

**Qualitative Determination of Syringaldehyde and
Dihydroconiferyl Alcohol in Maple Sirup**

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The combined amount of syringaldehyde and dihydroconiferyl alcohol in maple sirup was determined by extracting the sirup with methylene chloride, isolating the compounds by GLC (SE-30 column), and comparing the height of the single peak representing the two compounds with a standard of syringaldehyde. The procedure accounts for 97-98% of the SA-DHCA in the sirup with a standard deviation of $\pm 4\%$ of the value obtained. Two samples of sirup were found to contain 5 and 8 ppm SA-DHCA.

The present authors (1, 2) qualitatively isolated a number of aromatic compounds from maple sirup by gas-liquid chromatography (GLC). These components included vanillin, syringaldehyde, and dihydroconiferyl alcohol.

Quantitative measurement of these constituents would furnish information to guide research for improving the quality of maple sirup, developing sirup for specific uses, and establishing purity of these sirups. Work is, therefore, continuing on development of procedures for quantitative determination of such aromatic compounds in maple sirup.

Syringaldehyde was selected for investigation first, since it is more specific to the maple tree than vanillin and is more easily obtained and more stable than dihydroconiferyl alcohol. In trial runs on the Carbowax 20M column, under qualitative GLC conditions (1, 2), syringaldehyde and dihydroconiferyl alcohol could not be completely separated (Fig. 1); they were contaminated with a relatively large and varying amount of bleed which produced an indefinite baseline. In earlier studies, this bleeding of the

column substrate necessitated further purification of fractions. A loss of constituents occurred during the purification, and the Carbowax 20M column was replaced by a SE-30 column for quantitative fractionation. This silicone packing remains stable at the high column temperature necessary to volatilize these aromatic compounds. However, syringaldehyde and dihydroconiferyl alcohol are obtained as a single peak from the SE-30 column. Since the dihydroconiferyl alcohol is also a constituent of the flavor extract of maple sirup, determining the two phenolics as a single value could be as useful as determining syringaldehyde alone.

This paper presents a quantitative GLC method for measuring the combined amounts of syringaldehyde (SA) and dihydroconiferyl alcohol (DHCA) in maple sirup. The analytical results are best expressed as SA because DHCA cannot be readily obtained as a standard.

Experimental

Preparation of Sample for Chromatography

Since the syringaldehyde and dihydroconiferyl alcohol are present in trace amounts, they must be isolated from the sirup and concentrated before GLC analysis. Extraction with a nonpolar solvent, in which the sugars and water of the sirup are insoluble, was found suitable for qualitative isolation (1, 2). Therefore, chloroform, methylene chloride, and diethyl ether were tested as quantitative extractants of the SA and DHCA. A sample of

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U.S. Grade A maple sirup was divided into nine 900 ml portions. Three of these portions were extracted with diethyl ether, three with methylene chloride, and three with chloroform as follows:

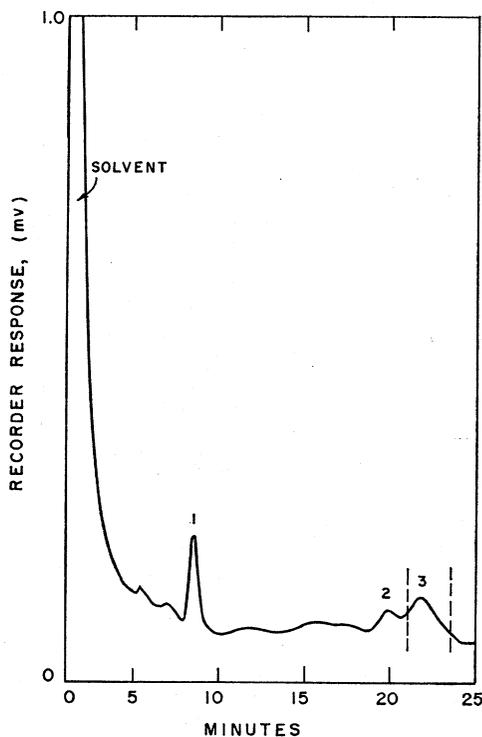


Fig. 1—Gas chromatogram of a chloroform extract of maple sirup on a Carbowax 20M column. Peak identification: 1, vanillin; 2, syringaldehyde; and 3, dihydroconiferyl alcohol.

To the 900 ml of sirup contained in a 2 L glass-stoppered bottle was added 900 ml of solvent. The bottle was closed and shaken in a precision Scientific Shaking Machine at moderate speed (120 oscillations per min.) for 10 min. This mixture was transferred to a 2 L separatory funnel equipped with a Teflon stopcock and allowed to separate into two layers. The sirup layer was returned to the 2 L bottle, and the solvent was transferred to a 2 L beaker. The sirup was then extracted with a second 900 ml of the solvent, and the extract, after separation, was added to the beaker containing the first extract. The combined extract was then reduced to about 100 ml in a Roto-Vap evaporator with a 1 L evaporator flask and then to 15 ml in a 100 ml

evaporator flask. This concentrate was further reduced to 2 ml in a 15 ml graduated centrifuge tube by blowing nitrogen over the surface of the solvent. Portions of the 2 ml concentrate of the extract were taken for GLC analysis.

The completeness of the extraction with methylene chloride and chloroform was tested by extracting 900 ml of sirup with three successive portions of the solvent and concentrating each solvent portion separately. When chloroform was used, 79, 17, and 4%, respectively, was extracted by the three solvent portions; corresponding peak heights were 37.5, 8.0, and 1.8. Results with methylene chloride were 83, 15, and 2% extracted, respectively, and peak heights of 43.7, 14.8, and 2.1. The physical disadvantages encountered in handling the diethyl ether were enough to warrant discarding it as a solvent.

The third extraction by either of the two solvents increased the amounts of SA and DHCA by only 2-4%, showing that 96-98% was recovered by the first two extractions. Since 2-3% of the total represents less than a tenth of a ppm, two extractions of the sirup by the solvent were considered adequate for this determination. Methylene chloride proved superior to chloroform because its lower boiling point made evaporation faster and because emulsification was much less than with chloroform. However, it was impossible to keep some of the solids extracted by the methylene chloride from precipitating when this solvent was concentrated from 1800 ml to 2 ml. Therefore, the extraction procedure was modified by concentrating the methylene chloride extract to 100 ml before adding 100 ml of chloroform and continuing the evaporation. By the time the extract was evaporated to 15 ml, the solvent mixture was largely chloroform and no precipitation occurred.

GLC Separation and Measurement of the Syringaldehyde-Dihydroconiferyl Alcohol

In the initial investigation of the quantitative gas chromatographic separation of the SA-DHCA fraction, the instrumental variables (such as flow rate and column temperature) were selected to give a peak height of 30-60 scale divisions (using a 1 mv recorder of 100 scale divisions). A 25 μ l aliquot of 2 ml concentrated solvent extract (see *Preparation of*

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Sample), was injected into an SE-30 column; the resulting chromatogram is shown in Fig. 2.

Standards

Different amounts of SA were passed through the SE-30 column by injecting varying amounts of a solution containing a known amount of syringaldehyde in chloroform. A 25 μ l portion

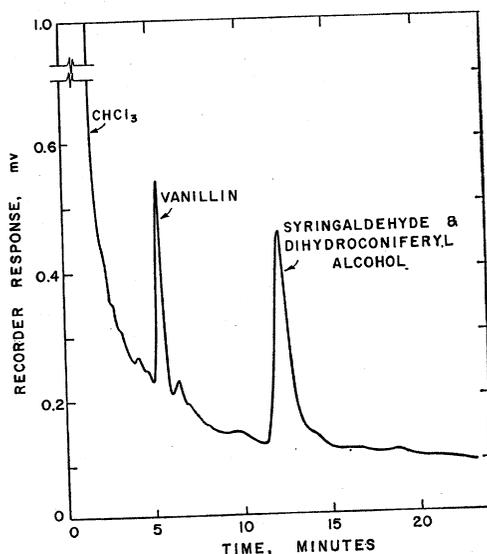


Fig. 2—Gas chromatogram of a chloroform extract of maple sirup on an SE-30 column.

of a chloroform solution containing 3.3 mg SA per ml produced a peak with a height equal to approximately 50% of the recorder scale. This compared closely to the GLC peak height obtained from a 25 μ l aliquot of the 2 ml concentrate of the sirup extract.

The reproducibility of the peak heights and of peak areas as measured by triangulation was tested. Triangulation appeared to offer no advantage over simple peak height as measured from the lowest point of the baseline immediately prior to the appearance of the peak. Later tests showed that samples of maple sirup were apparently free of interfering components in the ascendant slope. However, a component was present that accentuated the tailing effect in the descendant slope and made baseline determination for triangulation more difficult. Therefore, peak heights by direct measurement are used in this procedure to indicate the amount of SA-DHCA in the fraction isolated by the SE-30 column. The straight line relationship of peak

height to amount of SA over the range 30–130 μ g is shown in Fig. 3.

Factors Affecting Variation in Peak Height

A drift in detector response was often noted after four or five GLC separations of sirup extract portions. To determine the cause of this drift in detector response, a solution of diethyl phthalate (DEP) was added to an extract of sirup. GLC analysis of this mixture showed that much greater variance occurred in the SA-DHCA peak height than for the DEP. Many factors were checked to find the cause of this difference, such as (a) lowering the injection port temperature, (b) cleaning out deposits of carbonized solids in the injection port, (c) lowering the column temperature, (d) increasing elution time between injection of samples, (e) overnight conditioning of column at operating conditions, and (f) increasing injection temperature.

The results obtained for the first five determinations after overnight conditioning showed a consistent ratio of 0.9 between peak height response for SA-DHCA and DEP (Table 1). However, this ratio varied for the next four determinations. Raising the injection port temperature from 290° to 350°C eliminated this variation and raised the ratio to 1:3. This increased ratio was caused by an increase in the SA-DHCA peak height. Comparison of these ratios rather than peak heights compensated for slight variations in speed and volume of injection. A similar increase in peak height with increased injection temperature was not obtained for syringaldehyde.

Table 1. Peak heights for syringaldehyde-dihydroconiferyl alcohol (SA-DHCA) and diethyl phthalate (DEP, added standard) from 9 consecutive GLC separations of a CHCl_3 extract of maple sirup (injection temperature, 290°C)

Run	Recorder Response		Ratio SA-DHCA: DEP
	SA-DHCA	DEP	
1	40.2	42.6	0.944
2	40.1	42.6	0.944
3	41.5	44.0	0.943
4	39.7	43.4	0.915
5	42.1	44.4	0.948
6	33.6	44.5	0.755
7	35.6	45.0	0.791
8	36.7	46.5	0.789
9	40.2	45.8	0.872

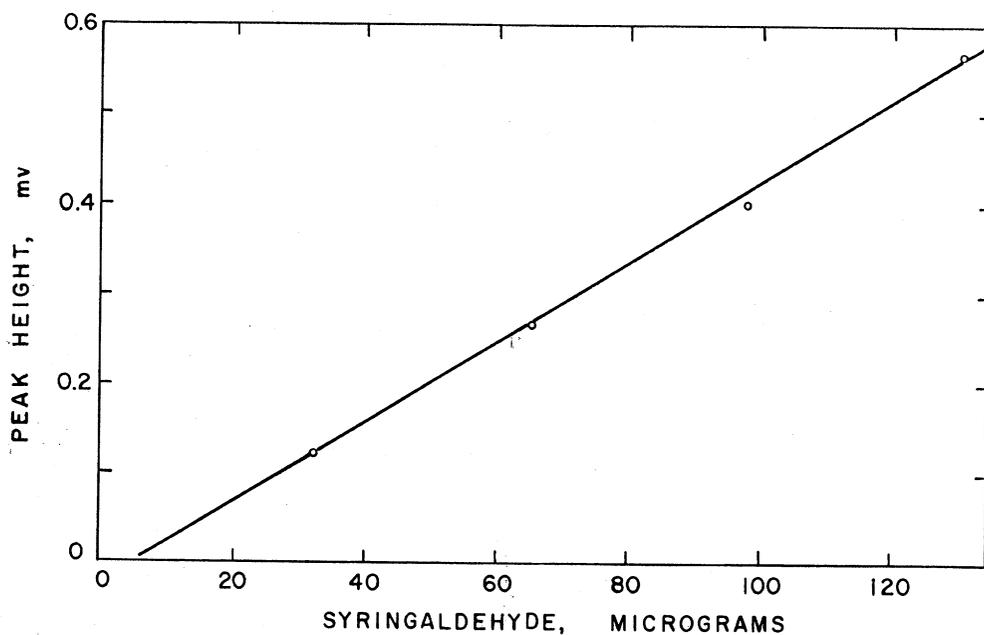


Fig. 3—Relationship of the amount of syringaldehyde to peak height on an SE-30 column.

From the results of these experiments GLC operating conditions were selected for an F&M Model 720 gas chromatograph as follows: flow rate, 50 ml/min. helium; injection port temperature, 352°C; column temperature, 204°C; detector temperature, 315°C; bridge current, 125 milliamperes; sensitivity, 1x; elution time, 24 minutes between injections. Reproducible values were obtained in the analysis of concentrated maple sirup extracts for SA-DHCA under these conditions. Recovery of a known amount of SA added to a maple sirup extract was tested by mixing equal volumes of a maple sirup chloroform extract with a chloroform solution containing a known amount of SA. Recoveries averaged 99%.

Two samples of pure maple sirup were analyzed in quadruplicate for SA-DHCA by the extraction procedure and the GLC separation. The values obtained are shown in Table 2.

Discussion

Preparation of Sample for Chromatography

From the earlier work of the authors (1, 2) and others (3-5) on the qualitative extraction of maple sirup flavoring materials we selected several good solvents to isolate the aromatics from the sirup and the conditions for their fractionation by gas chromatography. The detailed procedure for sample

preparation has been dictated by the trace amounts of syringaldehyde and dihydroconiferyl alcohol in the sirup and the amounts of them needed for the gas chromatographic analysis with the thermoconductivity detector. About one quart of sirup was required to furnish enough SA-DHCA to provide adequate material for GLC separations. About 2 liters of solvent was required to concentrate the extract to 2 ml. Both this large sample of sirup and volume of solvent (requiring a 1000:1 concentration) would be greatly reduced if a more sensitive GLC de-

Table 2. GLC analysis of maple sirup for syringaldehyde-dihydroconiferyl alcohol. (Amounts are expressed as peak heights (mv) in scale division of the chromatogram.)

	Sample A	Sample B	Standard ^a
No. Detns	12	9	3
Min.	44.0	32.0	41.8
Max.	49.8	37.1	43.9
Av.	46.9	34.6	43.1
Std Dev.	±1.8	±1.7	

^a Peak heights for 25 μ l of standard solution containing 83.2 μ g syringaldehyde/25 μ l chloroform.

tector, such as flame ionization, could be adapted to this determination.

Gas-Liquid Chromatographic Analysis

The instrumental conditions chosen for the gas chromatographic analysis of the SA-DHCA in the chloroform extract of maple sirup represent a compromise between speed in analysis and reproducibility of results. The combined concentration of SA-DHCA in the concentrated extract (2 ml) is only about 0.4%. Consequently, the instrument must be operated at maximum sensitivity because a low injection volume is necessary to minimize the tailing effect of the solvent. The column temperature was selected to give an elution point for SA at which tailing of the solvent was minimal. The time interval between injection of successive samples was chosen to permit elution of all observable peaks occurring after the SA-DHCA fraction had passed through the column.

The effect of increased injection temperature on the height of the SA-DHCA peak indicates that a thermal degradation product contributes to the calculated SA content. However, Underwood, *et al.* (4) have shown by other methods of isolation that syringaldehyde is in the chloroform extract.

Since the instrument must be operated at maximum sensitivity, slight variations in gas flow and other factors can cause significant changes in detector response. Therefore, the instrument must be calibrated each day by running a standard of SA selected to give a peak height of SA-DHCA near to that

obtained from the sample. The standard and sample should be determined in triplicate.

Analysis of Sirup Samples

The data in Table 2 show that reasonably good precision was obtained. The standard deviation of ± 1.75 scale divisions for peak heights (PH) representing the SA-DHCA in the two maple sirups obtained by this procedure is satisfactory considering the small amounts of SA-DHCA present in maple sirup. Sirups A and B contained only 8.04 and 5.90 ppm of SA-DHCA, respectively, calculated according to the equation

$$\text{ppm SA-DHCA} = \frac{\text{PH}_{\text{sample}} \times W_{\text{std}} \times 80}{\text{PH}_{\text{std}} \times V}$$

These two values indicate that these constituents exhibit the same wide variation as other maple sirup components.

Recommendation

While the method could have value in following sirup or sap treatments in research studies on the development of color and flavor, it is recommended that study of methods for syringaldehyde in maple sirup be continued with particular emphasis on the use of more sensitive detectors.

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The recommendation of the Associate Referee was approved by the General Referee and by Subcommittee D, and was accepted by the Association. See *This Journal*, **48**, 180 (1965).