Journal of Chromatography, 246 (1982) 313–316
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,980

Note

Rapid control of vanilla-containing products using high-performance liquid chromatography

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(Received April 22nd, 1982)

The flavour of vanilla is appreciated in a wide variety of foods. Natural vanilla extract, being extremely expensive, is being replaced more and more by the cheaper natural vanillin (isolated from lignin) or by synthetic ethyl vanillin. The use of vanilla extract is popular for various products, especially those used in confectionery, and must be controlled.

It is easy to separate ethyl vanillin from vanillin using gas[1,2] or thin-layer[3] chromatography. Their determination is also possible without performing a separation, using UV-absorption measurements[4]. Much more difficult is the differentiation between vanilla extract and natural vanilla isolated from lignin. The best way is to test for the presence of those by-products typically present in vanilla extract but not in synthetic vanillin, *e.g.* vanillyl alcohol, 4-hydroxybenzoic acid and, especially 4-hydroxybenzaldehyde[5]. This last compound is oxidizable and may be absent in aged extracts. Such a separation is possible using thin-layer[6] or gas[7,8] chromatography. An interesting method has been reported[9,10] in which a mass spectrometer is used to measure the ratios of the hydrogen and carbon isotopes which differ in trees and in vanilla fruits. The separation of some phenolic acids and aldehydes from plants using a high-performance liquid chromatographic (HPLC) method has been reported[11], as has also the separation of vanillin and syringaldehyde[12] using the same method.

Recently[13] an HPLC method has been reported for the measurement of 4-hydroxybenzaldehyde and vanillin but not of the other components of interest present, especially theobromine and coffeine. Our objective has been to develop a reliable HPLC method adapted especially for the control of vanilla products through the separation and identification of the basic components, *viz.* 4-hydroxybenzyl alcohol, aldehyde and acid, vanillin and vanillin alcohol, ethyl vanillin and coumarin.

EXPERIMENTAL

Preparation of references and samples

Vanilla extraction. As a reference for a typical composition of vanilla extract we applied the method described elsewhere[14] using two different charges of Bourbon vanilla: 10 g of vanilla pod were finely chopped and macerated for 12 h at 40°C with 20 ml of water in a closed vessel. Ethanol (20 ml) was then added, and the mixture was mixed thoroughly and macerated again for 3 days. The mixture was poured through a

0021-9673/82/0000–0000/$02.75

sintered filter funnel and the filtrate collected in a 100-ml flask. The filter cake was pressed and washed with ethanol until the total volume of filtrate and washings was 100 ml.

This extraction was also carried out in an alkaline medium in order to break down the possible glycosides of vanillin. This procedure gives *ca.* 14% more vanillin than shown in Table I for charge A and *ca.* 2% more for charge B.

TABLE I

MAIN COMPONENTS IN VANILLA POD

Component	*Vanilla A (%)*	*Vanilla B (%)*
4-Hydroxybenzyl alcohol	0.46	0.30
Vanillyl alcohol	0.06	0.06
4-Hydroxybenzoic acid	0.07	0.04
4-Hydroxybenzaldehyde	0.15	0.14
Vanillin	1.92	1.45

Standards. 4-Hydroxybenzoic acid, 4-hydroxy-3-methoxybenzyl alcohol (vanillyl alcohol) and 3-hydroxy-4-methoxybenzaldehyde (isovanillin) were obtained from Fluka (Buchs, Switzerland), 4-hydroxybenzaldehyde, 4-hydroxybenzyl alcohol, 3-ethoxy-4-hydroxybenzaldehyde (ethyl vanillin) and 1,2-benzopyrone coumarin from E. Merck (Darmstadt, G.F.R.) and 4-hydroxy-3-methoxybenzaldehyde (vanillin) from C. Roth (Karlsruhe, G.F.R.). All these substances were dissolved in methanol to 250 ppm of pure and mixed solutions, except for coumarin and 4-hydroxybenzaldehyde which were diluted to 50 ppm.

Samples. The commercial vanilla extracts, possibly containing added sugars, were diluted with 80% methanol (3 g in 100 ml). The sugars were dissolved in water (5 g in 10 ml) and 10–20 μl directly injected. Solutions of vanilla sugars must be filtered prior to injection.

Experimental conditions

The HPLC apparatus consisted of a gradient system employing two Altex 110 pumps controlled by a microprocessor, a Rheodyne injector (7105) and a UV detector (Kontron, Switzerland). The integration and the computation of the data were carried out using a central data system (Spectra-Physics 4000).

The column (250 × 4.6 mm I.D.) and the precolumn, (40 mm × 4.6 mm I.D.) (Knauer, Oberursel, G.F.R.) were filled with RP-18 (10 μm) (E. Merck). The eluent was a mixture of two solutions of 0.2 *M* acetic acid in water (A) and in water–methanol (2:8) (B). The analysis conditions were as follows: conditioning, 10% B in A; gradient, in 14 min up to 60% B in A, 8 min isocratic, in 1 min down to 10% B in A; flow-rate, 1 ml/min constant; detection, 275 nm; extinction range, 0.5.

RESULTS

Fig. 1 shows the chromatogram obtained under the conditions described, for an injection of 10 μl of a standard mixture. Ethyl vanillin is well resolved from vanillin making the distinction between natural and artificial products reliable and easy. The

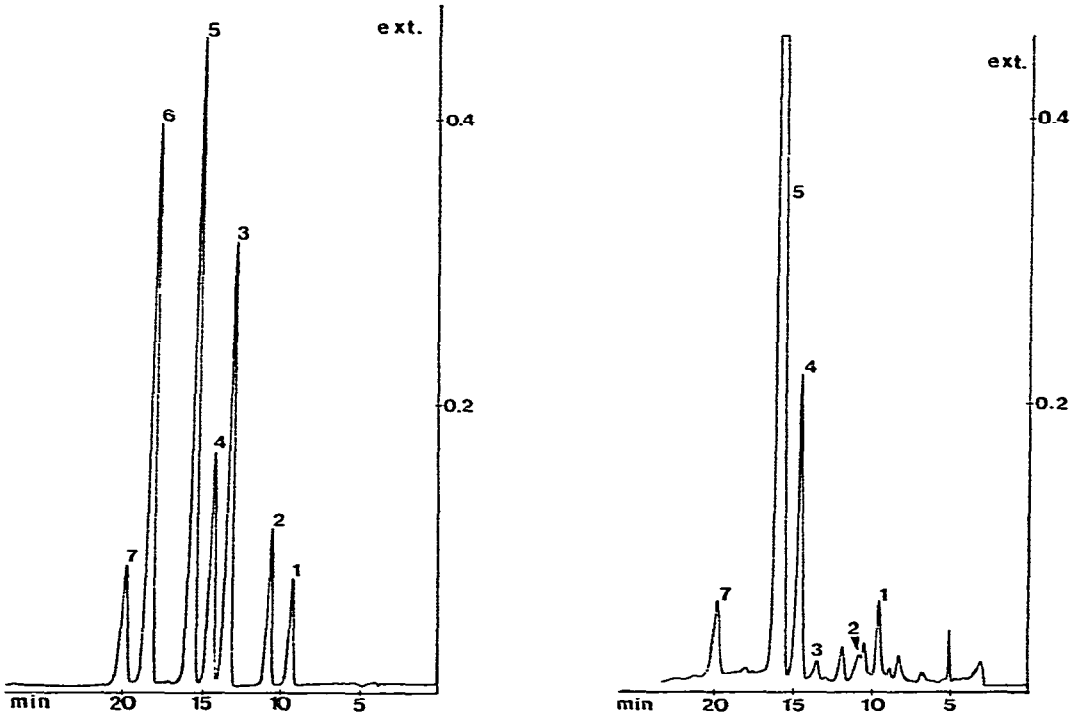

Fig. 1. Chromatogram of 10 μl of a standard mixture. Peaks: 1 = 2.5 μg of 4-hydroxybenzyl alcohol; 2 = 2.5 μg of vanillyl alcohol; 3 = 2.5 μg of 4-hydroxybenzoic acid; 4 = 0.5 μg of 4-hydroxybenzaldehyde; 5 = 2.5 μg of vanillin; 6 = 2.5 μg of ethyl vanillin; 7 = 0.5 μg of coumarin. Extinction range, 0.5.

Fig. 2. Chromatogram of 10 μl of vanilla extract; peaks as in Fig. 1.

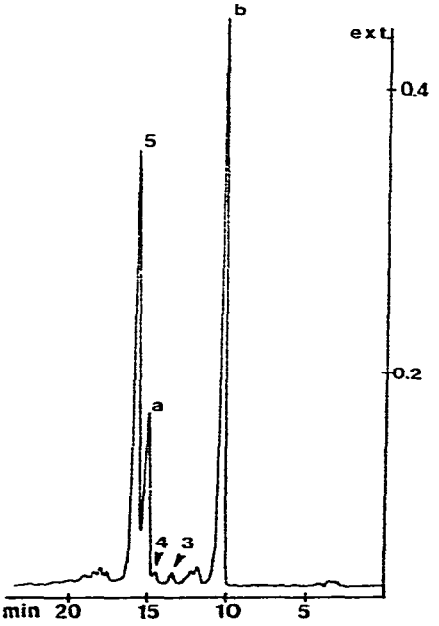

Fig. 3. Chromatogram of cocoa-drink showing caffeine (a) and theobromine (b) peaks. Other peaks as in Fig. 1.

other components are also well resolved so that small amounts of secondary components are detectable beside the main vanillin peak. Fig. 2 shows the curve obtained with 10 μl of our vanilla extract A and Table I gives the concentrations of the most important components in samples of vanilla obtained from two different sources (both "Bourbon"). It appears that both benzyl alcohol and benzaldehyde must be present in an authentic extract, the former even in aged samples owing to its stability against oxidation. Coumarin, probably derived from cinnamic acid, is also present in such samples, albeit in small quantities, but is easily detectable owing to its high extinction coefficient ($E_{1\,cm}^{0.1\,\%}$ in methanol) at 275 nm of 81.0 compared to that of 4-hydroxybenzyl alcohol under the same conditions (14.4).

Isovanillin is not resolved under these conditions, having a retention time a few seconds less than vanillin. Caffeine is often present in dessert preparations and appears between 4-hydroxybenzaldehyde and vanillin with a resolution of 60% (Fig. 3). This chromatogram also shows theobromine appears much earlier than the other constituents and may be quantitated if necessary.

CONCLUSION

The method proposed seems well suited to check on the authenticity of commercial vanilla extracts. It is also very easy to control the vanillin sugars that should contain at least 2% of natural or natural synthetic vanillin but no ethyl vanillin. It is not so reliable for the vanilla sugars: owing to the dilution of vanilla in these sugars (10%) only the major constituents will be measurable without extra concentration and these may be easily added in synthetic preparations. In doubtful cases it would be necessary to apply the mass spectrometry method[10].

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