Comparison of Gas-Liquid, Gas-Solid, Liquid-Liquid, and Liquid-Solid Chromatographic Techniques in Analysis of Vanillin and Ethyl Vanillin in Alcoholic Solutions

Glenn E. Martin,* G. Gale Guinand, and Donald M. Figert

Vanillin and ethyl vanillin can be qualitatively determined in alcoholic solution by gas-liquid, gas-solid, liquid-liquid, and liquid-solid chromatography. This study details the gas-liquid, gas-solid, liquid-liquid, and liquid-solid chromatographic procedures carried out to determine which of these techniques provides the best resolution and highest efficiency for vanillin and ethyl vanillin analyses. It was found that the best resolution and highest efficiency for both vanillin and ethyl vanillin were obtained using liquid-solid chromatography. The results of the quantitative analyses of several commercial vanillin preparations (only one of which contained ethyl vanillin) are also included.

Over the years, several methods have been developed for vanillin-ethyl vanillin determinations. For many years the Folin-Denis procedure was the official method of the Association of Official Analytical Chemists. Although it was reproducible, it was nonspecific. Ultraviolet photometric procedures such as those developed by Ensminger (1953) and Feeny (1964) have been found satisfactory but have the disadvantage of not distinguishing vanillin from ethyl vanillin without preliminary separation steps.

Gas-liquid chromatographic procedures such as those of Shaw and Waldock (1967), Fitelson and Bowden (1969), Johansen (1965), and Martin *et al.* (1964) have proven of value in both differentiating and quantitating vanillin and ethyl vanillin.

Gas-solid chromatography and the newer technique of liquid chromatography also appear useful for the analysis of vanillin and ethyl vanillin, and it is the purpose of this paper to show their potential for the simultaneous analysis of these compounds.

APPARATUS

A Hewlett-Packard Model 402 gas chromatograph with flame ionization detector and Minneapolis-Honeywell recorder of 1 mV range were used for both the gas-liquid and gas-solid chromatographic analyses. For the glc method, a 6 ft × 2 mm i.d. glass column was employed having a liquid phase of Carbowax 20M 8% (w/w) on a Chromosorb WAW support (mesh size 100/120). The helium flow rate was 60 ml/min and the column, detector, and injection port temperatures were 175° isothermal, 190°, and 180°, respectively.

For the gsc method, a 4 ft × 2 mm i.d. glass column packed with Chromosorb 101 (mesh size 100/120) was employed. The helium flow rate was 60 ml/min and the column, detector, and injection port temperatures were 260° isothermal, 270°, and 270°, respectively.

A Nester-Faust Model 1240 liquid chromatograph operated isothermally at 37° and having a uv detector at 254 mμ was used for both the liquid-liquid and liquid-solid chromatographic analyses.

For the llc method, the mobile phase consisted of 75% hexane (Mallinckrodt Nanograde) and 25% chloroform (Mallinckrodt Nanograde) at a flow rate of 1 ml/min and a flow pressure of 250 psi. The stationary phase was 1.8% Carbowax 400 on Porosil C with a mesh size of 36–75 μ diameter.

Bureau of Alcohol, Tobacco and Firearms, Department of the Treasury, Washington, D. C. 20226.

For the lsc method, the mobile phase consisted of 50% hexane and 50% chloroform at a flow rate of 0.8 ml/min and a flow pressure of 1250 psi. The stationary phase was sil-X of mesh size 37–74 μ diameter.

STANDARDS

A. Place 5 g of cinnamic alcohol (Eastman, reagent grade) into a 100-ml volumetric flask and bring to volume at room temperature with absolute alcohol. Cap flask and shake to ensure uniform solution.

B. Dry vanillin (USP, Fisher Scientific Co.) over concentrated H_2SO_4. To prepare gas chromatographic standards, place 0.05, 0.1, 0.15, and 0.2 g of vanillin in 100-ml volumetric flasks. To prepare liquid chromatographic standards, place 0.025, 0.050, 0.075, and 0.100 g of vanillin in 100-ml volumetric flasks. Bring to volume at room temperature with absolute alcohol and cap. Repeat procedure using ethyl vanillin (Matheson, Coleman and Bell, reagent grade). Pipet 25 ml of each of the vanillin and ethyl vanillin solutions into 25-ml volumetric flasks.

PROCEDURE FOR GLC-GSC

At room temperature, pipet 0.5 ml of solution A above (internal standard) into 25 ml of each of the vanillin and ethyl vanillin gas chromatographic standards prepared in Step B (above). Cap flasks, shake to ensure uniform solution, and replace cap with rubber septums.

Using a 5-μl sample, analyze these solutions by gas-liquid and gas-solid chromatography. Determine the peak height ratio of vanillin and ethyl vanillin to cinnamic alcohol for each of the eight solutions. Construct a graph with the peak height ratio of vanillin or ethyl vanillin to cinnamic alcohol as ordinate and the concentration of vanillin or ethyl vanillin (g/100 ml of standard solution) as abscissa.

PROCEDURE FOR LLC-LSC

Using a 4-μl sample, analyze the vanillin and ethyl vanillin liquid chromatographic standards (do not add internal standard) by liquid-liquid and liquid-solid chromatography. Calculate the peak height of vanillin and ethyl vanillin for each of the eight solutions. Construct a graph with the peak height of 4 μl of vanillin or ethyl vanillin standard solution as ordinate and the concentration of vanillin or ethyl vanillin (g/100 ml of standard solution) as abscissa.

The graphs thus made are used to determine the vanillin and ethyl vanillin content of a vanilla product, either natural or imitation.

For a onefold natural vanilla sample, place 5 ml in a

Table I. Relative Retention Times for Vanillin and Ethyl Vanillin Using gsc, glc, llc, and lsc

	gsc	glc	lsc	llc
Cinnamic alcohol	1.00	1.00		
Vanillin	1.52	2.72	1.00	1.59
Ethyl vanillin	2.08	2.34	1.19	1.00

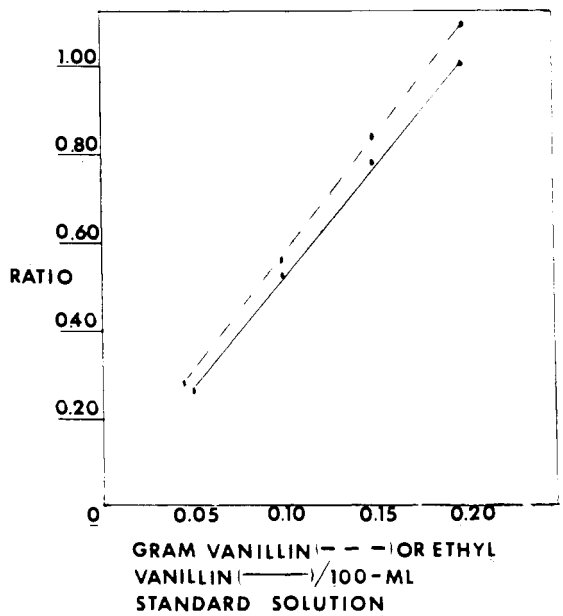

Figure 1. Ratio of the peak height of vanillin or ethyl vanillin to cinnamic alcohol as the ordinate and the gram vanillin or ethyl vanillin/100 ml of standard solution as the abscissa for the gas–liquid chromatographic analysis.

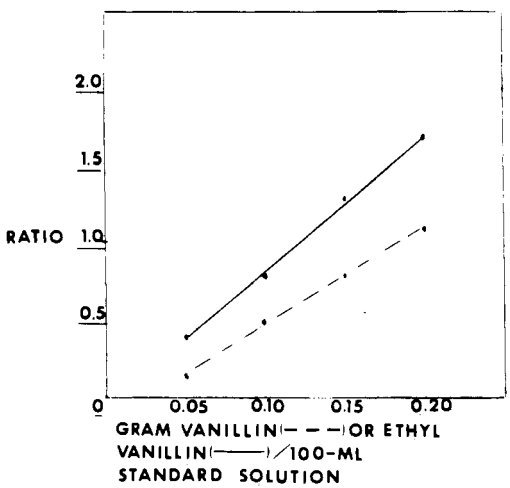

Figure 2. Ratio of the peak height of vanillin or ethyl vanillin to cinnamic alcohol as the ordinate and the gram vanillin or ethyl vanillin/100 ml of standard solution as the abscissa for the gas–solid chromatographic analysis.

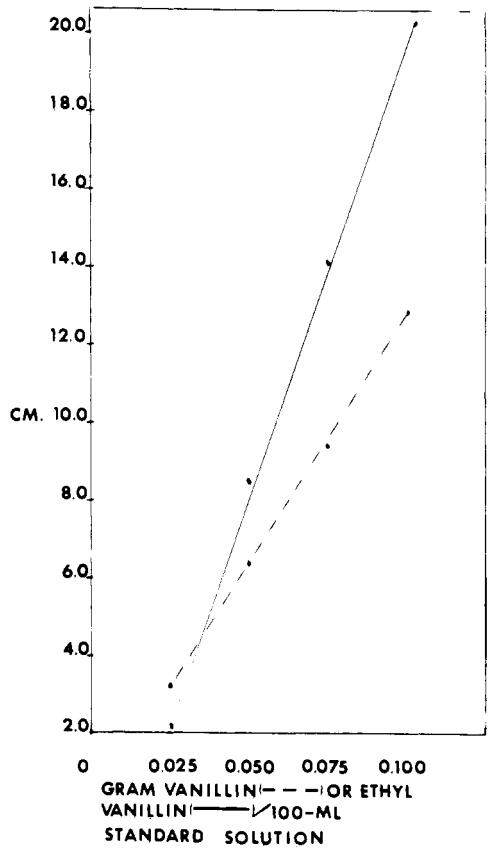

Figure 3. Peak height for 4 μl of vanillin or ethyl vanillin standard solution as the ordinate and gram vanillin or ethyl vanillin/100 ml of standard solution as the abscissa for the liquid–liquid chromatographic analysis.

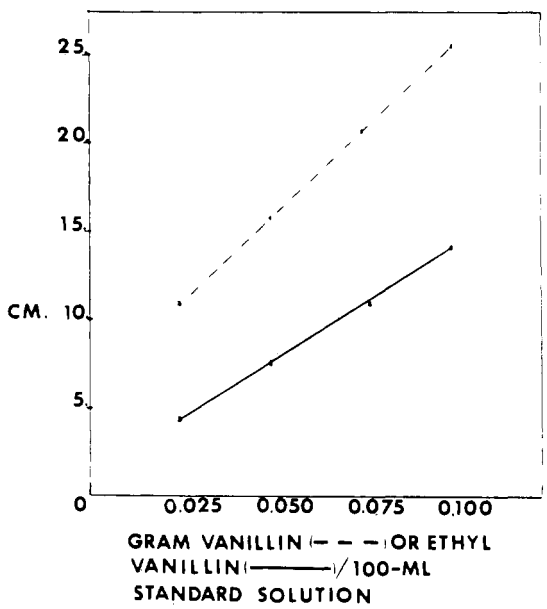

Figure 4. Peak height for 4 μl of vanillin or ethyl vanillin standard solution as the ordinate and gram vanillin or ethyl vanillin/100 ml of standard solution as the abscissa for the liquid–solid chromatographic analysis.

25-ml volumetric flask. Bring to mark at room temperature with alcohol. For imitation vanillas, high in vanillin and/or ethyl vanillin, use 1 ml rather than 5 ml of sample. Add 0.5 ml of internal standard if it is to be run by gsc or glc.

A 4-μl sample was used for the llc and lsc procedures, since 5 μl was found to be outside the response range of the recorder.

DISCUSSION AND RESULTS

Cinnamic alcohol was chosen as internal standard for the gsc–glc procedures because of its noninterfering properties and because of its good resolution under the conditions of analysis; however, the resolution for cinnamic alcohol under the lsc–llc procedure was very poor, and it was therefore omitted as an internal standard. The other compounds that were tried were also found unsuitable.

Table I gives the relative retention times for vanillin and ethyl vanillin to cinnamic alcohol for the gsc–glc pro-

Table II. Comparison for the Quantitative Determination of Vanillin and Ethyl Vanillin Using uv Spectrophotometry, glc, gsc, llc, and lsc

Sample no.	glc		gsc		lsc		llc		Ultraviolet, calcd as vanillin
	Vanillin	Ethyl vanillin	Vanillin	Ethyl vanillin	Vanillin	Ethyl vanillin	Vanillin	Ethyl vanillin	
A	0.3975		0.3650		0.3800		0.4650		0.4694
B	0.3500		0.3427		0.3250		0.3600		0.3636
C	0.3562		0.3210		0.3500		0.3450		0.3806
D	0.3320	0.0887	0.3000	N.D.[a]			0.3200	0.080	0.3886
E	0.1875		0.1562		0.1750		0.1850		0.1770
F	0.2250		0.2062		0.1900		0.2150		0.2004
G	1.020				1.300		1.000		0.9630
H	0.3100		0.3000		0.3000		0.3000		0.3100
I	0.2375		0.2250		0.2385		0.2000		0.2152
J	0.2125		0.2000		0.2000		0.1850		0.1900
K	0.2062		0.1942		0.1900		0.1875		0.2013
L	2.500		2.593		2.500				2.609

[a] Not detectable.

Table III. Separation/ft, Peak Sharpness/ft, and Resolution/ft for Vanillin and Ethyl Vanillin Using gsc, glc, lsc, and llc

	S(separation)/ft $\frac{t_{(R)}\text{ ethyl vanillin} - t_{(R)}\text{ vanillin}}{t_{(R)}\text{ vanillin}} / \text{ft}$	Q(peak sharpness)/ft $\frac{t_{(R)}\text{ of (X)}^a}{\text{Base width of (X)}} / \text{ft}$		R(resolution)/ft Q × S/ft	
		Vanillin	Ethyl vanillin	Vanillin	Ethyl vanillin
Liquid-solid chromatography	0.107	5.413	4.886	0.579	0.523
Gas-solid chromatography	0.069	2.180	2.375	0.150	0.164
	$\frac{t_{(R)}\text{ vanillin} - t_{(R)}\text{ ethyl vanillin}}{t_{(R)}\text{ ethyl vanillin}} / \text{ft}$				
Gas-liquid chromatography	0.023	1.226	1.575	0.028	0.023
Liquid-liquid chromatography	0.247	2.046	1.866	0.505	0.461

[a] X = vanillin or ethyl vanillin.

Table IV. NETP/ft and HETP (in.) for Vanillin and Ethyl Vanillin Using glc, gsc, llc, and lsc

Chromatography	NETP/ft		HETP (in.) = $\frac{\text{Column length (in.)}}{\text{number of equivalent theoretical plates}}$	
	Vanillin	Ethyl vanillin	Vanillin	Ethyl vanillin
Liquid-liquid	100	84	0.120	0.140
Liquid-solid	704	574	0.017	0.021
Gas-solid	304	361	0.040	0.033
Gas-liquid	144	238	0.083	0.050

cedures, vanillin to ethyl vanillin for the llc, and ethyl vanillin to vanillin for lsc.

Table II shows the results of a series of determinations of vanillin and ethyl vanillin on various natural and imitation vanilla products using glc, gsc, llc, lsc, and uv. Generalizations based on the results are difficult to make, since no standard deviation was determined. On the whole, however, the uv–glc analyses gave the highest values.

Table III indicates that the best separation of vanillin and ethyl vanillin was obtained using the liquid–liquid chromatographic method, while the best peak sharpness was achieved using liquid–solid chromatography.

Table IV shows the greatest NETP/ft was obtained using liquid–solid chromatography and similarly the HETP was best for liquid–solid chromatography. It is also interesting to note that although under the most ideal conditions in gas chromatography it is difficult to exceed 400 number equivalent theoretical plates/ft, using liquid–solid chromatography, it is relatively easy to obtain more than 700.

The values obtained for the efficiencies and resolutions were determined for the specific chromatographic system in the determination of vanillin and ethyl vanillin. The values obtained were not carried out to ascertain the most ideal conditions as set forth in the van Deemter equation, namely eddy diffusion, molecular diffusion, and resistance to mass transfer.

Figures 1 and 2 show the ratio of peak height of vanillin and ethyl vanillin to cinnamic alcohol as the ordinate and gram vanillin and ethyl vanillin/100 ml of standard solution as abscissa for the glc and gsc analyses, respectively. The graphs are linear, and the x and y axes, if extended, will pass through the origin.

Figures 3 and 4 show the peak height for 4 μl of vanillin and ethyl vanillin for a standard solution as the ordinate and gram vanillin and ethyl vanillin/100 ml of standard solution as abscissa for the llc and lsc chromatographic analyses. The graphs are linear; however, the vanillin curve for lsc fails to pass through the zero intercept.

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Liquid Chromatography Applications in Analysis and Quality Control of Flavor Chemicals

John P. Walradt* and Chi-Kuen Shu

A simple gel permeation chromatographic method has been applied to the determination of the extent of oligomer or polymer formation in flavor chemicals. The method is illustrated for Δ-dodecalactone, 2,4-pentadienal, and *p*-vinyl guaiacol. In addition, a procedure for calibration of the uv detector is described which requires the preparation of a pure sample component only once, after which the detector sensitivity may be calculated using a stable reference standard. The significance of the gel permeation method in relation to internal standard and area normalization gas chromatographic methods is discussed.

In the course of our evaluation of currently practiced liquid chromatographic techniques, we searched both for methods which could be used in the isolation of natural product constituents and for methods which could be made a routine part of our quality control procedures for pure flavor chemicals. For the latter application, a method was sought which would provide an estimate of chemical purity in addition to presently used gas chromatographic methods, especially for those compounds with a history of polymer formation. Gel permeation chromatography, which elutes components essentially in order of decreasing molecular weight, seemed particularly promising. With this paper, we wish to present some preliminary results which illustrate the type of information obtainable by a simple gel permeation method.

EXPERIMENTAL SECTION

Apparatus. *Lc System 1.* Initial experimentation and analyses, including Figures 1 and 2, were performed using a Varian Aerograph Model 4100 liquid chromatography system equipped with ultraviolet absorption (uv, 254 nm) and refractive index (ri) detectors. The column was ¼ in. o.d. × 6 ft stainless steel, packed with Poragel 60Å (Waters Associates).

Lc System 2. Subsequent analyses, including Figures 3–7, were done with a system consisting of a Waters C903 pumping system, a 5-μl volume high-pressure injection valve (Hamilton part no. 77503), and a Varian Aerograph uv detector. The column used with this system was ¼ in. o.d. × 12 ft stainless steel packed with Poragel 60Å. In both systems 1 and 2, the eluting solvent was reagent grade dichloromethane flowing at the rate of 100 ml/hr. At that flow rate, all analyses were complete within 30 min. Chart speed on the 1 mV Varian Model 20 recorder was 0.25 in./min for both systems 1 and 2.

Preparative gas chromatography was accomplished using a ⅜ in. o.d. × 12 ft stainless steel column packed with 20% SE-52 on 30–60 mesh Chromosorb W in a Varian Aerograph Model 712 preparative gas chromatograph.

Flavor Research Department, International Flavors and Fragrances, Inc., Union Beach, New Jersey 07735.

Analytical gas chromatography was performed using a 500 ft × 0.03 in. i.d. open-tubular column coated with SF-96 for 2,4-pentadienal. For *p*-vinyl guaiacol and Δ-dodecalactone, a ⅛ in. × 12 ft column packed with 12% SF-96 on 80–100 mesh Chromosorb W-HP was used. Both columns were operated in a Hewlett-Packard Model 5751B gas chromatograph with area measurements and internal standard calculations performed by an on-line gc computer system (Craven *et al.*, 1971).

Uv Detector Calibration. The relationship between peak area, A, and sample weight, W, may be expressed as $A = KW$, where K is the response factor. Since the detector measures absorbance at 254 nm, the response factor may be considered equal to a detector constant, X, divided by the absorptivity, a, at 254 nm: $K = X/a_{254}$. Since X reflects any changes in detector sensitivity and is independent of the compound being analyzed, it can be determined using a pure, stable reference standard such as 1,4-dimethylnaphthalene (Aldrich Chemical Co., λ_{max} = 289 nm), as in the present case. The absorptivities at 254 nm were determined using a Beckman DBG spectrophotometer. Peak area measurements from gel permeation chromatography were made using a planimeter. Sample and standard solutions were made on a weight/volume basis using dichloromethane as the solvent.

RESULTS AND DISCUSSION

The formation of polymeric material is often suspected on the basis of changes in properties such as solubility, color, viscosity, and flavor or aroma intensity. Among the first chemicals checked were two samples of Δ-dodecalactone, one of which had a lower aroma level than a fresh sample. Area normalization gas chromatography had indicated that the two samples were virtually identical, but it was observed that the suspected sample did not completely dissolve in propylene glycol at the 10% level as did the fresher sample. Figure 1 shows the gel permeation chromatogram obtained from the suspected sample using lc system 1. No attempt was made to calibrate the detector response at this time, but, for purposes of comparison, we assumed that absorptivities would not be very different for the higher molecular weight materials and calculated