and other lipids on a chromatogram.

## Experimental Procedures and Data

Glass fiber filter paper (Schleicher & Shuell No. 29) cut to 23 x 23 cm size, was coated with silica gel by a previously described method (1). Reagent grade
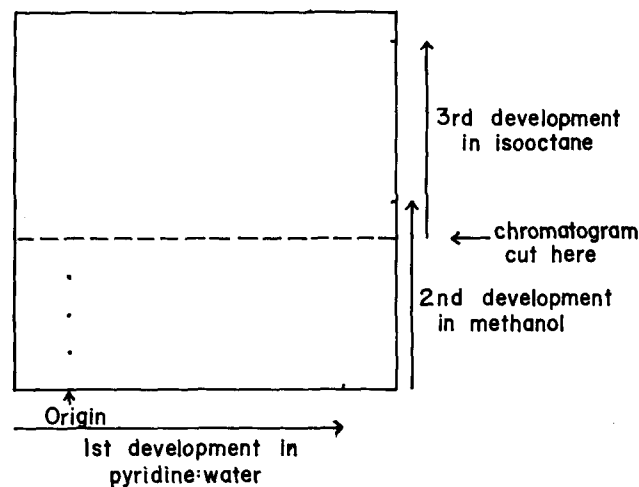


FIG. 1. Schematic drawing of the complete two-dimensional procedure. Elatography is done between the first and second development.