Journal of Chromatography, 462 (1989) 461–466
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 079

Note

Analysis of vanilla essences by high-performance liquid chromatography

ALAN W. ARCHER
Division of Analytical Laboratories, New South Wales Department of Health, P.O. Box 162, Lidcombe, New South Wales 2141 (Australia)
(Received October 20th, 1988)

Vanilla essence is prepared by the extraction of vanilla beans, the cured and dried fruit pods of *Vanilla planifolia* Andrews, grown in Madagascar and Indonesia, and *V. tahitensis* Moore, grown in Tahiti. Essences may be prepared by direct extraction of the beans with aqueous ethanol or by dilution of concentrated extracts or vanilla oleoresins. The authenticity of vanilla essences has been assessed by a variety of procedures[1,2] including stable-isotope ratio analysis[3]. The use of the vanillin/*p*-hydroxybenzaldehyde ratio has been proposed[4] for this purpose and it has been suggested that the other minor compounds present in vanilla extracts could also give an indication of authenticity[5]. Vanillin and related compounds present in vanilla beans have been determined by a variety of techniques[6] including high–performance liquid chromatography (HPLC)[5,7–12]; the published HPLC methods are summarised in Table I. Only two of these methods use an internal standard and both of the compounds used as internal standards are far from ideal as ethyl vanillin may occur as an artificial additive in vanilla essences and 3,4-dihydroxybenzaldehyde occurs naturally in vanilla[5]. This note describes the separation and determination of vanillin and related compounds in vanilla essences using an aqueous–organic mobile phase with a C_{18} stationary phase and phenoxyacetic acid as an internal standard.

EXPERIMENTAL

Chromatography

The apparatus used consisted of an Altex Model 321 liquid chromatograph with a Rheodyne 7125 sample injector fitted with a 10-μl loop, and an Erma Model ERC-7210 variable-wavelength detector set at 275 nm and 0.08 absorbance units. A Microsorb C_{18} reversed-phase column, 150 × 4.6 mm I.D., 5 μm particle size, Rainin, cat. No. 80-215, with a Brownlee RP-18 5-μm, 3-cm guard column, was used with a flow-rate of 1 ml/min. The mobile phase was a mixture of 50 ml of methanol, 100 ml of acetonitrile and 10 ml of acetic acid, diluted to 1 l with filtered, de-ionised water. All organic solvents were HPLC grade and acetic acid was analytical-reagent grade.

Reagents

Ethyl vanillin, vanillyl alcohol and *p*-hydroxybenzyl alcohol were purum grade

0021-9673/89/$03.50

TABLE I

CHROMATOGRAPHIC CONDITIONS USED FOR THE DETERMINATION OF VANILLIN AND RELATED COMPOUNDS

Column	*Particle size (μm)*	*Mobile phase*		*Detector wavelength (nm)*	*Flow-rate (ml/min)*	*Internal standard*	*Ref.*
		Aqueous phase modifier	*Organic component*				
Micropak MCH	10	Orthophosphoric acid	Methanol 10–100%	280	2	No	5
μBondapak Phenyl	10	Orthophosphoric acid	Methanol 25%	280	3	No	7
RP-18 Merck	10	Acetic acid	Methanol 8–48%	275	1	No	8
C_{18}	–	Orthophosphoric acid	Methanol 25%	254	–	2,4-Dihydroxybenzaldehyde	9
LiChrosorb C_{18}	5	Buffer, pH 2.4	Methanol 35%	254	1	Ethyl vanillin	10
LiChrosorb C_8	10	Acetic acid	Methanol 10%	254	2.5	No	11
μBondapak C_{18}	5	Nil	Methanol 40%	275	1	No	12
Microsorb C_{18}	5	Acetic acid	Methanol 5%, acetonitrile 10%	275	1	Phenoxyacetic acid	This work

(Fluka), vanillin was Unilab grade (Ajax Chemicals, Sydney, Australia), vanillic acid, *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, coumarin and 3,4-dihydroxybenzaldehyde were from Tokyo Kasei; phenoxyacetic acid was from Merck. Methanol for the standard and internal standard solutions was analytical-reagent grade from May and Baker.

A standard solution was prepared to contain 100 mg of vanillin, 10 mg vanillic acid, 10 mg *p*-hydroxybenzaldehyde and 5 mg of *p*-hydroxybenzoic acid in 100 ml of methanol. The internal standard solution contained 250 mg phenoxyacetic acid in 1 litre of methanol–water (50:50, v/v).

Procedure

Vanilla essences contain about 0.1% (w/v) vanillin and were analysed without further dilution; concentrates and oleoresins were diluted with methanol–water (50:50) to give a vanillin concentration of about 0.1% vanillin. Add 1 ml of standard solution to 10 ml of internal standard solution, mix, inject 10 μl and determine the peak area ratios of vanillin, *p*-hydroxybenzaldehyde, vanillic acid and *p*-hydroxybenzoic acid to phenoxyacetic acid. Add 1 ml of vanilla essence, or diluted concentrate, to 10 ml of internal standard solution and inject 10 μl. From the peak area ratios of vanillin, *p*-hydroxybenzaldehyde, vanillic acid and *p*-hydroxybenzoic acid to phenoxyacetic acid, calculate the concentration of these compounds in the sample.

RESULTS AND DISCUSSION

Vanilla essences contain vanillin as the major aromatic compound and this is accompanied by smaller concentrations of vanillic acid, vanillyl alcohol, *p*-hydroxybenzaldehyde, *p*-hydroxybenzoic acid, *p*-hydroxybenzyl alcohol and 3,4-dihydroxybenzaldehyde. Ethyl vanillin, a synthetic vanillin analogue, may be present as an additive in vanilla essences. The major compound vanillin has a UV absorption maximum in the mobile phase at 275 nm and this was chosen as the detector wavelength. Adequate separation of the compounds of interest was obtained with an aqueous mobile phase containing both methanol and acetonitrile. A number of aromatic acids were examined for use as an internal standard; phenoxyacetic acid has a suitable retention time and uv absorption spectrum, with a maximum in the mobile phase at 275 nm. It was necessary to dilute the samples before analysis and this was done by adding the sample to the internal standard solution. Vanilla essences may contain caramel as an added colouring and it was found that a precipitate was produced if these essences were added to the internal standard dissolved in 100% methanol. When this solvent was replaced with methanol–water (50:50), no precipitation occured. The separation of reference compounds is shown in Fig. 1A. Typical retention times (in minutes) were: *p*-hydroxybenzyl alcohol, 4.1; vanillyl alcohol, 4.9; *p*-hydroxybenzoic acid, 7.3; vanillic acid, 8.8; *p*-hydroxybenzaldehyde, 10.4; vanillin, 13.5; phenoxyacetic acid, 21.8; ethyl vanillin, 28.8. Coumarin, retention time 39.2 min, was not detected in the vanilla extracts, essences and oleoresins examined. In practice, the two earlier eluting compounds were not quantitated as interfering compounds with similar retention times are present in vanilla essences, as shown in Fig. 1B. The small peak X (Fig. 1B), retention time 6.2 min, was identified by co-chromatography as 3,4-dihydroxybenzaldehyde. The detector response for vanillin, *p*-hy-

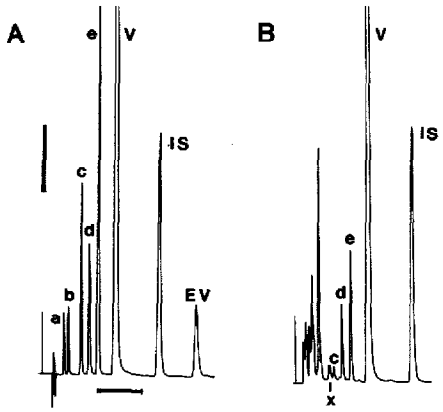

Fig. 1. HPLC traces of (A) reference compounds and (B) laboratory-prepared extract of vanilla beans. Peaks: a = *p*-hydroxybenzyl alcohol; b = vanillyl alcohol; c = *p*-hydroxybenzoic acid; d = vanillic acid; e = *p*-hydroxybenzaldehyde; V = vanillin; IS = phenoxyacetic acid, EV = ethyl vanillin; x = 2,4-dihydroxybenzaldehyde. Vertical bar = 0.01 a.u.; horizontal bar = 10 min. For chromatographic conditions see text.

TABLE II

HPLC ANALYSIS OF VANILLA OLEORESINS, VANILLA EXTRACTS AND RETAIL VANILLA ESSENCES

	p-Hydroxy-benzoic acid	*Vanillic acid*	*p-Hydroxy-benzaldehyde*	*Vanillin*
Retail vanilla essences (mg/100 ml)				
1	0.14	0.50	6.23	79.3
2	0.17	0.97	9.46	128.2
3	1.27	2.34	2.09	82.6
4	1.51	4.27	5.52	100.4
5	2.57	8.62	8.34	115.5
Vanilla extracts (mg/100 ml)				
1 Bourbon beans	1.33	3.49	6.49	85.4
2 Bourbon beans	0.90	3.90	5.90	85.7
3 Bourbon beans	1.77	7.04	7.38	129.3
4 Bourbon beans	1.63	7.16	7.83	134.0
5 Bourbon beans	1.85	9.30	11.2	163.2
6 Java beans	2.43	7.95	7.48	81.9
Vanilla oleoresins (%, w/w)				
1	0.028	0.089	0.093	1.58
2	0.058	0.192	0.183	3.32
3	0.009	0.017	0.305	3.98

TABLE III

RATIOS OF CONCENTRATIONS OF MINOR COMPOUNDS TO VANILLIN

	Ratio (mg compound/g vanillin)		
	p-Hydroxy-benzoic acid	*Vanillic acid*	*p-Hydroxy-benzaldehyde*
Retail vanilla essences			
1	2	6	78
2	1	8	73
3	15	28	25
4	15	42	55
5	22	75	72
Vanilla extracts			
1	16	41	76
2	11	46	69
3	14	55	57
4	12	53	58
5	11	57	69
6	30	97	91
Vanilla oleoresins			
1	18	57	59
2	17	58	55
3	2	4	77
U.S.A. values*	5–21	27–82	57–90 (n = 13)
F.R.G. values**	–	–	54–92 (n = 40)

* Calulated from the results in ref. 11.
** Calulated from the results in ref. 4.

droxybenzaldehyde, vanillic acid and *p*-hydroxybenzoic acid was linear up to concentrations of 150, 12, 10 and 5 mg/100 ml, respectively, in the sample. The recoveries of compounds added to a diluted vanilla essence to give concentrations within the linear response range were: vanillin 99.8–101%, vanillic acid 100.5–102%, *p*-hydroxybenzaldehyde 100.9–108.5% and *p*-hydroxybenzoic acid 99.0–100.7%

Samples of vanilla oleoresins, extracts of vanilla beans prepared in the laboratory with ethanol–water (50:50, v/v) and retail samples of vanilla essences were examined; the results are shown in Table II. Although the concentrations of all compounds vary widely, the ratios of the concentrations of the minor compounds to that of vanillin, expressed as mg of compound/g of vanillin, show less variation and these ratios can be used to assess the authenticity of vanilla extracts. These ratios are shown in Table III. The ratios for the three compounds present in the genuine, laboratory prepared vanilla extracts are similar to those calculated from the reported concentrations found in vanilla extracts by HPLC[11]. The ratio of vanillin to *p*-hydroxybenzaldehyde has been used to determine the authenticity of vanilla extracts and a range of 10.9–18.4 g vanillin/g *p*-hydroxybenzaldehyde was reported[4]. This is equivalent to a range of 54–92 mg *p*-hydroxybenzaldehyde/g vanillin, similar to those calculated from the U.S.A. results and the range of ratios found for the laboratory prepared

vanilla extracts (Table III). The following ranges of ratios (mg compound/g vanillin) are suggested for genuine vanilla essences: *p*-hydroxybenzoic acid, 5–30; vanillic acid, 27–97; *p*-hydroxybenzaldehyde, 54–92. The *New South Wales Pure Food Regulations*[13] require vanilla essence to contain "the quantity of soluble substances in their natural proportions that are extracted by an aqueous alcoholic solution", the ethanol content of which is 50–55% (v/v). A comparison of the results obtained from the retail samples of vanilla essences and the ratios proposed above, suggest that these requirements[13] are met only by retail essences 4 and 5.

ACKNOWLEDGEMENTS

The author is grateful to Bush Boake Allan Australia Ltd., Melbourne, Australia, Givaudan Pty. Ltd., Sydney, Australia and Allied Industries Pty. Ltd., Sydney, Australia, for gifts of vanilla beans and oleoresins and acknowledgement is made to the Director, Division of Analytical Laboratories, New South Wales Department of Health, for permission to publish this paper.

REFERENCES

1 G. E. Martin, M. W. Etheridge and F. E. Kaiser, *J. Food Sci.*, 42 (1977) 1580.
2 M. Ishiguro, S. Nanba and A. Nakatsu, *Kazei Chuo Bunseikishoho*, 26 (1986) 61.
3 G. E. Martin, F. C. Alfonso, D. M. Figert and J. M. Burggraff, *J. Assoc. Off. Anal. Chem.*, 64 (1981) 1149.
4 U. Jürgens, *Lebensmittelchem. Gerichtl. Chem.*, 35 (1981) 97.
5 F. Dalang, E. Martin and J. Vogel, *Mitt. Geb. Lebensmittelunters. Hyg.*, 73 (1982) 371.
6 J. S. Pruthi, *Spices and Condiments: Chemistry, Microbiology, Technology*, Academic Press, New York, 1980, p. 146.
7 U. Jürgens, *Deut. Lebens. Rundschau*, 77 (1981) 211.
8 A. Herrmann and M. Stöckli, *J. Chromatogr.*, 246 (1982) 313.
9 N. Arnaud, J. C. Bayle and M. Derbesy, *Parfums, Cosmet. Aromes*, 53 (1983) 99; *Chem. Abstr.*, 100 (1984) 101716b.
10 D. Fraisse, F. Maquin, D. Stahl, K. Suon and J. C. Tabet, *Analusis*, 12 (1984) 63.
11 P. A. Guarino and S. M. Brown, *J. Assoc. Off. Anal. Chem.*, 68 (1985) 1198.
12 R. D. Thompson and T. J. Hoffmann, *J. Chromatogr.*, 438 (1988) 369.
13 *New South Wales Pure Food Regulations*, 1937, regulation 49 (6), vanilla essences.