three curves for the 50-ton/day plant fall within or below the 42-46 cents/lb price range of soy protein concentrate).

For the 25-ton/day plant processing Texas seed with 44% classification yield, the selling prices of edible cottonseed flour for only the 4-yr payout period are competitive with the price of soy protein concentrate over the entire annual production range studied for that plant. Selling prices for the 2- and 3-yr payout periods are competitive over less of the production range.

Although selling prices of flour from Mississippi seed for the various payout periods and classification yields average 7.3 cents/lb higher than prices of flour from Texas seed, selling prices of flour from Mississippi seed from the 50-ton/day plant for the 2-, 3- and 4-yr payout periods are competitive with the price of soy protein concentrate over the entire production range studied for that plant except for 2-yr payout period and 150-day operations.

For the 25-ton/day plant, selling prices of flour from Mississippi seed for the 2-yr payout period are competitive only for 300- and 350-day operations. Selling prices remain competitive for operations down to 200 days only with a payout period of 4 years.

ENGINEERING PROSPECTUS

An engineering prospectus for the 25- and 50-ton/day cottonseed flour plants is available upon request from K.M. Decossas at the address given in the byline. It includes a flowsheet, material balances for various classification yields, a detailed equipment list with specifications and costs, a list of equipment suppliers, energy requirements, itemized manufacturing costs and general expenses for both size hypothetical plants operating for various lengths of season at two locations, and tables of profitability and selling prices of the edible flour product.

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Operating Variables in the Analysis of Tall Oil Acids by Capillary Gas Chromatography

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ABSTRACT

Factors influencing the analysis of fatty and resin acid methyl esters by capillary gas chromatography have been investigated. Methods to calculate equivalent chain length (ECL) values and their limitations are first discussed. Retention data, expressed as equivalent chain lengths (ECL) were determined on SE-30, SP-2100 and Carbowax 20M columns. The medium length (20-30-m) polysiloxane columns, SE-30 and SP-2100, provided overall better resolution and shorter retention times than the more polar Carbowax 20M column of similar length. The temperature dependence of ECL values was investigated for all three columns in the range 180-210 C. Retention times and ECL values were more temperature-dependent for the Carbowax 20M than for the other two stationary phases. The effects of split ratio and method of injection on the precision and accuracy of the analysis were also examined. Using optimal conditions of analysis established in this paper, the difference between measured and actual weights of an internal standard added to two tall oil samples was determined to be less than 3%.

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INTRODUCTION

In the pulp and paper industry, millions of dollars are lost yearly because of problems arising from the resinous material in the wood (1-3). Wood resin is usually defined as the component of the wood which is insoluble in water but soluble in nonpolar, organic solvents such as ether, benzene or acetone (4). This material, which comprises about 1-5% by wt of the wood, is partially liberated during the pulping process (1) and can deposit on the surfaces of process equipment. If such deposits break away from their surfaces of attachment, they can contaminate the final product with dirt or cause sticking problems on the paper machines. Other materials used in the process, such as defoamers and sizing agents, can cause similar deposition problems; to determine the source of deposition, it is highly desirable to be able to analyze accurately the fatty and resin acids which are major components of wood resin.

Gas chromatography (GC) with packed columns has been extensively used for the separation of wood resin into

its individual classes of components (5-9). Different fractions have been analyzed on a number of columns using a wide range of stationary phases and loadings. From the experience acquired in this laboratory with methylated extracts of pitch deposits, the separation on packed columns is sometimes too poor to provide a reliable identification and a good quantitative analysis.

To provide the resolution required for complex natural mixtures, GC with wall-coated, open, tubular (WCOT or capillary) columns is now the method of choice (10,11). Although the field of capillary chromatography is over 20 years old, it is only within the last few years that its use has become widespread. With the advent of new chromatographs specially designed for capillary columns and the availability of high-quality, glass columns coated with a broad range of stationary phases, the technique is now used in numerous laboratories for routine analysis. Capillary columns made of soda lime or borosilicate glass can be operated at temperatures nearly as high as those used with packed columns, with good inertness and long life. A recent advance (12,13) in the field has been the introduction of fused silica columns; with nonpolar coatings, exceptionally inert and thermostable columns of this kind can be prepared (14). When they are drawn with thin walls, these columns are flexible and can be easily fitted to the detector and injector with minimal dead-volumes and risk of breaking the ends during installation. Due to the low surface energy of fused silica, stationary phases with polarities greater than Carbowax 20M cannot be used at present (14).

Very few studies have appeared on the analysis of wood resin components by capillary chromatography. A notable exception is the work of Holmbom et al. (15-17) on the analysis of tall oil components by capillary GC-MS. Using a variety of stationary phases, they were able to identify nearly 100 neutral and acidic compounds in different tall oil fractions, and their results provided a valuable reference source for the present work.

The purpose of this initial paper was to establish optimal conditions for the analysis of fatty and resin acids of the type commonly found in wood resin. Crude and distilled tall oil, as well as standard mixtures of fatty and resin acids, were used. A number of factors, including methods for calculating equivalent chain length (ECL), type of column, temperature, solvent, split ratio and method of injection were critically examined to determine their effect on the nature of the chromatograms and the accuracy of results.

EXPERIMENTAL

Materials

Fatty and resin acid mixtures from two tall oil sources were used: (a) a doubly distilled tall oil (Sylvatal 40DD, Sylvachem Corp.) with a maximum of 2% neutrals and an acid number of 187 was used as received; (b) ca. 200 mg of crude tall oil from a western Canadian mill was dissolved in a 90:9:1 solution of ether, methanol and water and fractionated on a DEAE-Sephadex column according to the method of Zinkel and Rowe (18,19). The following composition of eluted compounds was obtained: 26.2% neutrals, 71.5% weak acids and 2.3% strong acids; 2.8% of highly colored materials remained in the column after elution of the three fractions.

After addition of 5-10% by wt of heptadecanoic acid as internal standard, the doubly distilled tall oil and the weak acid fraction of the crude tall oil were dissolved in a 9:1 solution of ether and methanol and methylated with freshly prepared diazomethane. Excess diazomethane and the solvent were removed under a small flow of prepurified nitrogen. Methylated samples (~15 mg) were then redissolved in chloroform or methylene chloride.

Standard fatty acid and fatty acid methyl ester mixtures (99+% purity) for identification and quantitative analysis were obtained from Applied Science Laboratories, Inc., and Supelco. Resin acids were obtained from Chemical Procurement Laboratories Inc. (pimaric), Eastman (dehydroabietic), and K & K Labs (abietic, neoabietic). Samples of fatty and resin acids were methylated before analysis. Methyl esters of heptadecanoic and/or stearic acids were used as internal standards.

Apparatus

The data were obtained on a Hewlett-Packard Model 5840A gas chromatograph with a flame ionization detector (FID) and a capillary inlet system (Model 18835B). The split injection technique was adopted in this study. A precolumn consisting of the glass liner tube (Hewlett-Packard, part no. 18740-60840) packed with 1 cm of GC packing (10% OV-1 on 80/100 Chromosorb W-HMDS-AW-DMCS) served the dual purpose of minimizing split discrimination and of retaining nonvolatile substances which could otherwise condense and accumulate at the head of the column.

Helium (high purity grade, further purified with two traps to remove traces of water and oxygen) was used as carrier gas. A linear flow rate of 20-30 cm/sec was maintained in all columns. Prepurified nitrogen (~40 mL/min) used as auxiliary gas was added to the air and hydrogen to optimize the flame ionization detection. The injector and detector port temperatures were maintained at 260 C.

Columns

The following glass capillary columns (WCOT) were used: 25-m SP-2100 (0.2-mm id) fused silica column deactivated with Carbowax 20M (Hewlett-Packard), 20-m SE-30 (0.25-mm id) fused silica column (J & W Scientific Inc.) and 25-m Carbowax 20M, grade AA (0.25-mm id), glass column (Supelco).

The straight ends of the fused silica columns were easily inserted directly into the injection port (1-cm) and into the detector jet (~1 mm from the flame). The curved ends of the Carbowax 20M column were straightened by feeding one end at a time through a 5-cm long (0.32 cm od) stainless steel tubing heated with a gentle flame (10).

Injection Techniques

Methylated samples were injected with Hamilton 701N syringes as dilute solutions in methylene chloride or chloroform using two injection techniques: the solvent flush and the hot needle (20). In the solvent flush method, the 10-μL syringe was successively filled with 1 μL of pure solvent and 1 μL of air (taking into account the internal volume of the needle itself, ~0.85 μL). Between 0.9 and 1.2 μL of sample was then introduced into the syringe followed by 1.5 μL of air. Without delay, the whole plug (solvent-air-sample-air) in the barrel was then injected through the septum. The hot needle injection technique consisted of filling the syringe with ~0.9-1.2 μL of sample, followed by 1.8 μL of air. The needle was then inserted through the septum and held 3 sec in the injection chamber before the plunger was rapidly depressed.

Identification

Common fatty and resin acids were identified by injection of reference compounds. Other, naturally less abundant, compounds were identified by comparison of their relative retention times or ECL with literature values (9,15,16,

21,22) using the same stationary phases under similar conditions. Although most of the peaks in the tall oil samples were identified, some minor components (e.g., isomers of fatty and resin acids, cyclic acids formed during pulping or tall oil distillation) were either not detected in the chromatograms or could not be positively identified.

For a reliable interlaboratory exchange of chromatographic results, it is important that the retention data be reported with a high degree of accuracy. Methods for the calculation of ECL values and their limitations are discussed in the next section.

RESULTS AND DISCUSSION: QUALITATIVE ANALYSIS

Determination of Equivalent Chain Length

In the concept of ECL, the retention of a compound is expressed relative to the retention of the normal saturated fatty acid methyl esters (FAME) (23,24). A scale is set up by assigning to each member of the saturated FAME series an ECL value equal to its carbon number. Thus, for example, methyl palmitate and stearate have ECL values of 16.000 and 18.000, respectively. Using this definition together with the well known linear relationship (Eq. I) under isothermal conditions, between the logarithm of the adjusted retention time t_R' and the number of carbon atoms in a member of a homologous series, allows the retention of any compound to be expressed on this scale:

$$\log t_R' = \log(t_R - t_M) = aN + b, \qquad [I]$$

where t_R is the uncorrected retention time of the member of the homologous series having N carbon atoms; t_M is the dead-time of the column and can be determined experimentally from the time required to elute an unretained substance (e.g., helium, argon, air) from the column; a and b, the slope and intercept, respectively, of a straight line plot of log t_R' vs N, vary with the nature of the stationary phase and the column temperature. So, from t_R' values of two or more saturated FAME already present or added to the mixture to be indexed, a and b values can be determined. The ECL of any unknown compound X can be calculated from its $(t_R')_X$ value by solving for N_X in Equation II.

$$N_X = (ECL)_X = \frac{\log(t_R')_X - b}{a} \qquad [II]$$

A compound with an ECL value of 18.250 on a given stationary phase would then elute somewhere after methyl stearate. With modern chromatographs, retention times can be measured with a high degree of precision and accuracy, and hence, it would be expected that ECL values could also be reported with a high degree of accuracy. Unfortunately, this is not the case because: (a) the relationship in Equation I may sometimes not be strictly linear over the whole range of carbon numbers in a homologous series, and (b) controversy remains over what is the most accurate way to determine t_M in Equation I when an FID detector is used.

There is now increasing evidence (25,26) that the log plot is not strictly linear when applied to a wide range of carbon atoms. In fact, it is believed that the relationship log t_R' = f(N) would be better represented by a quadratic (25) or a cubic (26) equation.

The determination of t_M is troublesome because truly unretained substances such as helium do not produce a signal on an FID detector. For this reason, the methane peak is frequently used as a substitute for helium or air. Although some workers believe that it is a good approximation (27), others (28,29) maintain that methane is retained on most stationary phases (particularly on nonpolar phases such as SE-30) and, therefore, should not be used to estimate t_M. As an alternative, various mathematical methods, recently reviewed by Wainwright and Haken (30), have been proposed. All the methods are based on Equation I whereby t_M can be calculated from simple equations using three homologs or from more elaborate statistical and/or iterative techniques performed on four or more homologs. In this laboratory, an iteration technique was adopted (31,32). The principle is to iterate t_M in Equation I until the best fit of the experimental t_R' values of four or more homologs to a straight line is achieved. The value of t_M so obtained is termed the "mathematic dead-time." The major drawback of the concept of mathematical dead-time is that it is based on the assumption that the log is linear, but this linearity has not yet been clearly established.

It was found in this work that use of the mathematical dead-time produced more accurate ECL values than those obtained by taking a t_M equal to the retention time of methane (Table I). From the t_R values of six saturated FAME (which bracket the fatty and resin acids included in Table II), linear regression analyses of the log t_R' vs N were performed assuming t_M values to be equal to the retention time of methane or to the mathematical dead-time, respectively. From the slopes and intercepts obtained, ECL values of the six standards were calculated. It is evident from the results in Table I that the difference between calculated and theoretical values (i.e., ECL = N) is smaller when the mathematical dead-time is used. These results suggest that, at least in this carbon range (14-24), the methane peak should not be used for the calculation of ECL values because the accuracy of ECL values obtainable is unacceptably low.

The fact that, in this case, the mathematical dead-time yields accurate ECL values does not imply that it is a better

TABLE I

Effect of the Dead-Time on the Accuracy of ECL Values

Saturated FAME	t_R[a] (min)	$t_M = t_R$ (methane)[b]	t_M = mathematical[c] dead-time
14:0	2.95	13.967	14.004
16:0	4.85	16.007	15.990
18:0	8.73	18.027	18.000
20:0	16.58	20.025	20.007
22:0	32.40	22.008	22.009
24:0	63.92	23.966	23.991

[a] t_R values measured in triplicate on a 20-m, SE-30 column at 200 C.
[b] t_R (methane) = 1.17 min; correlation coefficient (r) = 0.99997.
[c] Mathematical dead-time = 1.067 min; correlation coefficient (r) = 0.999997.

estimation of the column dead-time than the methane retention time. Parcher and Johnson (29) have recently compared the retention times of inert gases and methane on a methyl polysiloxane stationary phase using a mass spectrophotometer as the detection system. It was found that the capacity ratio k (where $k = [t_R - t_M]/t_M$) of methane is ca. 0.04 at 30 C. If the mathematical dead-time in Table I is assumed to be equal to t_M, the value of k for methane would then be 0.10 at 200 C. This is clearly an unreasonable value because the solubility (and hence the retention) of methane in SE-30 decreases as the temperature increases, so that at 200 C, k should rather decrease and approach zero. Thus, it would appear that at 200 C, methane is almost completely unretained on the column and its retention time is a far better approximation of the column dead-time than the calculated t_M.

Moreover, when the mathematical dead-time was calculated using saturated FAME with carbon chain length between 10 and 16, a value of 1.165 min (±0.018) was obtained. This value is considerably higher than the previous estimate (1.067 min) but is now in good agreement with the methane retention time (1.17 min). Thus, the mathematical dead-time appears to vary with the range of carbon atoms used for the calculation. The discrepancy is further evidence that the relationship between log t_R' and ECL might not be perfectly linear. To verify this possibility, an *n*-alkane series with a very broad range of carbon atoms (9-32) was used to calculate the column dead-time. In the case of the lower members of the series (9-15) with short retention times, the mathematical dead-time agreed within experimental error with the methane retention time, whereas the use of higher homologs yielded progressively lower values (0.900 min using the range 22-32 carbon atoms). A likely explanation for this effect is a slight deviation from linearity of the log plot.

It is evident from the previous discussion that the calculation of ECL values requires a great deal of caution. In the range of saturated FAME (14-24) used in this study, the mathematical dead-time is a poorer approximation of t_M than the methane retention time but it use yields more accurate ECL values. This paradox may be explained by the slight deviation from linearity of the log t_R' plot which results in erroneous ECL values when using the correct t_M (t_R of methane). However, when one is dealing with a limited range of carbon atoms, the mathematical dead-time can be used to obtain more accurate ECL values because it acts as an adjustment parameter to linearize the log t_R' plot. With a broader range of carbon atoms, the effect of nonlinearity should be taken into account in calculating ECL values.

Effect of Stationary Phase

The retention data at 200 C, expressed as ECL values, for fatty and resin acid methyl esters on SE-30, SP-2100 and Carbowax 20M columns are listed in Table II. Although the elution order was the same on both SE-30 and SP-2100, ECL values were consistently higher on the SP-2100 column. This was particularly pronounced with resin acids where differences in ECL of up to 0.08 were observed between the two columns. Such a large discrepancy was unexpected since both columns are made up of 100% methyl polysiloxane. Different diffusivities of the solutes in the two stationary phases (SP-2100 is a fluid [MW = 600] and SE-30 is a gum [$MW = 1\text{-}2.5 \times 10^6$]) may partly account for this effect (33), although a more likely explanation is that the Carbowax 20M undercoating of the SP-2100 causes it to act as a mixed stationary phase (14). It should be noted that the ECL values obtained on the SE-30 column are in close agreement with those of Holmbom (16). All major fatty acids were reasonably well separated on the SE-30 and the SP-2100 columns and were sufficiently distant from resin acids to be easily recognized. The resolution of resin acids was also good, with the exception of palustric and levopimaric acids which were not separated from each other. For illustration, the chromatogram of distilled tall oil on SP-2100 is presented in Figure 1.

On the more polar Carbowax 20M stationary phase, the elution order of the acids is markedly altered as evidenced by large differences in ECL values compared to the other two stationary phases. The fatty acids were even better resolved on the more polar Carbowax 20M column than on the polysiloxane columns. On the other hand, the resin acids were not all well separated from each other and often overlapped with the fatty acids. However, as indicated in the next section, such overlaps can be eliminated by varying the column temperature.

Thus, for routine analysis, the medium length (20-30-m) SE-30 and SP-2100 columns appear to be better than the Carbowax 20M. Other advantages of the polysiloxane columns include shorter times of analysis (20-30 min compared to 70-90 min for Carbowax 20M), high accuracy (±0.01 ECL), high reproducibility of ECL values even after extensive use of the columns (a precision of ±0.005 ECL was obtained on the SE-30 column compared to ±0.03 on Carbowax 20M) and high thermal stability which also makes them suitable for the analysis of neutrals. It should be noted that due to the Carbowax undercoating, the column performance of SP-2100 deteriorates rapidly when exposed for a few hours at temperatures higher than 250 C. The SE-30 column was exposed to temperatures as high as 320 C without significant deterioration.

Effect of Temperature

The effect of temperature on ECL values was investigated for the three stationary phases in the temperature range 180-210 C. It was found that, on SE-30 and SP-2100 columns, the temperature dependence of ECL values of fatty acids was not large enough to alter the elution order. The values decreased with temperature and typical variations of ECL for a 10 C interval were 0.004-0.005 for branched, 0.008-0.015 for monounsaturated, 0.012-0.020 for diunsaturated and 0.019-0.027 for triunsaturated fatty acids. The temperature dependence for resin acids was more pronounced (0.100-0.130), causing some overlap of peaks with fatty acids. For example, at 180 C, the peaks of abietic and arachidic acids overlap.

Because Carbowax 20M is a more polar stationary phase, the temperature dependence of ECL values for both fatty and resin acids was larger on this column (by a factor of 1.2-2.0) than on the others. Thus, the elution order varied significantly with small temperature changes, leading, in some cases, to major overlaps. Some of these can be eliminated with a proper choice of temperature, or by temperature programming. For example, at 200 C, 8,15-IP, 5,11,14-20:3 and 7,11,14-20:3 form a single peak. However, at 195 C, 8,15-IP (ECL = 20.65) can be separated from 5,11,14-20:3 and 7,11,14-20:3 (ECL = 20.78), whereas, at 210 C, the three components become separated (ECL of 8,15-IP = 20.95, of 5,11,14-20:3 = 20.82 and of 7,11,14-20:3 = 20.90). Also, at 210 C, the peaks for abietic and dehydroabietic acid overlap but as the temperature is lowered, the resolution increases. The temperature dependence of ECL values of resin acids on the Carbowax 20M was also found to be much larger than that of the fatty acids, which presents a way to distinguish between the two. The exact temperature dependence on the Carbowax 20M was more difficult to determine than on the other two columns because ECL values at a given temperature

TABLE II

ECL Values at 200 C of Fatty and Resin Acid Methyl Esters on SE-30, SP-2100 and Carbowax 20 M Capillary Columns

Acid[a]		SE-30	SP-2100	Carbowax 20M
14:0	(myristic or tetradecanoic)	14.00	14.01	14.00
15:0 ai	(12-methyl tetradecanoic)	14.73	14.73	14.66
15:0	(pentadecanoic)	15.00	15.00	15.00
9-16:1	(palmitoleic)	15.74	15.73	16.28
7-16:1		15.79	15.79	16.21
16:0	(palmitic or hexadecanoic)	15.99	16.00	15.99
17:0 ai	(14-methylhexadecanoic)	16.72	16.72	16.67
17:0	(heptadecanoic)	17.00	17.00	16.99
5,9,12-18:3	(pinolenic)	17.43	17.46	18.85
5,9-18:2		17.51	17.53	18.65
9,12-18:2	(linoleic)	17.62	17.64	18.77
9,12,15-18:3	(linolenic)	17.66	17.69	19.26
9-18:1	(oleic)	17.71	17.72	18.21
11-18:1	(vaccenic)	17.76	17.77	18.39
conj 18:3		17.86	17.88	–
18:0	(stearic)	18.00	18.00	18.00
9*c*,11*t*-18:2		18.07	18.09	19.54 (?)
10*t*,12*c*-18:2		18.15	18.17	19.62 (?)
conj 18:3		18.20	18.21	–
9-19:1		18.41	18.43	–
(10*t*,12*t* + 9*t*,11*t*)-18:2		18.49	18.53	20.11
8,15-IP	(8,15-isopimaradien-18-oic)[b]	18.59	18.64	20.79
8,15-P	(8,15-pimaradien-18-oic)[b]	18.83	18.87	21.12
P	(pimaric)	18.97	19.01	21.21
Sa	(sandaracopimaric)	19.12	19.16	21.50
5,11-14-20:3		19.34	19.37	20.79
7,11,14-20:3		19.39	19.43	20.79
IP	(isopimaric)	19.46	19.51	22.26
Pal	(palustric)	19.54	19.60	22.23
Lev	(levopimaric)	19.54	19.60	22.19
11,14-20:2		19.60	19.63	20.63
11-20:1	(eicosenoic)	19.69	19.71	20.20
DeAb	(dehydroabietic)	19.77	19.84	23.50
20:0	(arachidic or eicosanoic)	20.00	20.00	20.01
Ab	(abietic)	20.23	20.29	23.46
Neo	(neoabietic)	20.66	20.74	23.99
13-22:1	(erucic)	21.69	–	22.18
22:0	(behenic or docosanoic)	22.01	22.00	22.01
15-24:1	(nervonic)	23.68	–	24.17
24:0	(lignoceric or tetracosanoic)	23.99	–	23.99

[a]In this shorthand system for fatty acids A,B-N:Y, the fatty acid chain length is denoted by N, the number of double bonds by Y and their positions by A, B . . . ; *c* and *t* denote *cis* and *trans* but unless specified, the unsaturated acids have a *cis* configuration; ai (anteiso) indicates a methyl branched acid.
[b]Resin acid isomers formed during tall oil distillation. Nomenclature as in ref. 15.

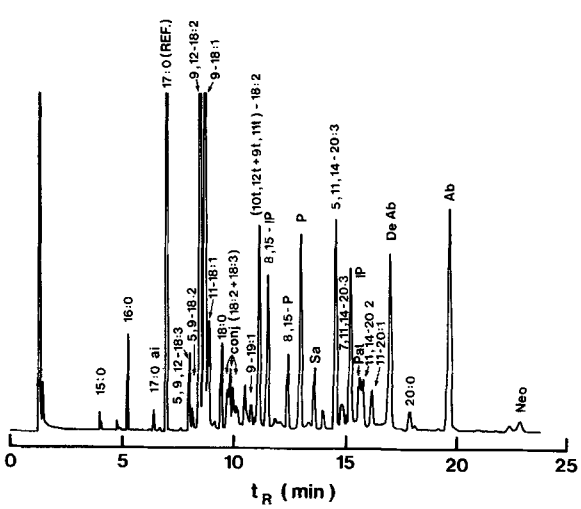

FIG. 1. Chromatogram at 200 C of a distilled tall oil sample on a SP-2100 (25-m) column (carrier gas linear velocity = 26 cm/sec); cf. Table II for names of acids.

changed systematically toward lower values with time, particularly after exposure of the column to a temperature of 215 C for several hours.

QUANTITATIVE ANALYSIS

When performing the analysis of a mixture of fatty acid methyl esters on a packed column connected to a flame ionization detector, it is frequently assumed that the relative peak area of a particular ester is equal to its relative weight in the mixture. This is not perfectly correct (34) because the detector response depends on the degree of unsaturation and on the chain length of the ester (i.e., the wt % of active carbons in the chain). If a factor of unity is assumed for methyl oleate, the area correction factor (factor by which the area of a given peak must be multiplied to equal a weight) varies theoretically from 1.05 to 0.95 going from methyl palmitate to methyl lignocerate (34).

In capillary chromatography using a split mode, the quantitative analysis is further complicated by discrimination at the split point and in the syringe needle which could result in a mixture composition in the column different

from the one injected. Several criteria to minimize discrimination in the splitter have been proposed (10,11,35-39). The more important ones include a good volatilization of all components in the mixture, a high degree of mixing to produce a homogeneous mixture at the split point, and minimal pressure and flow changes during splitting. Therefore, the geometry of the splitter, the flow of carrier gas (split ratio), the injection temperature, the buffer volume, the sample volume, the presence of a packing in the liner, the boiling point of the solvent, may all affect the precision and accuracy of the analysis.

An additional source of discrimination is the syringe needle itself. In capillary chromatography, sample volumes are usually kept small (0.2-2 μL) to avoid overloading effects and large pressure changes in the injector. However, the internal volume of the syringe needle is of the same order as the sample size which could cause a large error in the quantitative analysis if part of the sample remains in the needle. Grob and Neukom (20,40) have recently discussed the mechanisms of transfer of samples from the syringe to the vaporizing injector by the combined action of evaporation and rinsing provided by air and/or vaporized solvent. Since the less volatile components could be incompletely evaporated, it is important that their removal from the syringe by solvent or air be efficient to minimize fractionation. The "solvent flush" and "hot needle" injection techniques did not completely eliminate the discrimination, but at least yielded reproducible results (20).

The effect of split ratio and injection techniques on the precision and accuracy of the analysis of fatty and resin acid methyl esters was examined and Table III shows the effect of split ratio on the area correction factor f using the "solvent flush" method of injection. The correction factors were obtained by injecting mixtures of methyl esters of known weight composition. Since oleic acid is usually the most abundant fatty acid in wood, methyl oleate was selected as the reference ester. The standard deviation from the mean was calculated from the data of five to 10 runs. It should be noted that the two resin acid methyl esters were only 85% pure, with 15% isomers, and that equal response was assumed for the impurities.

The results in Table III indicate that at very low split ratios (1:15 and 1:35), the less volatile esters tend to be discriminated at the expense of the more volatile ones, whereas at higher split ratios, the reverse is true. On the other hand, the two resin acid methyl esters appear to maintain a higher concentration in the column stream than in the bypass flow. Compared to the results of Slover and Lanza (41) who report correction factors from 0.93 to 1.35 for fatty acids of similar chain lengths at a split ratio of 1:100, the correction factors obtained here are closer to unity, reflecting a better linearity of the splitter combined with an efficient transfer of samples from the syringe. However, the reproducibility was not very good; the standard deviation was as high as 0.06 for some compounds with high boiling points.

The "hot needle" injection technique was attempted at three split ratios to determine its effect on the reproducibility of the results (Table IV). There is a significant improvement in the precision and the correction factors are much closer to unity, particularly at the 1:55 and 1:150 split ratios. In fact, at a split ratio of 1:150, a response factor of one could be assigned for all the components without introducing significant errors into the analysis. This was demonstrated by recording the chromatograms of samples of crude and distilled tall oil with an added internal standard (methyl heptadecanoate). The measured relative peak area of the internal standard was compared to its actual relative weight in the mixture. A response factor of unity was used for all the fatty and resin acid methyl esters. The relative peak areas (expressed as percentages of total peak area) of methyl heptadecanoate in the chromatograms of distilled and crude tall oil were 4.92% ± 0.11 and 11.25% ± 0.20%, respectively, compared to actual weight percentages of 5.05 and 10.95. The agreement is very good (~3% deviation), considering the complexity of the mixtures to which the internal standard was added. It is important to note that with both injection techniques, the accuracy and precision will be affected by the sample size. Constant volumes should be injected (1.0 μL) and large volumes should be avoided (e.g., 3 μL) because they cause large discrimination.

Effect of Solvent

It was found in this work that methylene chloride and chloroform could be used as solvents, since both provided similar precision and accuracy of analysis. However, it is advisable to use fresh solutions since the conversion of levopimaric to dehydroabietic in dichloromethane has been observed (42) after overnight standing in a refrigerator.

TABLE III

Area Correction Factors (f)[a] and Their Standard Deviations (σ) for the "Solvent Flush" Injection Technique at Various Split Ratios

Split ratio	1:15		1:35		1:55		1:110		1:150	
methyl esters	f	σ	f	σ	f	σ	f	σ	f	σ
16:0	0.970	0.026	1.015	0.027	1.010	0.022	1.022	0.022	1.021	0.016
17:0	0.980	0.015	0.995	0.011	0.992	0.016	0.992	0.002	1.007	0.005
18:0	1.002	0.017	1.001	0.002	1.007	0.002	1.002	0.011	0.999	0.013
20:0	0.995	0.020	0.996	0.015	0.970	0.003	0.952	0.020	0.954	0.016
22:0	1.030	0.026	0.986	0.018	0.971	0.024	0.931	0.015	0.924	0.013
9-16:1	0.960	0.016	0.970	0.025	0.993	0.029	1.005	0.010	1.019	0.014
9-18:1	1.000	–	1.000	–	1.000	–	1.000	–	1.000	–
11-20:1	1.041	0.026	1.034	0.028	1.006	0.038	1.003	0.015	0.986	0.016
13-22:1	1.063	0.033	1.042	0.046	0.980	0.050	0.975	0.030	0.945	0.023
15-24:1	1.064	0.045	1.030	0.050	0.953	0.052	0.955	0.050	0.925	0.036
5,9-18:2	1.006	0.022	0.992	0.005	1.019	0.003	1.003	0.011	1.020	0.014
9,12,15-18:3	1.010	0.020	1.025	0.003	1.006	0.010	1.010	0.024	0.993	0.007
P	0.975	0.029	0.961	0.025	1.002	0.030	0.981	0.009	0.987	0.005
DeAb	0.950	0.025	0.952	0.030	0.940	0.035	0.930	0.031	0.910	0.030

[a]f = area of reference/weight of reference × weight of ester/area of ester.

TABLE IV

Area Correction Factors (f) and Their Standard Deviations (σ) for the "Hot Needle" Injection Technique at Three Split Ratios[a]

Split ratio methyl esters	1:15		1:55		1:150	
	f	σ	f	σ	f	σ
16:0	0.981	0.010	0.972	0.006	0.980	0.007
17:0	1.008	0.006	1.001	0.009	0.997	0.004
18:0	1.002	0.012	1.001	0.006	0.998	0.007
20:0	0.980	0.006	0.985	0.004	0.985	0.017
22:0	1.040	0.020	1.025	0.019	0.991	0.023
9-16:1	0.954	0.009	0.958	0.003	0.979	0.005
9-18:1	1.000		1.000		1.000	
11-20:1	1.032	0.008	1.024	0.004	1.016	0.010
13-22:1	1.040	0.020	1.028	0.006	0.996	0.014
15-24:1	1.060	0.024	1.044	0.012	0.992	0.015
5,9-18:2	0.950	0.045	1.023	0.007	1.010	0.007
9,12,15-18:3	1.080	0.050	0.980	0.008	0.986	0.009
P	0.985	0.025	1.050	0.025	1.021	0.018
DeAb	1.002	0.011	1.035	0.025	1.007	0.005

[a]Column: SE-30, 20-m. Injector and detector temperature: 260 C.

Diethyl ether was also tested as a possible solvent, but led to nonreproducible results and was not used any further.

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