Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 107 (2008) 1701–1709
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical Methods

Comparison of headspace-SPME-GC–MS and LC–MS for the detection and quantification of coumarin, vanillin, and ethyl vanillin in vanilla extract products

Lowri S. De Jager *, Gracia A. Perfetti, Gregory W. Diachenko

Center for Food Safety and Applied Nutrition, US Food and Drug Administration, 5100 Paint Branch Parkway, College Park, MD 20785, United States

Received 14 August 2007; received in revised form 25 September 2007; accepted 26 September 2007

Abstract

A headspace-solid phase micro-extraction (HS-SPME) GC–MS method has been developed for the determination of coumarin, vanillin and ethyl vanillin in vanilla products. Limits of detection ranged from 1.33 to 13.2 ng mL^{-1}. Accuracy and precision data for the method were measured and compared to those obtained using LC-ESI-MS. A survey of 24 commercially available vanilla products was completed using both techniques. No coumarin was detected in any of the samples. Examination of the GC–MS chromatograms revealed the presence of 18 other flavor related compounds in the samples. The method validation and sample analysis data using HS-SPME-GC–MS were comparable to those obtained using the LC–MS method. Because the two methods are conceptually different from one another, both methods would not be subject to the same interferences. This would allow them to be used as confirmatory methods for each other. Published by Elsevier Ltd.

Keywords: Coumarin; Vanilla; HS-SPME; GC–MS; LC–MS; Headspace

1. Introduction

Solid phase micro-extraction (SPME) is a relatively new sample preparation technique that has been steadily increasing in popularity since its development in 1990 (Arthur & Pawliszyn, 1990). SPME is particularly promising for the sampling of complex mixtures because it often allows direct extraction and concentration of the analytes from the original matrix without lengthy intermediate steps. The use of SPME for the analysis of foods was reviewed in 2000 (Kataoka, Lord, & Pawliszyn, 2000). Although SPME can be used with LC instruments, it is more commonly coupled with GC separation. In the past, the main disadvantage of SPME was that it was mainly a manual system. This increased the amount of manpower required per sample and reproducibility problems could occur if the extraction times were not carefully monitored. Recently, several companies have introduced autosamplers compatible with SPME. Automation of the extraction process gives SPME the potential to become more widely used.

Vanilla flavoring is one of the most popular flavorings in the world. Authentic vanilla extracts are prepared from the pods of *Vanilla planifolia*. Cultivation of the pods is expensive and synthetic or artificial vanilla extracts are widely used. Although the major flavor constituent of vanilla extract is vanillin (4-hydroxy-3-methoxybenzaldehyde), many other volatile compounds such as guaiacol, *p*-anisaldehyde, and methyl cinnamate have been reported to contribute to its flavor (Lamprecht, Pichlmayer, & Schmid, 1994; Perez-Silva et al., 2006). Artificial vanilla extracts are generally less complex and can contain vanillin, ethyl vanillin and other related compounds produced using inexpensive starting materials. Coumarin (2*H*-1-benzopyran-2-one) has a sweet herbaceous odor and has been detected in some imported vanilla products (Thompson & Hoffmann, 1988). Coumarin has been shown to cause hepatoxicity in animals and has been banned for use as a food additive

* Corresponding author. Tel.: +1 301 436 2555; fax: +1 301 436 2634.
E-mail address: lowri.dejager@fda.hhs.gov (L.S. De Jager).

0308-8146/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2007.09.070

in the US since 1956 (Code of Federal Regulations, 2006; Hazleton, Tusing, Zeitlin, Thiessen, & Murer, 1956). Several methods for the detection of vanillin, ethyl vanillin and/or coumarin in vanilla extract have been published in the literature including TLC (Belay & Poole, 1993; Mckone & Chambers, 1988) HPLC-UV (Ehlers & Bartholomae, 1993; Jagerdeo, Passetti, & Dugar, 2000; Kahan & Krueger, 1997; Martin, Guinand, & Figert, 1973; Waliszewski, Pardio, & Ovando, 2007)and GC–MS (Sostaric, Boyce, & Spickett, 2000). Each of these methods has drawbacks and no single method has been reported for the quantitative determination of vanillin, ethyl vanillin and coumarin in vanilla extract that provides qualitative mass spectral confirmation.

Recently, a new LC–MS method for the quantification of coumarin, vanillin and ethyl vanillin in vanilla products was reported (de Jager, Perfetti, & Diachenko, 2007). In this method, a diluted vanilla sample is directly analyzed and quantified using external calibration. LC–MS gives good reproducibility and low limits of detection but fragmentation patterns provide only limited analyte confirmation and no identification of unknown peaks. In the LC–MS study, there were several samples in which extraneous peaks were thought to cause errors in quantification.

A previous publication has reported a headspace-SPME-GC–MS method for the identification of volatile components in vanilla extracts and flavorings (Sostaric et al., 2000). This study optimized SPME extraction conditions in order to determine nine compounds which occur in vanilla products. This method was able to detect coumarin, vanillin, and ethyl vanillin from a variety of samples with the goal of determining the type of vanilla product. This method was purely qualitative and no attempts at quantification were reported. Stanfill et al., used HS-SPME-GC–MS for the determination of coumarin and other volatile flavor compounds in tobacco products (Stanfill & Ashley, 1999; Stanfill et al., 2003). In this paper, a quantitative method for the determination of vanilla components using headspace-SPME-GC–MS is presented and the results compared with those obtained in our previous LC–MS study.

Table 1
Descriptions of the 24 samples, the measured concentrations using HS-SPME-GC–MS and LC–MS signals (mg mL^{-1}) and the calculated differences between the two methods

Sample	Labeled artificial	Origin	SPME-GC–MS	LC–MS	Difference (%)
+Vanillin					
1	Yes	USA	2.56	2.46	4.0
2	No	USA	1.20	1.12	6.9
3	Yes	USA	4.15	3.68	12.0
4	Yes	USA	4.36	4.25	2.6
5	Yes	USA	1.93	1.95	1.0
6*	Yes	USA	8.30	8.59	3.4
7	Yes	Peru	6.04	5.62	7.2
8	Yes	USA	6.86	6.42	6.6
9	Yes	Dominican Republic	1.49	1.43	4.1
10	No	Mexico	1.31	1.40	6.6
11	No	USA	1.60	1.47	8.5
12	Yes	Mexico	2.17	2.54	15.7
13	No	USA	1.51	1.61	6.4
14	No	Mexico	2.63	2.27	14.7
15	No	Mexico	4.76	4.84	4.0
16*	No	Mexico	9.18	8.39	6.9
17	No	Mexico	1.12	1.46	12.0
18	No	Mexico	6.61	6.79	2.6
19*	No*	Mexico	8.93	7.22	1.0
20*	No*	Mexico	8.28	8.20	3.4
21	No	Mexico	1.91	2.50	7.2
22	Yes	Mexico	0.68	0.733	6.6
23	Yes	Mexico	3.52	3.94	4.1
24	No	Mexico	2.09	2.48	6.6
Ethyl vanillin					
4	Yes	USA	0.318	0.330	3.7
5	Yes	USA	0.352	0.394	11.3
7	Yes	Peru	1.39	1.29	7.5
12	Yes	Mexico	0.651	0.651	0.0
14	No	Mexico	0.793	0.78	1.7
15	No	Mexico	0.888	0.950	6.7
16*	No	Mexico	2.37	2.27	4.3
18	No	Mexico	0.683	0.682	0.1
20*	No	Mexico	2.39	2.16	10.1
23	Yes	Mexico	0.671	0.767	13.4
24	No	Mexico	0.213	0.202	5.3

* Denotes that samples were diluted 1:300.

2. Experimental

2.1. Samples

Twenty-four vanilla extract products were purchased from local and internet retail stores (Table 1). Products which were labeled as imitation or artificial will be referred to as artificial products and those which are labeled as pure, real extract or authentic or did not declare artificial content will be referred to as authentic products. All products were water soluble.

2.2. Materials

Vanillin (99%), and ethyl vanillin (99%) were obtained from Aldrich (Milwaukee, WI, USA) and coumarin ($\geqslant$99%) was obtained from Sigma (St. Louis, MO, USA). All solvents used were HPLC grade or better. Water was purchased from J.T. Baker (Phillipsburg, NJ, USA). 3′,4′-(Methylenedioxy)acetophenone (98%) was obtained from Aldrich and was used as an internal standard.

2.3. Instrumentation

2.3.1. SPME-GC–MS

A Varian (Walnut Creek, CA, USA) 3800 GC with a 1200 L single quadrupole mass spectrometer was used. A Combi PAL autosampler was used to allow automated SPME analysis. Data was collected using Varian Star® software version. Ultra pure helium (99.999%) passed through a GasClean GC/MS filter (Varian) was used as the carrier gas at a flow of 1.2 mL min^{-1}. Injections were

made with an initial split ratio of 10:1 followed by a 100:1 split after 2.5 min with the injection port temperature held at 250 °C. A Varian Factor FOUR™ VF-5MS 30 m 5% phenyl methylpolysiloxane copolymer column (Varian) with an inner diameter of 0.25 mm and a 0.250 μm film thickness was used. The initial oven temperature was set to 100 °C with an initial 1.0 min hold followed by a programmed temperature ramp of 10 °C min^{-1} to 153 °C and a hold of 1.0 min. The temperature was then increased to 154 °C at a ramp of 0.2 °C min^{-1} followed by a final temperature ramp of 40 °C min^{-1} to 250 °C.

The MS signal was collected over 50–300 *m/z*. Standard solutions of the individual analytes and the internal standard were analyzed to determine their retention times and identify quantification (quant.) ions. 3′,4′-(Methylenedioxy)acetophenone was chosen as the internal standard at a concentration of 0.5 μg mL^{-1} because it had been successfully used in previous SPME-GC–MS studies of semi-volatile alkylbenzenes including coumarin (Stanfill & Ashley, 1999; Stanfill et al., 2003). The retention times and quantification (quant.) ions used are as follows: Vanillin 7.18 min, 152.1 *m/z*; 3′,4′-(Methylenedioxy)acetophenone 7.86 min, 149, 164 m/z; coumarin 7.98 min, 146, 118 *m/z*; and ethyl vanillin 8.12 min, 137.0 *m/z*. All quantification was based on a peak area ratio of the signal of the analyte and the signal of the internal standard. The scan signal was used to verify the identities of the analyte peaks and determine identities of extraneous peaks.

The final procedure was as follows: six (6) mL diluted sample was placed in a 10 mL screw top vial with PTFE/silicon cap (Supelco). The sample was incubated at 75 °C for 10 min. After incubation an 85 μm polyacrylate fiber (Supelco) was exposed to the headspace of the heated vial for 30 min. The fiber was then desorbed in the injection port for 5 min.

2.3.2. LC–MS

An Agilent (Palo Alto, CA, USA) 1100 series quadrupole LC–MS with electrospray ionization (ESI) interface was used in the positive ionization mode. Data was collected using Chemstation software version A.09.03. A Luna 5 μm ODS C-18 250 × 2.0 mm column (Phenomenex, Torrance, CA, USA) was used for separation with a flow rate of 0.25 mL min^{-1}. The column temperature was held at 30 °C and a 10 μL injection volume was used in all analyses. Separation was achieved using an isocratic elution of 35% acetonitrile and 65% (aqueous 0.1% formic acid) solution and was followed by a gradient to 80% acetonitrile to wash the column. The MS signal was collected in both the scan and selected ion monitoring (SIM) mode. The scan signal was collected over 125–250 *m/z* and the SIM signal contained the quasi-molecular ions of the analytes and the internal standard (147, 153, 165, 167 *m/z*). All LC–MS quantification was based on a peak area ratio of the SIM signal of the analyte and the internal standard. The scan signal was used to verify the identities of the chromatographic peaks. Complete experimental conditions, method development and validation data have been described previously (de Jager et al., 2007).

2.4. Standard solutions

Stock solutions of the analytes were made by accurately weighing 0.05 g (±0.0025 g) of the standard and dissolving it in 50 mL of ethanol in a volumetric flask. Characteristic *m/z* signals and retention times were determined by analyzing the individual standards. Calibration standards were prepared by diluting the stock solution with water in 25 mL volumetric flasks. Five calibration standards were made containing coumarin at concentrations between 1 and 25 μg mL^{-1} and five calibration standards were made containing vanillin and ethyl vanillin at concentrations between 2 and 50 μg mL^{-1}. The internal standard (100 μg mL^{-1}) was added to each standard to give a final concentration of 0.5 μg mL^{-1}.

2.5. Sample preparation SPME

Sample preparation consisted of a 1:100 dilution of the vanilla extract in water. Vanilla extract (250 μL) and internal standard (125 μL) were pipetted into a volumetric flask (25 mL) and water was added to volume. After initial analysis, some of the samples had peak area ratios greater than those obtained in the calibration procedure. For these samples 1:300 dilutions were made in 50 mL volumetric flasks. These samples are denoted with an asterisk (*) in Table 1.

3. Results and discussion

3.1. Method development HS-SPME-GC–MS

Initial method development was based on the method reported by Sosatric et al. (Sostaric et al., 2000). In their work manual extractions were performed using a polyacrylate fiber with an extraction time of 40 min at room temperature. Their work described more efficient extraction at higher temperatures but they chose to use room temperature and longer exposure time in order to improve the reproducibility. Use of an automated SPME system should reduce this type of error so the effects of extraction time and temperature were reexamined. A bivariate optimization strategy was used with extractions done at 40, 50, 60, 75 °C and extraction times between 5 and 45 min. Three extractions were performed for each extraction time/temperature combination and the mean areas and percent relative standard deviations (%RSD) values were calculated (Fig. 1). For coumarin, as extraction temperature and times were increased, peak areas also increased. In addition, increasing extraction temperature resulted in lower %RSD. The increased reproducibility compared to that reported in the previous study is probably due to the reduced error provided by the use of an autosampler. The calculated %RSD values show that there are no significant differences in reproducibility between extraction times

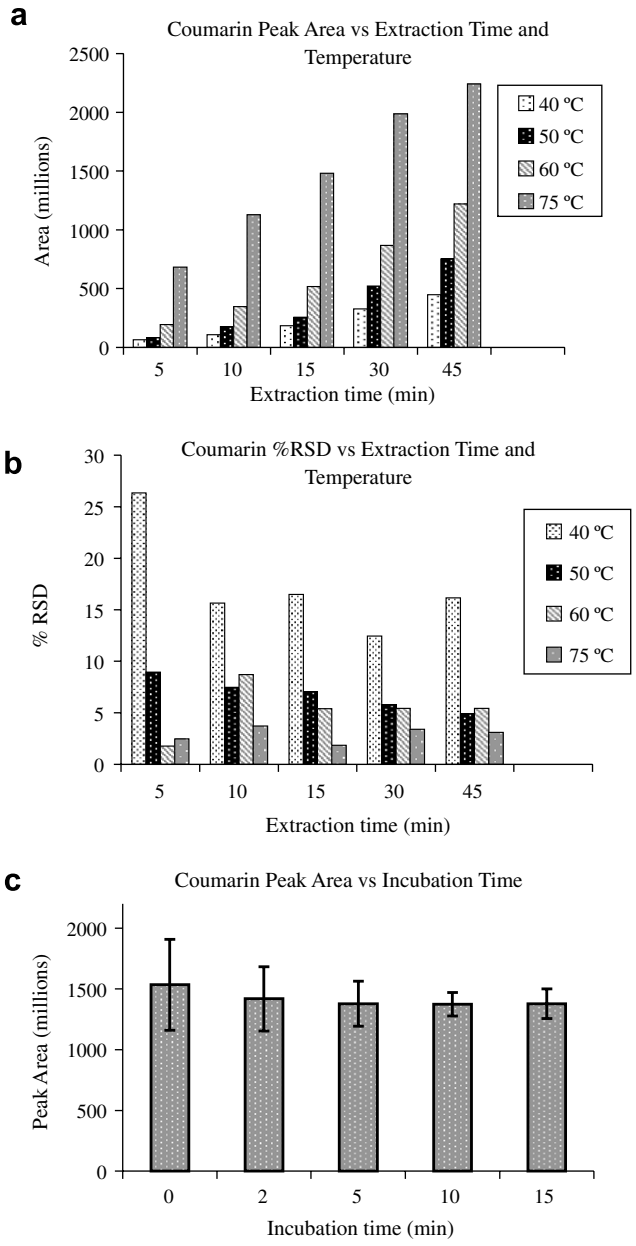

Fig. 1. HS-SPME optimization for coumarin in aqueous samples. Extractions done on 4 mL of a 5 μg mL^{-1} analyte solution after a 5 min incubation time using a 5 min desorption time. (a) Peak area versus extraction time and temperature, (b) %RSD versus extraction time and temperature and (c) peak area versus incubation time at 75 °C ($n = 3$). Error bars represent 95% confidence intervals with $p = 0.05$.

of 30 and 45 min at 75 °C which have %RSD values of 3.4 and 4.9%, respectively. Although extractions of 45 min gave larger peak areas than those obtained at 30 min, it was decided that a 30 min extraction time at 75 °C provided adequate sensitivity with low %RSD while providing extraction times parallel to the GC–MS run time. Extraction profiles for vanillin and ethyl vanillin gave similar results.

The influences of sample volume and incubation times were also considered. Analyses of a 5 μg mL^{-1} solution were performed using 1, 2, 4 and 6 mL of sample in a 10 mL vial. Three extractions were done at each volume and the mean areas and %RSD values were calculated. Peak areas gradually increased with increasing sample volume and the highest reproducibility was achieved using a 6 mL sample volume. Extraction volumes greater than 6 mL caused the fiber to be partially immersed in the aqueous solution and were not used. Incubation time was optimized by performing extractions with 0, 2, 5, 10, and 15 min of sample incubation prior to fiber exposure. There was no significant effect on signal intensity (Fig. 1) but reproducibility was improved using an incubation time of 10 min.

The final extraction conditions were as follows: 6 mL sample volume, 10 min incubation time, 75 °C extraction temperature, and 30 min extraction time.

3.2. SPME method performance characteristics

Peak area ratios of the analyte to the internal standard were used to construct calibration curves. Regression analysis was used to assess the linearity of the analytical method. Five point calibration curves were constructed which produced correlation coefficients (R^2) of greater than 0.99. Five point calibration curves were constructed over three consecutive days to determine the reproducibility of the extraction and chromatographic method. Percent relative standard deviations (%RSD) of the peak area ratios were calculated and pooled data calibration curves were constructed ($R^2 > 0.99$). %RSD values for the SPME-GC–MS data ranged between 1.1 and 5.4 for coumarin, 2.7–6.6 for vanillin, and 2.2–7.9 for ethyl vanillin. These values were comparable to those found with LC–MS analysis. Daily calibration curves were constructed during sample analysis. Limits of detection (defined as a peak giving a response equal to a blank signal plus three times the standard deviation of the noise) were calculated to be 1.33, 13.2 and 4.76 ng mL^{-1} for coumarin, vanillin and ethyl vanillin, respectively. These values are significantly lower than those found with the LC–MS method (45, 72 and 14 ng mL^{-1}, respectively). The increase in sensitivity can be attributed to concentration of the analytes on the SPME fiber during the exposure time. No quantification of analytes was made at concentrations lower than the least concentrated standard of the daily calibration line.

3.3. Accuracy and precision

The effect of the matrix on the quantification of coumarin was determined by spiking a vanilla sample that was found to have no coumarin with known amounts of a coumarin standard (0.0011–5.4 mg mL^{-1}). Coumarin concentrations were determined by interpolating the resulting peak area ratios from calibration lines. The results of the study and a comparison to the LC–MS results are shown in Table 2. In the 1984 survey, adulterated products were found to contain between 0.20 and 2.56 mg mL^{-1} coumarin (Thompson & Hoffmann, 1988)

Table 2
Comparison of accuracy and precision for the analysis of coumarin using HS-SPME-GC–MS and LC–MS

Coumarin concentration		SPME-GC–MS			LC–MS		
Original ($mg\ mL^{-1}$)	Diluted extract ($\mu g\ mL^{-1}$)	Average measured value	% Recovery[a]	%RSD ($n = 3$)	Average measured value	% Recovery[a]	%RSD ($n = 3$)
5.40	54.0	50.0	92.6	4.8	41.4	76.7	2.0
2.16	21.6	21.5	99.5	3.0	19.7	91.2	0.7
1.08	10.8	10.8	100	2.3	10.9	101	1.4
0.540	5.40	5.45	101	4.3	5.50	101	4.0
0.108	1.08	0.994	92.0	2.9	0.901	83.4	0.3
0.0108	0.108	bc			bc		
0.00540	0.0540	bc			bc		
0.00108	0.0108	bc			nd		

bc = Below calibration standards, nd = not detected.
[a] 100% Recovery represents the amount recovered from a laboratory water sample spiked at the same level as the vanilla sample.

and at these levels the accuracy and precision of the headspace-SPME-GC–MS method were excellent. An approximation of the limits of detection for coumarin was done by analyzing samples of decreasing concentrations. At a concentration of $0.018\ \mu g\ mL^{-1}$ the signal to noise ratio of the coumarin peak was 74. Comparison of the data obtained using SPME-GC–MS to those measured using the LC–MS method shows that precision of the two methods are generally similar but the accuracy data, represented as % recovery, suggested that there might be a significant difference. In order to determine if there was a statistically significant difference between the two methods, a Student's *t*-test analysis was performed using a *p* value of 0.05. At this confidence level, there is a significant difference between the two methods for the samples fortified at 5.4 and $0.108\ mg\ mL^{-1}$. This study shows that the SPME-GC–MS method provides more accurate results at the highest and lowest coumarin concentration levels and greater sensitivity than the LC–MS method.

Since all of the vanilla products contained vanillin, the effect of the matrix on the quantification of vanillin and ethyl vanillin was approached in a different way. Standard addition experiments were used to access the accuracy of the SPME-GC–MS method for vanillin and ethyl vanillin. Samples containing moderate levels of vanillin (samples 5 and 11) and ethyl vanillin (sample 5) were used. Both samples were spiked with six levels of vanillin and sample 5 was also spiked with levels of ethyl vanillin prior to analysis. The resulting standard addition curves had high degrees of linearity with R^2 values of 0.98 and greater. Concentrations of the analytes in the unfortified extract were calculated by extrapolating the standard addition calibration curve to the *x* intercept. In order to establish the accuracy of the measurement, concentrations were also determined by interpolating the peak area ratios produced by the unfortified samples from the external calibration lines. The percent difference (Eq. (1)) between the two quantification methods (Table 3) is shown as a metric of the accuracy of the method for the quantification of vanillin and ethyl vanillin

Table 3
Comparison of concentrations calculated ($mg\ mL^{-1}$) using standard addition and external calibration methods for HS-SPME-GC–MS and comparison of standard addition calibration and LC–MS analysis

Sample	External calibration	Standard addition	Difference (%)	LC–MS analysis
Vanillin 5	2.17	2.32	7.0	2.04
Ethyl vanillin 5	0.394	0.333	16.8	0.390
Vanillin 11	1.46	1.38	5.6	1.39

$$\%\ \text{Difference} = \frac{|\text{measured}_1 - \text{measured}_2|}{\left(\frac{\text{measured}_1 + \text{measured}_2}{2}\right)} * 100 \qquad (1)$$

Examination of the results in Table 3 shows that for SPME-GC–MS there is good agreement between the two quantification methods. A Student's *t*-test with a *p* value of 0.05 was performed and there are no statistically significant differences between the results obtained from the SPME-GC–MS methods and LC–MS results.

3.4. Sample analysis

Vanilla samples were prepared as described in the experimental section prior to analysis. Samples were analyzed using the optimized SPME-GC–MS method and concentrations of the analytes were calculated from the resulting GC–MS signals. The results are compiled in Table 1. Unlike the 1988 survey, no sample tested positive for coumarin (Thompson & Hoffmann, 1988). All samples contained vanillin at concentrations ranging between 0.68 and $9.18\ mg\ mL^{-1}$ ($\bar{x} = 3.88$). The concentrations of vanillin in domestic artificial products are 1.4–5.9 times higher than those in authentic domestic products. Eleven (11) samples contained ethyl vanillin at concentrations ranging between 0.213 and $2.39\ mg\ mL^{-1}$ ($\bar{x} = 0.975$). Since ethyl vanillin is only present in artificial vanilla products, the presence of ethyl vanillin indicates that these products are not authentic vanilla extracts. The data also shows that samples with the highest ethyl vanillin concentrations also had very high levels of vanillin. Two examples are samples

16 and 20 which have two of the highest vanillin and ethyl vanillin levels. The concentrations of vanillin and ethyl vanillin found in this study are comparable to those reported in a similar survey by Thompson and Hoffman in 1988 (Thompson & Hoffmann, 1988).

The results of the SPME-GC–MS sample analysis were compared to those reported using the LC–MS method. The percent difference of the concentrations determined using the two methods were calculated using the formula shown in Eq. (1). There was little difference in the concentrations obtained using the two methods with the average difference being 6.23%. Because of this, the SPME-GC–MS method could be used as an alternate to or a confirmatory analysis for the LC–MS method.

In the previous study (de Jager et al., 2007), many extraneous peaks were seen in the LC–MS scan signal. Because of the low levels of fragmentation associated with LC–MS analysis, the identity of these peaks could not be determined. The increased fragmentation afforded by GC–MS allows identification of unknowns by searching a mass spectral database. Twenty-one of the twenty-four samples contained at least one extraneous peak which could be identified using the NIST MS Search 2.0 (Table 4). Eighteen different compounds were identified using the NIST database with R_{match} values greater than 0.85. All of the compounds were on the FDA's "everything added to food in the US" (EAUFS) list except 3,4-methylenedioxybenzyl alcohol, which is a product of the chemical reduction of piperonal. Piperonal was the most common constituent, being present in 10 samples. This is not surprising as piperonal or 1,3-benzodioxole-5-carboxaldehyde has been reported in several other studies of vanilla products (Jagerdeo, Passetti, & Dugar, 2000; Lamprecht et al., 1994; Sostaric et al., 2000) including the qualitative headspace-SPME study of authentic vanilla extracts reported by Sostaric et al (Sostaric et al., 2000). Methyl cinnamate, and *p*-4-methoxybenzaldehyde, seen in a total of eight samples, were also found in both this study and the previous SPME study on authentic vanilla extracts. Seven (7) products were found to contain γ-nonalactone and/or γ-octalactone which do not occur naturally in vanilla beans. All of these products were imported from Mexico; six contained ethyl vanillin and only two were labeled as artificial. The presence of these lactones provides additional evidence that the products not labeled artificial could be deemed to be misbranded.

In the LC–MS survey of vanilla products, a large unknown peak was present in 11 of the 24 products. Inspection of the SPME-GC–MS chromatograms of these products does not uncover a common analyte which would account for the LC-UV peak. Because the unknown is not detected using the SPME-GC–MS method, no conclusions could be made about its identity.

Table 4
Peaks identified in the mass spectral chromatograms of vanilla product samples

Name	Rt	Alternate name	Sample	Use/origin
Benzaldehyde	2.53		6, 9, 24	Found in plants and essential oils
2-Methoxyphenol	3.49	Guaiacol	3, 9	Isolated from plant oils and wood distillates
Benzoic acid	4.11		10	Preservative and seen in essential plant oils
Phenyl-3-buten-2-one	4.37	Benzalacetone, acetocinnamone	10	Flavoring ingredient found in some natural products
Gamma-octalactone	5.40		24	Flavoring agent, for coconut, fruity, and peach
4-Methoxybenzaldehyde	5.46	*p*-Anisaldehyde	18, 19, 21, 24	Found in anise oil and many other essential oils
Benzoic acid, 2-hydroxy-, ethyl ester	5.50	Salicylic acid, ethyl ester	1	Flavoring agent with wintergreen odor, present in various fruits
1-Methoxy-4-(1-propenyl)benzene	5.72	*p*-Propenylanisole, anethol	9	Extensively used in flavor industry Found in anise, fennel and other plant oils
Menthyl acetate	5.75	Menthol acetate	18	Component of peppermint oil
1,3-Benzodioxole-5-carboxaldehyde	6.46	Piperonal, piperonaldehyde	5, 7, 10, 12, 15, 16, 18, 20, 22, 23	Flavoring agent and extensively used in perfumery industry
4-Phenyl-3-buten-2-one	6.63	Benzylideneacetone, benzalacetone	10	Flavoring ingredient,
Gamma nonalactone	6.65	Dihydro-5-pentyl-2(3*H*)-furanone	12, 15, 16, 20, 21, 23, 24	Flavoring ingredient with coconut odor and fatty taste
2-(Ethoxymethyl)phenol	6.59	Hydroxybenzyl ethyl ether	2, 9, 13	Flavoring ingredient
2-Propenoic acid, 3-phenyl-, methyl ester	6.91	Methyl cinnamate	1, 9, 13, 17	Flavoring agent, occurs naturally in essential oils and various fruits
3,4-Methylenedioxybenzyl alcohol	7.03	Piperonol, piperonyl alcohol	14, 22	Flavoring agent, Sweet odor, reminiscent of vanilla
3,4-Dihydro-2*H*-1-benzopyran-2-one	7.06	Dihydrocoumarin, *O*-Hydroxycinnamic acid	5	Fragrance and flavoring ingredient Naturally found in sweet clover and *Artemisia compacta*
4-(Ethoxymethyl)-2-methoxyphenol	8.12	Ethyl vanillyl ether	3, 9, 13, 17	Flavoring agent
6-Methyl-2*H*-1-benzopyran-2-one	10.66	Methyl coumarin	22	Flavoring ingredient coconut, vanilla

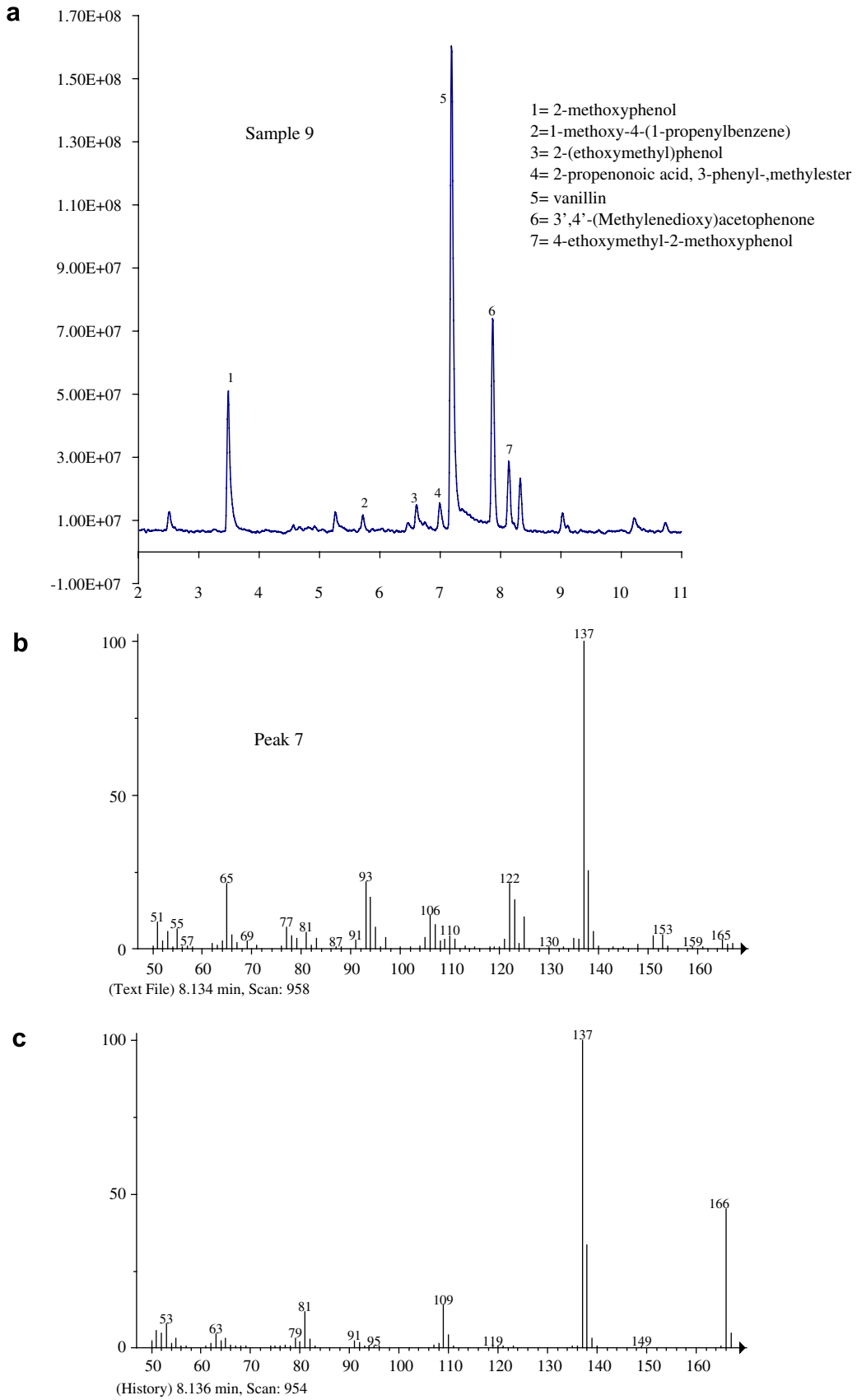

Fig. 2. HS-SPME-GC–MS analysis of sample 9. (a) MS scan chromatogram, (b) mass spectra of peak found at 8.134 and (c) mass spectra of ethyl vanillin standard.

Previous SPME studies show that some compounds frequently found in vanilla products (*p*-hydroxybenzaldehyde, 3,4-dihydroxybenzoic acid, and vanillic acid) were not extracted using a polyacrylate SPME fiber (Kahan & Krueger, 1997; Lamprecht, Pichlmayer, & Schmid, 1994; Sostaric et al., 2000 yet were known to absorb in the UV region. Structurally similar chemicals could be the cause of the extraneous UV peak but there is not enough evidence to determine the identity.

Four sample chromatograms have peaks in the TIC mass spectra which have the same retention time as ethyl vanillin (8.1 min) and are seen in the extracted ion chromatogram used for ethyl vanillin quantification (137 *m*/*z*). The chromatogram for sample 9 (Fig. 2) shows this co-eluting peak as well as other peaks identified in the sample. Examination of the MS of this peak identifies the compound as 4-(ethoxymethyl)-2-methoxyphenol or ethyl vanillyl ether, which has been previously reported in authentic vanilla products (Galetto & Hoffman, 1978). Because of its co-elution with ethyl vanillin, it could easily be misidentified if non-mass spectral GC detection methods were used. Misidentification of the peak could cause investigators to conclude that the products containing this peak are artificial, which may not be the case.

4. Conclusions

The HS-SPME-GC–MS method described in this paper provides an accurate and precise method for determination of vanillin, ethyl vanillin and coumarin in vanilla products. This method provides greater sensitivity and in some cases greater accuracy and precision than the recently reported LC–MS method. Because of the increased fragmentation afforded by GC–MS analysis, this method provides increased specificity and higher confidence in analyte identification. Analysis of the vanilla products using the two methods gave comparable results. The HS-SPME-GC–MS method has the additional capability to identify other components of the vanilla compounds which gives further evidence for determining if a product is adulterated or misbranded. One drawback to the HS-SPME-GC–MS method is the relatively long sample analysis times which include 10 min incubation, 30 min extraction and an 18 min GC cycle. This allows an analysis rate of one sample per 30 min. Because the LC–MS method requires no sample preparation, the total analysis time is significantly lower (16 min). The decreased analysis time allows higher sample throughput and potentially lower costs. Because of the similar accuracy and precision of the two methods, a preference can be made based on the availability of instrumentation and the specific requirements of the laboratory. Given the advantages and disadvantages of the two methods, they can be considered to be complementary techniques for the detection of coumarin and either technique could be used for confirmation of positive samples. Furthermore, the two methods are conceptually different from one another, ensuring that both methods would not be subject to the same interferences.

References

Arthur, C. L., & Pawliszyn, J. (1990). Solid-phase microextraction with thermal-desorption using fused-silica optical fibers. *Analytical Chemistry, 62*(19), 2145–2148.

Belay, M. T., & Poole, C. F. (1993). Determination of vanillin and related flavor compounds in natural vanilla extracts and vanilla-flavored foods by thin-layer chromatography and automated multiple development. *Chromatographia, 37*(7–8), 365–373.

Code of Federal Regulations (2006). Title 21 Part 189.160. US Government Printing Office, Washington, DC.

de Jager, L. S., Perfetti, G. A., & Diachenko, G. W. (2007). Determination of coumarin, vanillin, and ethyl vanillin in vanilla extract products: Liquid chromatography mass spectrometry method development and validation studies. *Journal of Chromatography A, 1145*(1–2), 83–88.

Ehlers, D., & Bartholomae, S. (1993). Analysis of Co2 vanilla extracts by high-performance liquid-chromatography – Comparison with the usual alcoholic vanilla extracts. *Zeitschrift fur Lebensmittel-Untersuchung Und-Forschung, 197*(6), 550–557.

Galetto, W. G., & Hoffman, P. G. (1978). Some benzyl ethers present in extract of vanilla (*Vanilla-planifolia*). *Journal of Agricultural and Food Chemistry, 26*(1), 195–197.

Hazleton, L. W., Tusing, T. W., Zeitlin, B. R., Thiessen, R., & Murer, H. K. (1956). Toxicity of coumarin. *Journal of Pharmacology and Experimental Therapeutics, 118*(3), 348–358.

Jagerdeo, E., Passetti, E., & Dugar, S. M. (2000). Liquid chromatographic determination of vanillin and related aromatic compounds. *Journal of AOAC International, 83*(1), 237–240.

Kahan, S., & Krueger, D. A. (1997). Liquid chromatographic method for determination of vanillin and ethyl vanillin in imitation vanilla extract (modification of AOAC Official Method 99025): Collaborative study. *Journal of AOAC International, 80*(3), 564–570.

Kataoka, H., Lord, H. L., & Pawliszyn, J. (2000). Applications of solid-phase microextraction in food analysis. *Journal of Chromatography A, 880*(1–2), 35–62.

Lamprecht, T., Pichlmayer, F., & Schmid, E. R. (1994). Determination of the authenticity of vanilla extracts by stable-isotope ratio analysis and component analysis by HPLC. *Journal of Agricultural and Food Chemistry, 42*(8), 1722–1727.

Martin, G. E., Guinand, G. G., & Figert, D. M. (1973). Comparison of gas–liquid, gas–solid, liquid–liquid, and liquid–solid chromatographic techniques in analysis of vanillin and ethyl vanillin in alcoholic solutions. *Journal of Agricultural and Food Chemistry, 21*(4), 544–547.

Mckone, H. T., & Chambers, T. E. (1988). Identification of coumarin in vanilla extracts by Tlc and Hplc. *Journal of Chemical Education, 65*(7), 628.

Perez-Silva, A., Odoux, E., Brat, P., Ribeyre, F., Rodriguez-Jimenes, G., Robles-Olvera, V., et al. (2006). GC–MS and GC-olfactometry analysis of aroma compounds in a representative organic aroma extract from cured vanilla (*vanilla planifolia* G Jackson) beans. *Food Chemistry, 99*(4), 728–735.

Sostaric, T., Boyce, M. C., & Spickett, E. E. (2000). Analysis of the volatile components in vanilla extracts and flavorings by solid-phase microextraction and gas chromatography. *Journal of Agricultural and Food Chemistry, 48*(12), 5802–5807.

Stanfill, S. B., & Ashley, D. L. (1999). Solid phase microextraction of alkenylbenzenes and other flavor-related compounds from tobacco for analysis by selected ion monitoring gas chromatography–mass spectrometry. *Journal of Chromatography A, 858*(1), 79–89.

Stanfill, S. B., Calafat, A. M., Brown, C. R., Polzin, G. M., Chiang, J. M., Watson, C. H., et al. (2003). Concentrations of nine alkenylbenzenes, coumarin, piperonal and pulegone in Indian bidi cigarette tobacco. *Food and Chemical Toxicology, 41*(2), 303–317.

Thompson, R. D., & Hoffmann, T. J. (1988). Determination of coumarin as an adulterant in vanilla flavoring products by high-performance liquid-chromatography. *Journal of Chromatography, 438*(2), 369–382.

Waliszewski, K. N., Pardio, V. T., & Ovando, S. L. (2007). A simple and rapid HPLC technique for vanillin determination in alcohol extract. *Food Chemistry, 101*(3), 1059–1062.