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A simple and rapid HPLC technique for vanillin determination in alcohol extract

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Abstract

This paper describes a simple and rapid HPLC technique for vanillin determination in alcohol vanilla extract. Vanillin was separated on a Nucleosil C18 column by using water and methanol (40:60) as the mobile phase and retention time was only 2.2 min. The measurements were made by using a photodiode array detector of the most adequate maximum wavelength absorbance at 231 nm. This method has been successfully applied for the determination of vanillin in some commercial extracts.

Keywords: Vanillin; HPLC determination; Vanilla commercial extracts

1. Introduction

Vanilla is one of the world's popular flavor extracts obtained primarily from *Vanilla planifolia,* a specie of tropical climbing orchid native to Mexico, but currently cultivated in many countries, principally in Madagascar, Indonesia, Tahiti, and Tonga. It is one of the most widely used flavor resources in confectionery, food, and beverages. The high demand for natural vanillin far exceeds the supply from all sources. Natural vanillin is extracted from vanilla pods by a suitable ethyl alcohol extraction process. The FDA defined a vanilla extract as that of a solution containing not less than 35% ethyl alcohol extracted from 13.35 oz of vanilla beans and containing more than 25% moisture in one gallon of finished product. No addition of synthetic vanillin is permitted in products designed as vanilla extract (Code of Federal Regulations, 1987).

The most often used quantification method by the flavor industry for routine analysis is the AOAC 964.10 method (AOAC, 1990), which determines vanillin through a time-consuming and laborious technique. Quantification proceeds by absorbance measurement of an alkaline solution of the extract at 348 nm and the vanillin absorbance depends on the final pH of the sample. If by simple error, the measurement sample of vanillin extract has pH below 11.0, the result of quantification will be significantly lower there expected. It is also known that some compounds are present in the vanilla extract such as *p*-hydroxybenzaldehyde, which interferes in this method and leads to greater (10–20%) than normal vanillin values than those to be expected.

Many HPLC techniques have been developed during the past 20 years focusing on quantification not only of vanillin, but also other important chemicals present in vanilla extract such as *p*-hydroxybenzyl alcohol, *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, ethyl vanillin and others less important (AOAC, 1995; Archer, 1989; Ehlers, 1999; Guarino & Brown, 1985; Herrmann & Stockli, 1982; Jagerdeo, Passetti, & Dugar, 2000; Khan, 1989; Lamprecht, Pichlmayer, & Schmid, 1994; Scharrer & Mosandi, 2001; Voisine, Carmichael, Chalier, Cormier, & Morin, 1995; Wallace, 1983). From revised literature, only Ehlers (1999) developed a rapid technique for vanillin quantification of 6.7 min of retention time but only because other vanilla extract com-

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pounds were included in quantification. The determination wavelength was considered 278 nm, which is not the maximum wavelength for vanillin, and one can expect that this quantification procedure is not very sensitive and accurate. Recently, Scharrer and Mosandi (2001) developed HPLC technique for vanillin and other important components present in vanilla extract but relating to vanillin its retention time was 26 min and this component was quantified at 275 nm.

The purpose of this study was to develop a simple and rapid HPLC technique only for vanillin determination in ethyl alcohol extract of vanilla pods. The simplicity and rapidity of the method encouraged us to determine the vanillin in available in Mexico some commercial extracts.

2. Materials and methods

2.1. Apparatus

Spectral measurements of vanillin standard were made with a Beckman Coulter 7500 spectrophotometer with a slit of one nm recording in 1-cm quartz cells. A Waters HPLC instrument consisted of a 626 model pumping system and 996 model photodiode array detector, 600S model controller with a gradient system, Empower software, and a manual sample injector up to 50 μL were used. After evaluation of different columns, some Nucleosil C18 columns manufactured by MetaChem Technologies (actually Varian) were employed to develop the quantification technique.

2.2. Reagents and solvents

Vanillin was obtained from ICN Biochemicals, and methanol HPLC grade came from J.T. Baker and ultra pure water was obtained from Millipore Simplicity equipment. Vanillin stock solution (1 mg/mL) was prepared in mobile phase solution and stored in an amber bottle at 4 °C. The following mobile phases were studied: water:methanol, acidified water with phosphoric acid:methanol, water:acetonirile, acidified water with phosphoric acid:acetonitrile. These mobile phases were selected due to separation properties of the columns. Acidified water had not effect in improvement of retention time of vanillin. The shortest retention time of vanillin was found when water:methanol was used as a mobile phase comparing to water:acetonirile.

Finally, the mobile phase of pure water and methanol was considered most adequate. The wavelength of 231 nm was determined as the most adequate for vanillin quantification because of the maximum absorbance at this wavelength. Ethanolic vanilla extracts were diluted 100–500 times in mobile phase and trace amount of ethanol had practically no effect on retention time and no distortion of vanillin peak occurred. The vanillin peak was confirmed by comparison with its specified uv spectrum. No interference of other compounds present in vanilla ethanol extract was observed probably due to a short time of chromatography.

3. Results and discussion

The available HPLC methods for vanillin quantification are summarized in Table 1. Vanillin elution time varied from almost 7 min (Ehlers, 1999) to 36 min (Voisine et al., 1995). Flow rate of the mobile phase was mostly 1 mL/min but some authors used flow rate as high as 2.5 and 2.7 mL/min. Even with high volume pumping, the elution time was still long (17 and 18 min). Many authors employed acidified water by acetic or hydrochloric acid mixed with methanol. Others have used methanol enriched with acetonitrile or tetrahydrofuran. There is variety in the maximum wavelength considered most adequate for vanillin quantification. Three authors considered 275 nm the optimum wavelength, but others quantified vanillin at 254, 270, 278, and even 340 nm. In fact, vanillin diluted in a mobile phase of water–methanol (40:60) and acidified pH of (4.8–6.3) has shown 231 nm as the wavelength of maximum absorption (Fig. 1). Pure vanillin shows four peaks; 204, 231, 279, and 310 nm. When results of vanillin absorbance were compared at 231 and 254 nm, the results of absorbance at 231 nm were almost five times higher. It

Table 1
HPLC conditions for vanillin quantification

Name of column	Mobile phase		Detector (nm)	Flow rate (mL/min)	Vanillin elution time (min)	Reference
	Aqueous phase modifier	Organic phase				
Reverse phase C 18	Acetic acid 0.2 M	Water–methanol (20:80)	275	1	16	Herrmann and Stockli (1982)
LiChrosorb C 8	Diluted acetic acid (10:800)	Water–methanol (90:10)	254	2.5	18	Guarino and Brown (1985)
Microsorb C18	Diluted acetic acid (10:990)	Water–methanol–acetonitrile (100:5:10)	275	1	13	Archer (1989)
LiChrospher RP18	Diluted hydrochloric acid	Water–methanol (70:30)	340	2.7	17	Lamprecht et al. (1994)
Spherisorb C 18	Acetic acid 1.25%	Water–methanol (65:35)	270	1	36	Voisine et al. (1995)
LiChrospher RP	Phosphoric acid (1:10000)	Water–acetonitrile (14:86)	278	1	7	Ehlers (1999)
Nova Pack C 18	Acetic acid 0.05 %	Water–methanol–tetrahydrofuran (70:30:0.2)	275	1	31	Jagerdeo et al. (2000)
LiChrospher 60	Phosphoric acid 1%	Acetonitril–methanol–water (2:3:95)	275	1	22	Scharrer and Mosandi (2001)
Nucleosil C 18	None	Water–methanol (40:60)	231	1	2	This study

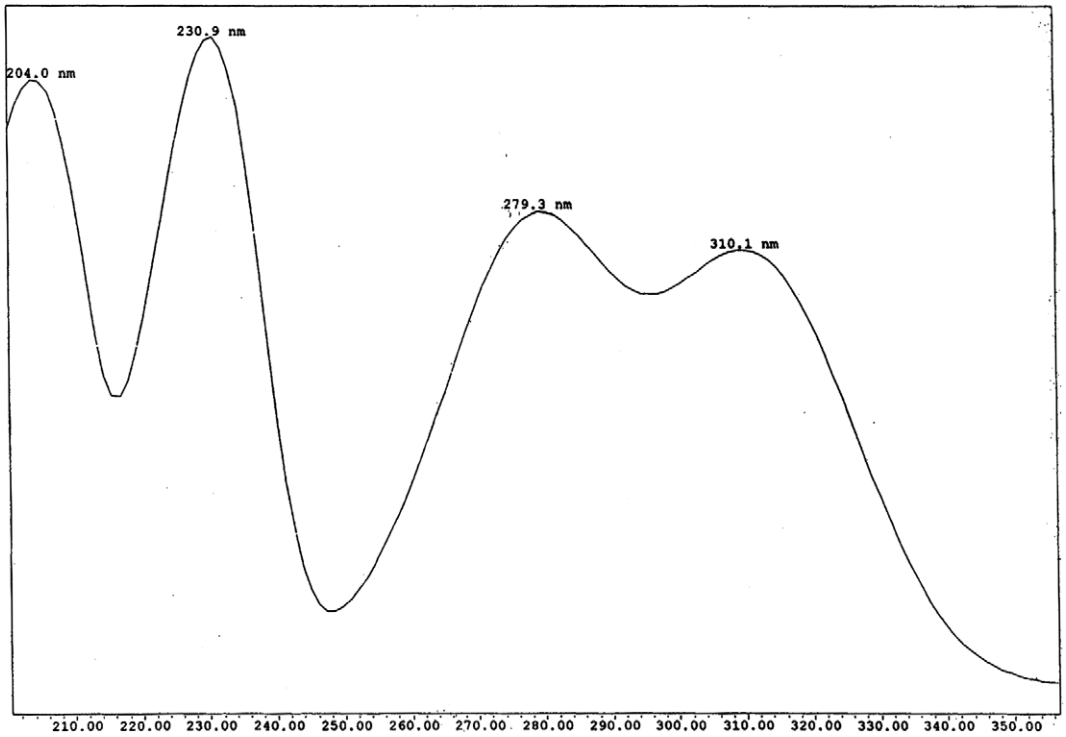

Fig. 1. Spectrum of vanillin in water–methanol solution.

means that if vanillin is quantified at 231 nm, the sensitivity of the method is five times higher.

The following columns were studied in the preliminary evaluation: Rostek Ultra C8 column 150 × 4.6 mm, 5 μm (Rostek Corp), Nova-Pak C18 column 150 × 4.6 mm, 4 μm (Waters Corp) and Nucleosil C18 (Meta Chem). Finally, a Nucleosil C18 150 × 4.6 mm with Meta guard column was selected. The mobile phase consisted of 40% ultra pure water and 60 % HPLC-grade methanol as the most adequate. Standard solution of vanillin was dissolved in the mobile phase. The following mobile phase gradient scheme was used: 0 time water–methanol (40:60), 5 min time methanol (100), 5 min time water–methanol (40:60). Each run lasted 15 min. The mobile phase flow rate was 1 mL/min. and injection volume was 20 μl. The retention time for vanillin was only 2.21 min and at 231 nm, no interference of other compounds present in ethanol vanilla extracts was detected (Fig. 2). The lowest detection limit was below 0.1 μg/20 μL of the injected sample.

To evaluate the quality of the analytical technique, a fortification study at 0.05, 0.1 and 0.15 g/L of vanillin dissolved in mobile phase was performed. The standard solution was added to ethanol vanillin extracts. The mean recovery for vanillin standard oscillated between 95.8%

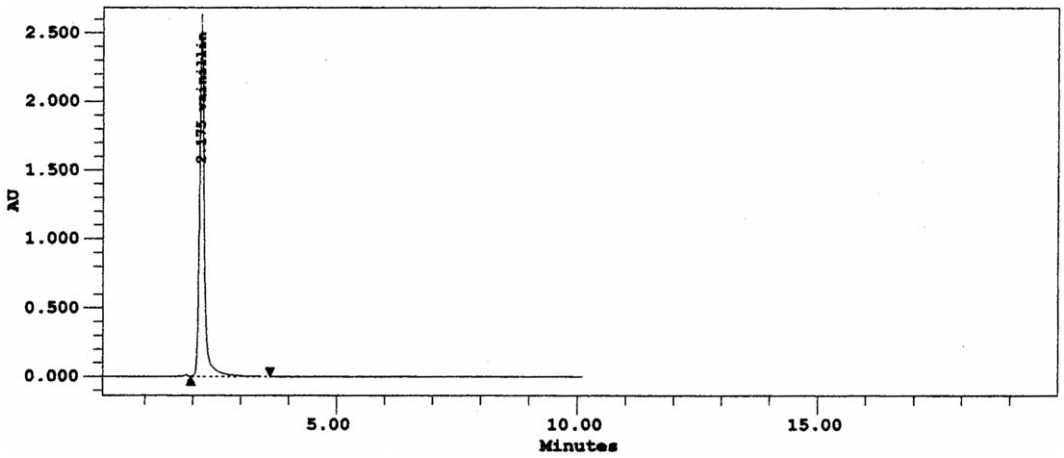

Fig. 2. A chromatogram of vanillin.

Table 2
Mean results and range of vanillin content in retail vanilla extracts

Vanilla extract	Mean result (mg/100 mL)	Range of vanillin content
National 1	85.8	82.1–90.2
National 2	94.7	92.6–98.7
National 3	92.3	88.3–93.6
National 4	108.1	102.0–111.4
National 5	102.5	98.5–107.6
National 6	109.3	105.9–120.3
US producer	92.1	88.6–100.2

and 100.6% with a 3.2 % standard deviation indicating the excellent analytical respond.

This simple and easy technique can be applied satisfactory to any vanillin quantification in vanilla extracts (Table 2). Due to FDA procedure of vanilla extraction, one can expect that it contains about 100 mg/100 mL. In fact, vanillin content in six different Mexican commercial extracts and one USA made extract varied from 82.1 to 120.3 mg/100 mL. In all practicality, only three retail products met requirements of the vanillin content of 100 mg/100 mL. The results are of three replicates and of a minimum three different sets of vanilla extracts.

4. Conclusions

This study contributes to the establishment of easy HPLC conditions for rapid and sensitive vanillin quantification in vanilla extract and for routine purpose analysis. This technique can be used by vanilla extract manufactures and consumers. By analyzing the vanillin content in some commercial products, only three extracts of seven met the requirements for the vanillin content of 100 mg/100 mL.

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