

A COMPARISON OF THE AOAC SPECTROPHOTOMETRIC METHOD FOR VANILLIN AND A GLC METHOD FOR VANILLIN AND ETHYL VANILLIN IN ALCOHOLIC BEVERAGES

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ABSTRACT

A comparison of the results of analyses of alcoholic beverage samples for vanillin by the AOAC spectrophotometric method and a GLC method are presented for 25 samples. Overall, the results were in good agreement. The GLC method distinguishes between vanillin and ethyl vanillin and eliminates interfer-

ences associated with the spectrophotometric method. The average of 12 determinations indicated a 97% recovery for added ethyl vanillin and 90% recovery for added vanillin. A mass spectrometer confirmation of ethyl vanillin and vanillin separated by the GLC was carried out.

Traditionally, there has been considerable interest in determining the vanillin content of vanilla extracts. Due to the increasing use of vanillin as a flavor in alcoholic beverages, it has become necessary to quantitatively determine their vanillin content.

The purpose of this paper is to compare results using the present AOAC spectrophotometric method (AOAC 12th ed. section 19.011-.012) for vanillin in vanilla extracts and results from a gas liquid chromatographic (GLC) determination of a methyl isobutyl ketone (MIBK) extract of an alcoholic beverage sample.

Vanillin and ethyl vanillin can be readily separated and quantitatively determined by GLC; whereas, with the AOAC spectrophotometric method no distinction can be made between ethyl vanillin and vanillin. The spectrophotometric method also suffers from interferences caused by coumarin and similar compounds. Matrix interferences can also be present. Due to the relatively small amount of vanillin and other parameters associated with alcoholic beverages, a rapid and accurate method for the determination of vanillin in alcoholic beverages was needed.

Other researchers (1,2,3,4,6) have determined ethyl vanillin and vanillin in vanilla products by GLC using various column packings and experimental conditions; whereas, this paper focuses on the determination of vanillin and ethyl vanillin in alcoholic beverage samples. Most single fold vanilla extracts (Madagascar bean) contain about 1800 mg/L vanillin (5) and alcoholic beverage samples usually contain around 100 mg/L vanillin. Of the common alcoholic beverages, vanilla extract may be added to special natural wines,

wine specialties, liqueurs, and distilled spirits specialties.

EXPERIMENTAL

Gas chromatography: The analysis of the ethyl vanillin and vanillin standard and sample solutions were carried out using the following GLC conditions: Hewlett-Packard Model 5710A gas chromatograph equipped with a flame ionization detector and a 1 mv recorder. A 6 ft. X 2 mm. i.d. glass column was packed with 10% SP-1000 (carbowax 20M-TPA) on chromosorb W(HP) 80/100 mesh. The column temperature was maintained at 200°C and the inlet and detector temperatures were 210°C. Helium was the carrier gas with a flow rate of 40 mL/min.

Reagents: Ethyl vanillin	MCB 6896
Vanillin	Fisher V-9
Methyl isobutyl ketone	Fisher M 213

Preparation of standard curves: Standard solutions of 10, 20, 40, 60, 80, and 100 mg ethyl vanillin and vanillin per liter of 10% ethanol were prepared. Similar solutions of ethyl vanillin and vanillin using 20%, 30%, and 40% ethanol were also prepared. Ten mL of each standard were transferred to respective ground glass stoppered 50-mL graduate cylinders and 30 mL of water were added and mixed. Ten mL of methyl isobutyl ketone were added and this mixture was shaken vigorously for two to three minutes and the layers allowed to separate. To prepare standard curves for the respective ethanol concentration used, five μ L of the clear MIBK extract was chromatographed and the results were plotted as peak height (in centimeters) versus ethyl vanillin and vanillin concentration (10-

100 mg/L).

Determination of ethyl vanillin and vanillin in samples: After considering the ethanol content, 10 mL of sample was extracted as described above using the appropriate ethanol concentration standard curve for reference. Inject 5 μ L of the clear MIBK extract and read the ethyl vanillin and vanillin concentration from the appropriate prepared standard curve.

AOAC spectrophotometric method 19.011 and 19.012: The AOAC spectrophotometric method 19.011 and 19.012 was used to determine the vanillin concentration of the samples listed in Table 1. The method was modified to increase the alkali content by adding 1 mL of 1N NaOH for each mL of sample used in the sample dilutions.

Table 1. Comparison of GC and AOAC methods.

Sample #	Description	GLC		AOAC 19.011
		Ethyl vanillin ^a	Vanillin ^a	Vanillin ^a
1	DS	ND	69	69
2	DS	ND	19	Interference
3	DS	ND	25	30
4	WS	ND	44	42
5	DS	ND	13	12
6	DS	ND	629	655
7	DS	ND	52	50
8	DS	7	57	50
9	WS	ND	63	60
10	DS	ND	78	78
11	WS	ND	93	90
12	DS	7	ND	7
13	DS	ND	91	95
14	WS	ND	20	23
15	DS	ND	17	Interference
16	DS	ND	33	36
17	WS	ND	44	45
18	DS	ND	206	Interference
19	DS	ND	58	60
20	WS	ND	73	73
21	DS	ND	308	330
22	DS	ND	103	320 (spectral interference)
23	WS	ND	87	85
24	DS	ND	53	50
25	DS	ND	129	116

^a = mg/L.
DS = Distilled spirits.
WS = Wine specialty.
ND = Not detected.

Mass spectrometer confirmation: For the confirmation of ethyl vanillin and vanillin, a GLC-MS coupled by a dual stage Biemann-Watson molecular separator to remove the carrier gas was employed. The chromatograph was operated at similar conditions as described above. The effluent peaks from the GLC were introduced into a Varian Model CH7, single focusing mass spectrometer having an electron bombardment source. The ionizing section of the mass spectrometer was operated at 200°C. The mass spectra were generated using an electron impact energy of 70 electron volts. The spectrum was scanned by varying the magnetic field. The spectra were tabulated using the Varian Spectra System-100 computer.

RESULTS AND DISCUSSIONS

MIBK is an excellent extraction solvent for separating ethyl vanillin and vanillin from the interfering aqueous components in the samples prior to the chromatography. The GLC conditions described under experimental provide an efficient and rapid separation of ethyl vanillin and vanillin from other organic com-

pounds in the samples. The ethyl vanillin and vanillin peaks are sharp and no interfering peaks have been observed. The retention times for the ethyl vanillin and vanillin are 11 minutes and 13 minutes, respectively, using the described conditions. Fig. 1 shows the linearity of ethyl vanillin and vanillin after MIBK extraction from a 30% ethanol solution. To ascertain the quantity of ethyl vanillin and vanillin recovered utilizing the extraction procedure, 20% ethanol-water samples were prepared with known amounts of ethyl vanillin and vanillin added (10-100 mg/L). These samples were extracted and chromatographed and the quantity of ethyl vanillin and vanillin was determined from a standard curve. These standard curves were based upon ethyl vanillin and vanillin solutions prepared in ethanol directly and not undergoing the extraction process. The average of 12 determinations indicated a 97% recovery value for the added ethyl vanillin and 90% recovery for added vanillin.

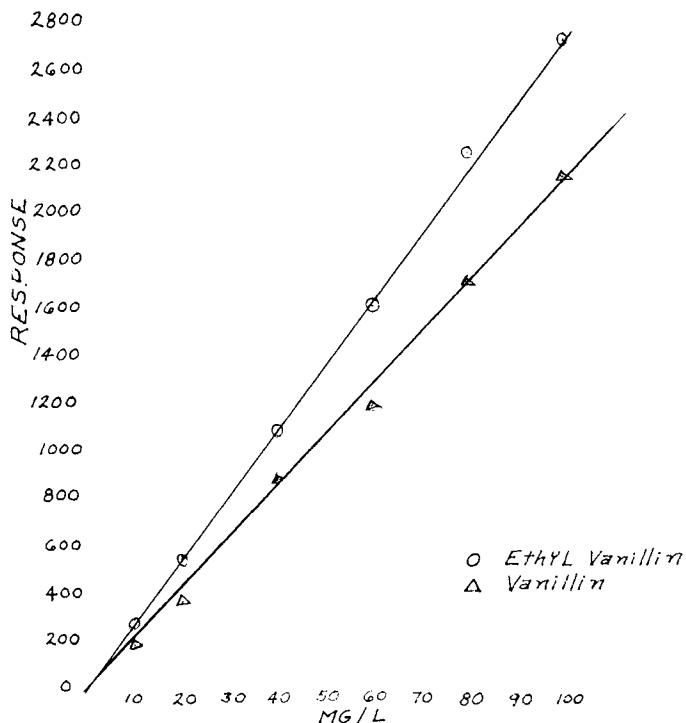


Fig. 1. Response linearity of ethyl vanillin and vanillin after MIBK extraction from 30% ethanol.

A wine sample was analyzed to ensure the absence of ethyl vanillin and vanillin, then known amounts of ethyl vanillin and vanillin (10-100 mg/L) were added. Next, the sample was analyzed by the described method and the data from 12 determinations averaged 101% recovery of the added ethyl vanillin and 101% recovery of added vanillin with a standard deviation of 2.4% for ethyl vanillin and 3.1% standard deviation for vanillin. The data from this experiment indicate accurate results can be obtained when the ethyl vanillin and vanillin standards are carried through the described extraction procedure and the appropriate percentage of ethanol has been used to prepare the standard reference solutions.

The MIBK extraction procedure is very sensitive to ethanol concentration, and the samples and standards must be very similar (within $\pm 5\%$ of the alcohol content; for example, if a sample is 18%, then the standard should be the 20% ethanol standard). For most of the alcoholic beverage samples encountered, the addition of 30 mL of water reduces the alcohol content to 10% or less. At this level, no significant volume changes occur with the MIBK extraction process. The addition of water to bring the alcohol below 10% avoids significant volume changes after equilibration of the immiscible phases and negates any need for calculations to correct for the changes.

Since the wine samples are more acidic and are diluted less than the flavor samples described in 19.011-.012, the alkali content should be increased to avoid obtaining low vanillin values.

A series of mass spectrometer tests was carried out to confirm the identity of the ethyl vanillin and vanillin separated by the gas-liquid chromatography. The standard ethyl vanillin and vanillin solution was injected into the gas chromatograph which was directly coupled to the mass spectrometer and identification of the separate GLC peaks was verified by the characteristic mass spectra for each compound.

Using the described extraction procedure and gas chromatographic conditions, the limit of detection for vanillin and ethyl vanillin is 2 mg/L.

By the described method, our laboratory has

analyzed 25 samples of alcoholic beverage samples for the determination of ethyl vanillin and vanillin and the results are summarized in Table 1. The agreement between the AOAC spectrophotometric method and the GLC method is good. However, the advantages of the GLC method are: 1) the method is specific for ethyl vanillin and vanillin without spectral or matrix interferences; 2) the method is rapid and straightforward requiring only a simple extraction and a gas chromatograph; 3) the method is accurate with results comparable to the AOAC procedure.

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