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NOTE

Determination of Vanillin in Milk Powder by Rapid Column High Performance Liquid Chromatography

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A rapid column high performance liquid chromatography method for the determination of vanillin in milk powder was studied. The vanillin was extracted from the sample with 40 % methanol by the ultrasonic extraction and then was purified by solid phase extraction with C_{18} cartridge. Vanillin was separated on a ZORBAX stable bound (4.6 × 50 mm, 1.8 mm) rapid chromatographic column with water-methonal (65:35) as mobile phase at a flow-rate of 2 mL/min and monitored with the photodiode array detector at 309 nm. The recoveries of the vinillin are 98-103 % and the relative standard deviations are 0.46-0.58 %. This method was applied to the determination of vanillin in milk powder with good results.

Keywords: High performance liquid chromatography, Vanillin, Milk powder.

Food additives monitoring was an important part of food safety monitoring. Vanillin, the world's largest production and consuming synthetic fragrances, was harmful when adding too much so that the determination of vanillin in food had important significance[1]. Liquid chromatography was widely used method for the determination of vanillin, but the ordinary high-performance liquid chromatography always take too much time (about 10 min)[2-7]. To reduce the analysis time, we studied the HPLC with fast separation column to analyze the vanillin from milk powder. The vanillin in the samples can separate baseline in only 1 min using this method, which was much faster than conventional liquid chromatography analysis.

Agilent 1100 HPLC, including: HP 1100 quaternary pump, HP1100 UV diode array detector, HP 1100 autosampler and Chem Station chromatography workstation.

Methanol (HPLC, produced by the fisher company); Vanillin (purity of at least 99 %, produced by the SIGMA company); Double distilled water (produced by the Milli-Q50 instrument of USA Millipore Corporation, which the resistance could get 18 MΩ cm or above) and the rest of reagents were analytical grade or above.

Chromatographic conditions: Components were separated on a ZORBAX Stable Bound (4.6 × 50 mm, 1.8 mm). The mobile phase was 35 % percents of methanol at a flow rate of 2 mL/min. Sample injection volume was 5 mL and the detection wavelength was 309 nm. The chromatogram of sample and standard sample in the above chromatographic conditions were shown in Fig. 1.

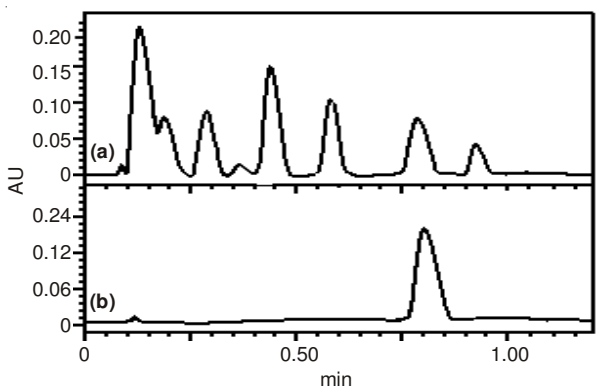

Fig. 1. Chromatograms of vanillin (b) and milk sample (a)

Sample preparation: 2 g sample was withdrawn into a 100 mL beaker add 40 mL 40 % methanol and then was ultrasonically extracted for 10 min at 40 °C. After the beaker cooled down, the liquid was withdrawn into a flask with 40 % methanol to 50 mL. With 5 mL solution through the pre-activation Waters Sep-Pak-C18 solid phase extraction column at a flow rate 10 mL/min, discard the first 3 mL solution and then filter the rest of 2 mL solution collected with 0.45 mm syringe filters. The injection volume was 5 mL.

Water and methanol were chosen as the mobile phase for separating vanillin. When the ratio of water and methanol was 35:65, the vanillin in the samples could be completely separated from the other coexisting components. As we know, conventional high performance liquid phase separation column needed a long time to separate vanillin, so we chose the ZORBAX Stable Bound (4.6 × 50 mm, 1.8 mm) C_{18} fast separation column to short the experiment time in this study. With this column, the vanillin could be separated in 1 min which was 85 % faster than the conventional column.

The spectra of each vanillin could be found between 200-400 nm wavelengths by the UV diode array detector and vanillin had a greater absorption and the less background interference at 309 nm, so we chose the 309 nm wavelength as the experiment condition. The vanillin of the sample was identified by the retention time, UV spectra and standard sample. In addition, we also analyzed the peak purity to confirm that the vanillin was completely separated from the coexistence components of the sample.

Sample preparation: Vanillin had a greater solubility in aqueous methanol solution and methanol concentration of 40 % was acceptable. With 40 mL 40 % aqueous methanol solution and ultrasonic oscillation 10 min, vanillin in the sample could be completely dissolved in methanol. Therefore, this method was applied in sample preparation.

Milk contains vanillin, also contain oily substances. Such substances can't be completely eluted in this mobile phase conditions. As we know, oily substances would residue accumulate on C_{18} column, increase chromatographic system backpressure and loss of efficiency, so degreasing treatment is indispensable before experiment. So our group carried out Study on the processing method above. With a waters SPE vacuum extraction device, 20 samples simultaneously per treatment, flow rate were 10 mL/ min when extraction column activation and sample pre-separation. Used 3 mL of methanol for extraction column activation and 10 mL water for methanol washing and took 40 % methanol extract of the sample pass through the extraction column so that oily substances retained in the column. Vanillin, not retained in the extraction column, could be directly determination by HPLC.

Regression equation, correlation coefficient and detection limit: Prepared series of standard solution of vanillin and the concentrations were 100, 20, 4, 0.8 g/mL). According to the peak area of different concentrations, calculated the regression equation after injection. Regression equation is: A = 4.18 × 105 C + 368 (A, the peak area; C, the concentration; units, g/mL), Correlation coefficient r = 0.9999. Because the letter dry ratio S/N = 3, detection limit was 10 mg/mL.

Results of sample analysis: Milk samples were determined by selected chromatographic conditions after sample pretreatment. When measured, accurately weighed in duplicate, one was standard, another one was of added standard vanillin of known quantity. Each sample was measured under the same condition 5 times in parallel. Recovery was calculated with the amount of sample measured divided by the amount of added standard sample. And the relative standard deviation was calculated with the 5 times results in parallel (Table-1).

TABLE-1
RESULTS OF SAMPLE ANALYSIS

Sample	Measured values (μg/g)	RSD (%)	Recovery of standard sample % (added amount was 200 μg)
Milk -1	287	0.46	98
Milk -2	412	0.52	103
Milk-3	185	0.48	99

Conclusion

This method used solid-phase extraction pre-separation, rapid column high performance liquid chromatography (HPLC) method for the determination of vanillin in milk powder. Vanillin was separated within the 1.0 min. and the recoveries of the vinillin were more than 85 %. The result showed that the method is simple, rapid, reliable and to a certain extent, for the rapid determination of vanillin present in milk.

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