the samples. I thank Dr. Möllhoff from Bayer AG for the mass spectra and IR spectral data.

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Received for review January 31, 1989. Accepted June 6, 1989.

Derivative Spectrophotometric Determination of Vanillin and *p*-Hydroxybenzaldehyde in Vanilla Bean Extracts

Cristóbal Carnero Ruiz,[†] Antonio Heredia Bayona,[‡] and Francisco García Sánchez*[,†]

Department of Analytical Chemistry and Department of Biochemistry, Faculty of Sciences, The University, E-29071 Málaga, Spain

Vanillin (4-hydroxy-3-methoxybenzaldehyde) and *p*-hydroxybenzaldehyde were determined by spectrophotometric derivative measurements in vanilla bean extracts. The method tolerates *p*-hydroxybenzaldehyde to vanillin ratios of 3:1 (w/w) with relative error below 5% in the determination of vanillin. This method compares favorably with the official AOAC spectrophotometric procedure. Relative errors of 0.70% and RSD's of 1.0% are obtained.

The importance of vanillin for the flavor industry and, consequently, its economic implications justify the numerous analytical criteria proposed for the determination of this and other compounds present in it. Moreover, the amount of vanillin found in vanilla extracts is an index as much of the product quality as of its origin. One author showed a correlation between the vanillin and *p*-hydroxybenzaldehyde ratio contained in vanilla beans in relation to their geographical origin (Juergens, 1981a).

Most of the analytical methods previously published use one of several chromatographic methods: thin-layer chromatography (Meili and Chaveron, 1976; Courcelles et al., 1978; Rey et al., 1980; Jiang et al., 1987), gas chromatography (Lhuguenot, 1978; Tabacchi et al. 1978), liquid chromatography (Guarino and Brown, 1985; Fayet et al., 1987), and HPLC (Juergens, 1981b; Dalang et al., 1982; Herrmann and Stoeckli, 1982). Particularly remarkable is the work of Fraisse et al. (1984) who reported a comparative study of three different analytical techniques, liquid chromatography, gas chromatography, and mass spectrometry, for the quantitative determination of the vanillin contained in vanilla beans.

On the other hand, the fraudulent adulteration of vanilla with synthetic vanillin (4-hydroxy-3-methoxybenzaldehyde), derived from wood pulp lignin, has been shown by a method consisting of the determination of the relative isotopic composition of ^{13}C (Krueger and Krueger, 1983; Fayet et al., 1987).

The current AOAC method (1984), mainly used by the flavor industry to determine vanillin, consists of the spectrophotometric determination of the alkaline extract solution at 348 nm, where *p*-hydroxybenzaldehyde interferes. This interference leads to greater vanillin values that those expected.

The aim of the present work is the determination of vanillin in the presence of *p*-hydroxybenzaldehyde without a previous separation. To achieve it, a graphic model based on the interference-free character of the derivative amplitudes measured from an isodifferential point in the base line to the break with the actual derivative curve is proposed. This graphic model has been previously used with satisfactory results for the spectrofluorometric and spectrophotometric determination of metal chelates (Gracia Sánchez et al., 1987; Márquez Gomez, 1988), pesticides (García Sánchez and Cruces, 1988), and phenolic acids (García Sánchez et al., 1988).

This paper describes a rapid assay for vanillin and *p*-hydroxybenzaldehyde extracted from vanilla beans. The quantitative determination of vanillin and *p*-hydroxybenzaldehyde in binary mixtures and comparative data of the results of the determination of vanillin in vanilla beans by the AOAC method are also given.

EXPERIMENTAL METHODS

Apparatus. Spectral measurements were made with a Shimadzu UV-240 Graphicord recording spectrophotometer in 1-cm quartz cells. Instrument parameters: slit, 2 nm; scanning speed, 3 nm/s; recording chart speed, 10 nm/cm. First-derivative ultraviolet spectra were obtained with a Shimadzu derivative spectrum with optional program/interface (Model OPI-2), giving first to fourth derivatives, and λ values of 1, 2, and 4 nm.

Reagents and Solvents. Vanillin and *p*-hydroxybenzaldehyde were obtained from Sigma Chemical Co. Stock solutions were prepared in ethanol and stored in amber bottles at 4 °C. Dilute aqueous solutions were prepared daily from these solutions. Water was both distilled and demineralized. The $NaHCO_3$–NaOH buffer solution (pH 11.0) was prepared by mixing appropriate volumes of 0.1 M $NaHCO_3$ and 0.1 M NaOH. The pH 5 buffer solution was prepared from 1 M acetic acid and 1 M sodium acetate. All solvents used were of analytical reagent grade.

Analytical Procedure. An aliquot of standard solution containing 1–5 μg/mL of vanillin and 0.5–4 μg/mL of *p*-hydroxybenzaldehyde was added to a 10-mL calibrated flask, followed

[†] Department of Analytical Chemistry.
[‡] Department of Biochemistry.

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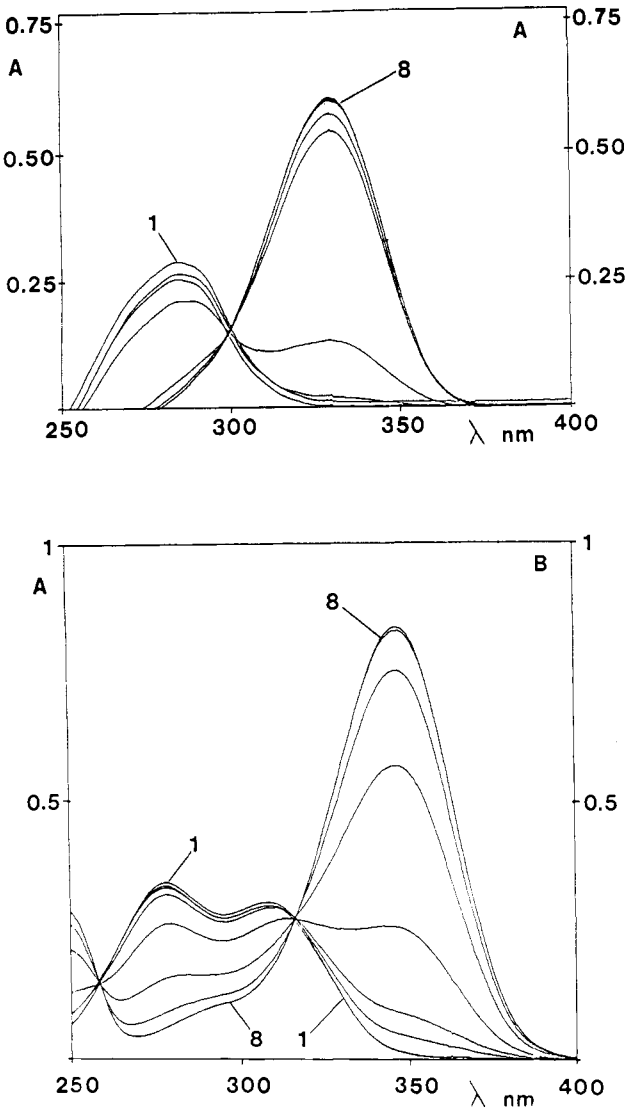

Figure 1. Absorption spectra at various pH values: (A) *p*-hydroxybenzaldehyde (2×10^{-5} M), (1) pH 3.00, (8) pH 11.02; (B) vanillin (3×10^{-5} M), (1) pH 3.70, (8) pH 11.12.

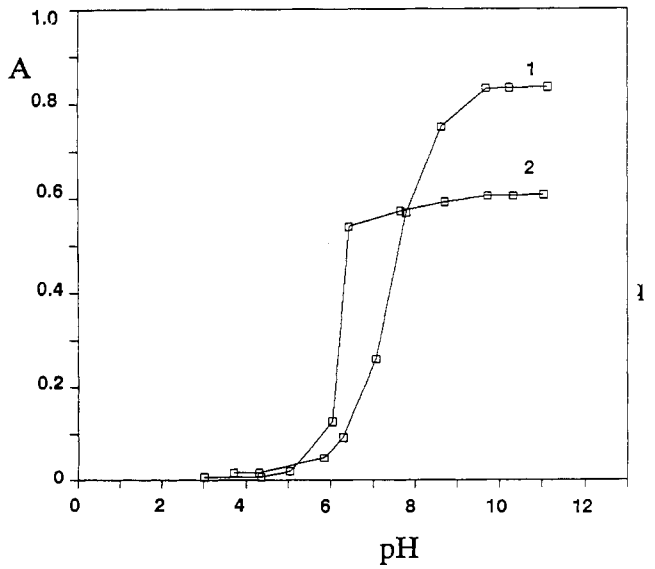

Figure 2. Absorbance–pH dependence of (1) vanillin (348 nm) and (2) *p*-hydroxybenzaldehyde (330 nm).

by the addition of 3 mL of pH 11.0 buffer solution, and diluted to the mark with deionized water. The first-derivative spectrum between 275 and 400 nm was recorded against a reagent

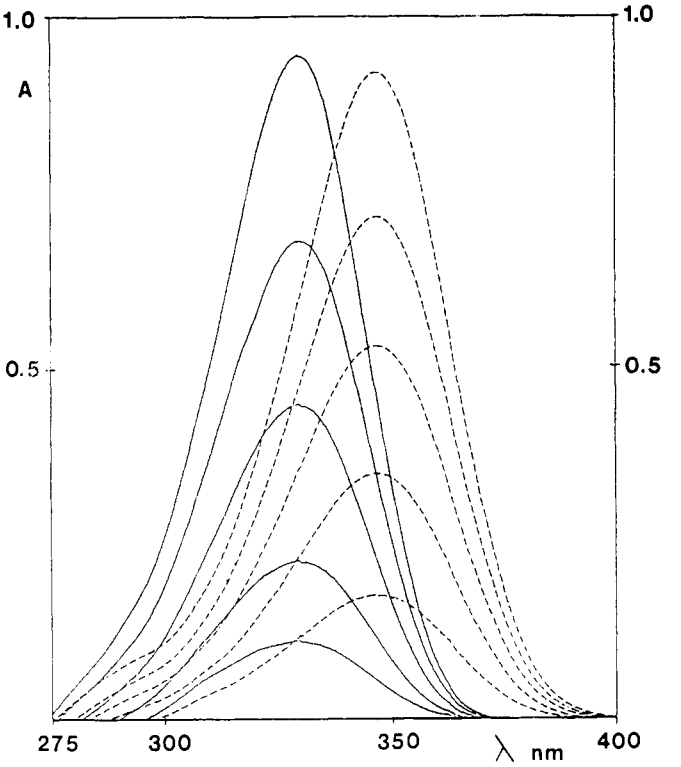

Figure 3. Absorption spectra of vanillin (- - -) and *p*-hydroxybenzaldehyde (—) at several different concentrations: vanillin, 1, 2, 3, 4, and 5 μg/mL; *p*-hydroxybenzaldehyde, 0.5, 1, 2, 3, and 4 μg/mL.

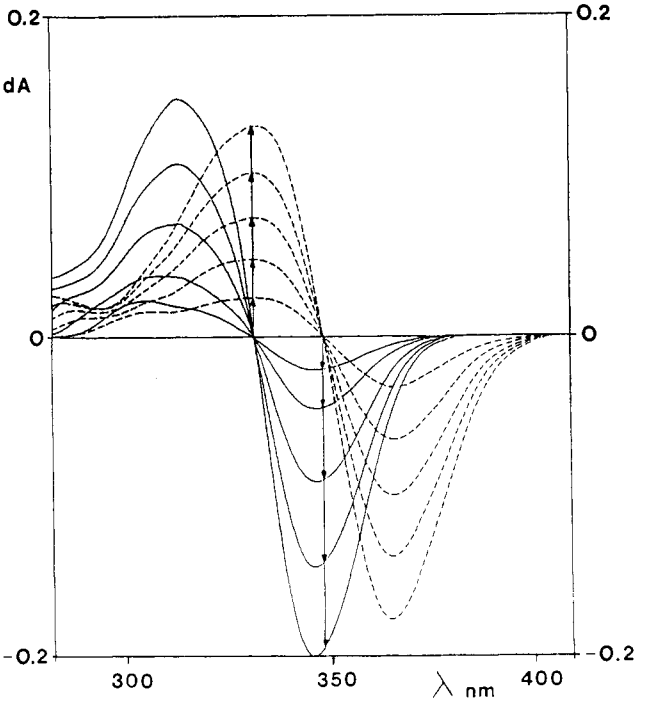

Figure 4. First-derivative spectra of vanillin (- - -) and *p*-hydroxybenzaldehyde (—). Concentrations as in Figure 3.

blank with $\Delta\lambda$ = 4 nm, at a scanning speed of 3 nm/s. The first derivative was measured in the absorbance change (d*A*) scale from the corresponding isodifferential point (330 nm for vanillin and 348 nm for *p*-hydroxybenzaldehyde) to the intersection with the first-derivative curve. The concentration of vanillin and *p*-hydroxybenzaldehyde in a sample is found from calibration graphs previously run under the same conditions.

Extraction Procedure. The vanilla beans were sliced and weighed (1.32 and 1.49 g, respectively) before being placed in the extraction cartridge. This was placed in a Soxhlet extractor with 250 mL of ethanol at 96 °C. After 2 h of flow-back,

Table I. Characteristics of the First-Derivative Isodifferential Procedure

compound	s_S, dA	S_A, ng/mL	C_L, ng/mL	C_Q, ng/mL	error,[a] %	RSD,[b] %
vanillin	8.0×10^{-3}	30	78	262	0.69	1.05
p-hydroxybenzaldehyde	1.4×10^{-3}	28	55	184	0.71	1.00

[a] Error = $100ts/\bar{x}\sqrt{n}$. [b] RSD = $100s/\bar{x}$.

Table II. Analysis of Synthetic Mixtures of Vanillin (A) and *p*-Hydroxybenzaldehyde (B)

	vanillin, μg/mL			*p*-hydroxybenzaldehyde, μg/mL		
ratio A/B (w/w)	taken	found	error,[a] %	taken	found	error,[a] %
10:1	5.00	5.00	0.0	0.50	0.48	−4.0
7:1	3.50	3.60	+2.8	0.50	0.48	−4.0
5:1	5.00	5.04	+0.8	1.00	1.09	+9.0
4:1	4.00	3.95	−1.2	1.00	1.07	+7.0
3:1	3.00	2.87	−4.3	1.00	1.02	+2.0
2:1	4.00	3.95	−1.2	2.00	2.10	+5.0
1:1	3.00	2.94	−2.0	3.00	3.06	+2.0
1:2	2.00	2.00	0.0	4.00	4.09	+2.2
1:3	1.00	0.95	−5.0	3.00	2.91	−3.0
1:4	1.00	1.10	+10.0	4.00	3.93	−1.7
1:5	1.00	1.10	+10.0	5.00	5.12	+2.4
1:7	0.50	0.58	+16.0	3.50	3.81	+8.9
1:10	0.50	0.60	+20.0	5.00	5.04	+0.8

[a] Relative error.

Table III. Analysis of Vanillin Beans

		vanillin[a]		
bean	vol of extr, μL	A	B	*p*-hydroxybenzaldehyde A
1st	30	1.91	2.28	0.201
	50	1.82	2.07	0.193
	80	1.86	2.02	0.181
2nd	30	1.39	1.71	0.202
	50	1.45	1.64	0.177
	80	1.38	1.55	0.187

[a] Percent calculated on fresh beans. A = Isodifferential procedure. B = AOAC method.

the ethanolic extract was evaporated to dryness under reduced pressure at 35 °C. Then, pH 5 buffer solution was added, and after having been filtered, the resultant solution was diluted to give 100 mL with the same buffer solution. From this solution was taken 25 mL and added to 25 mL of ethyl acetate; this mixture was shaken vigorously for 3 min and left to stand for 10 min. This extraction was repeated three times. The organic phase was evaporated to dryness under reduced pressure at 35 °C. Then, ethanol was added to give 10 mL. Aliquots of this solution were then analyzed according to the proposed analytical procedure.

Recoveries. To determine recoveries, simulated natural vanilla was prepared by mixing small amounts of vanillin, *p*-hydroxybenzaldehyde, vanillic acid, and *p*-hydroxybenzoic acid to obtain a total sample value in the range of natural vanilla.

RESULTS AND DISCUSSION

Because both spectral shape and relative absorbance are affected by pH of the medium (Figure 1), a study to determine absorbance–pH dependence was carried out. From Figures 1 and 2 it may be inferred that greatest sensitivity would be obtained between pH 10.0 and 11.0 and at wavelengths of 348 nm for vanillin and 330 nm for *p*-hydroxybenzaldehyde. Satisfactory results were obtained by buffering both solutions at pH 10.8.

Figure 3 shows the absorption spectra of two series of solutions containing increasing concentrations of vanillin and *p*-hydroxybenzaldehyde. As seen in Figure 3, the absorbance maxima of both compounds are separated only by 18 nm, and overlapping clearly occurs, from which a separate quantitation is impeded.

The model used in the paper to discriminate between the overlapping bands needs Beer's law to be obeyed in the full concentration range studied. This fact was proven with both compounds.

Assuming that the derivative of a spectral band is equivalent to the sum of the derivatives of its individual bands, when $dA_1/d\lambda_1 = 0$, the contribution of component 1 on the overall derivative amplitude is zero and, consequently, component 2 may be measured free of component 1 interference. The same applies to the component when $dA_2/d\lambda_2 = 0$.

From Figure 4 one gathers that the maximum sensitivity would be obtained when the first-derivative amplitude of component 1 is zero at the same wavelength at which the first-derivative amplitude of component 2 is maximal. This is satisfied when the first derivative of component 1 is zero and the second derivative of component 2 is zero at the same wavelength.

These considerations are of analytical interest because, by an easy mathematical treatment, it becomes possible to predict if two overlapping bands can be measured satisfactorily by using the isodifferential derivative approach (García Sánchez et al., 1987).

Calibration Graphs, Sensitivity, and Precision. Although the fulfillment of Beer's law was of 0–10 μg/mL for vanillin and of 0–7 μg/mL for *p*-hydroxybenzaldehyde, the calibration graphs were prepared by plotting absorbance and dA against standard vanillin between 0 and 5 μg/mL and *p*-hydroxybenzaldehyde between 0 and 4 μg/mL. The equations obtained by the least-squares method were $dA = -0.0035 + 0.0267c$ (r = 0.999) for vanillin and $dA = -0.0028 + 0.0490c$ (r = 1.000) for *p*-hydroxybenzaldehyde, where c is the concentration (μg/mL).

The sensitivity of the method is expressed as analytical sensitivity $S_A = s_s/m$, where s_s is the standard deviation of analytical signal and m is the slope of the calibration graph (Navas and Sánchez, 1984). The precision of the method was determined by measuring the dA of 11 separate samples, each containing 2.50 μg/mL of vanillin or *p*-hydroxybenzaldehyde.

The detection limit, C_L (k = 3), and determination limit, C_Q (k = 10), are reported as defined by IUPAC (Long and Winefordner, 1983). These analytical characteristics are summarized in Table I.

Applications of the Method to Synthetic Mix-

tures. To test the discriminatory power of the method, this has been applied to the determination of both compounds in synthetic mixtures with different concentration ratios. The results are indicated in Table II. It can be concluded that *p*-hydroxybenzaldehyde can be determined in the presence of a 10-fold concentration excess of vanillin, whereas vanillin can only be quantitatively determined in the presence of a 5-fold concentration excess of *p*-hydroxybenzaldehyde.

Analysis of Vanilla Beans. Two vanilla bean pods acquired in a commercial food establishment were separately submitted to the extraction procedure and further analyzed by the proposed method. In the same manner these samples were analyzed by the AOAC method for the determination of vanillin only. In both cases, three aliquots with different volumes (30, 50, 80 μL) of extract have been used. The results are indicated in Table III.

The values obtained for vanillin by the AOAC method are systematically greater that those obtained by using the isodifferential procedure. This fact agrees well with the results found by other authors using chromatographic techniques (Guarino and Brown, 1985), and of course, this is because of the *p*-hydroxybenzaldehyde positive interference. However, this interference has been effectively avoided by the application of the proposed isodifferential method.

On the other hand, although the amounts of vanillin and *p*-hydroxybenzaldehyde change according to their origin, the values found are in agreement with those previously reported by Fayet et al. (1987).

Recovery Assay. To evaluate the extraction procedure, a simulated natural vanilla was prepared. Three aliquots were separately submitted to the extraction procedure and analyzed by the proposed method. The results showed that a recovery percentage of 85.98 ± 5.76% for vanillin and of 102.22 ± 2.94% of *p*-hydroxybenzaldehyde had been obtained.

CONCLUSION

The results obtained by application of the isodifferential photometric method to the determination of vanillin and *p*-hydroxybenzaldehyde in mixtures minimize cross-interferences between these compounds, especially when vanillin to *p*-hydroxybenzaldehyde ratios are in the 1:3 and 10:1 ranges. Spectral shape specifications of vanillin and *p*-hydroxybenzaldehyde provide an adequate maximum absorption location for obtaining good results by the application of the proposed procedure (García Sánchez et al., 1987). The analytical performances offered by the method make it an alternative to the chromatographic and AOAC recommended procedures.

ACKNOWLEDGMENT

We thank the Dirección General de Investigación Científica y Técnica for supporting this study (Project PB86-0247).

Registry No. Vanillin, 121-33-5; *p*-hydroxybenzaldehyde, 123-08-0.

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Received for review December 29, 1988. Accepted July 10, 1989.