Optimization and Validation of HPLC for the Analysis of Rhodamine-B in Sponge Cake

Novi Yantih*, Zuhelmi Aziz, Aditya Dicky Prasetya

Abstract

A new HPLC method is described for sensitive and selective determination of trace level of rhodamine-B in sponge cake. The method is based on the partition of rhodamine B on C18 column in reverse phase HPLC system. HPLC method has been optimized and validated for the analysis of rhodamine-B in a sponge cake. The effects of different parameters such as solvent, the wavelength of maximum absorption, and the composition of mobile phase and flow rate were optimized. Rhodamine-B in methanol can be detected at a retention time of about 4.8 min by using a mobile phase of acetonitrile–phosphate buffer pH 3.5 (70: 30) with a flow rate 1.2 ml/min and detection wavelength of 554 nm. The concentration of simulation sponge cake solution (6-24 ppm) showed a linear relationship with the peak area (correlation coefficient of 0.999). The limit of detection and quantitation were 0.7 and 2.1 ppm, respectively. The accuracy and precision methods met the requirement of ICH. This method has been successfully applied to screening of rhodamine-B in commercial sponge cakes, and it is valuable to ensure the safety of food.

Keywords: Rhodamine-B, sponge cake, reverse phase HPLC

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Introduction

Food colouring is usually contained in food products to improve their aesthetics. More recent studies showed the use of non-permitted colours like rhodamine-B in street food, like sponge cake (1). Although more evidence in recent years indicates that the abuse of rhodamine-B may cause liver damage in mice and cancer (2,3), many kinds of non-permitted dyes are still widely used because of their low price, high effectiveness and excellent stability (4).

To protect public health, many countries have established strict regulations for the allowable kinds and concentrations of dyes. In Indonesia, the regulations of dyes that are allowed and prohibited for food and beverages was regulated by the Minister of Health (5). However, some food producers may still add banned dyes to their products putting sensitive population in health risk. Therefore, it is necessary to develop a sensitive and accurate method to screen banned dyes in foods to ensure food safety. This research has been carry out to develop a high performance liquid chromatography (HPLC) method for the analysis of rhodamine B in a sponge cake.

Various methods for the determination of rhodamine B in food have been reported, including capillary electrophoresis (6), TLC (7), ion pair chromatography (8), visible spectrophotometry (9), and LC-MS (10). However, these methods are not carried out for screening of rhodamine B in sponge cake matrix. Rhodamine B in cosmetics and food was analysed by using RP-column and modification the mobile phase of a previous study. They used the mobile phase of acetonitrile-phosphate buffer pH 3.05-water (70: 7: 3), detection at λ 546 nm, flow rate of 1.2 ml/min, injection volume of 20μl (11). In this study, the simple sample preparation was reached by decreasing various of the mobile phase. Partial method validation was be done by testing the linearity, accuracy, precision, and sensitivity of the method according to the International Conference On Harmonization (ICH) (12,13).

Methodology

Commercial red and white sponge cake were used as matrix of samples. Rhodamine B were obtained from PT Galic Bina Mada. Reference standard of rhodamine B was carefully weighed approximately 100 mg, then put into a 100 mL volumetric flask and diluted with water. Simulation sponge cake made of commercial white sponge cake and rhodamine B were added at various concentrations in sponge cake.

Analysis was performed by high performance liquid chromatograph (Shimadzu LC-20 AD). The
optimization of method included selection of solvent, determination of maximum absorption wavelength, and the selection and composition of the mobile phase flow rate.

Matrix of the sponge cake on the linearity experiments were used from commercial white sponge cake that were added rhodamine B at the concentration of 60, 80, 100, 120, and 140%, i.e. 9, 12, 15, 18, and 21 ppm. Limits of detection and quantitation were determined based on a linear line equation. Testing of precision and accuracy were performed by preparing sponge cake simulation which contain rhodamine B of 80, 100, and 120%.

Sponge cake samples suspected to contain rhodamine B were weighed approximately 200 mg, dissolved in methanol, and shaken until homogeneous. Samples were centrifuged, diluted with water, and then shaken, filtered and sonicated. Afterwards, 20μL the solution were injected into the chromatograph.

**Results and Discussion**

The selection of extraction solvent was carry out to obtain a solvent that can extract rhodamine B optimally from the matrix of sponge cake. Although hydrochloric acid can also extract rhodamine-B, but the solution becomes turbid because the starch was contained in sponge cake, was degraded by acid and formed colloids in solution. Methanol was used as solvent to produce clear solution.

Based on the absorption spectrum, a solution of rhodamine B in methanol had maximum absorption at a wavelength of 553.8 nm. Spectrum profile can be seen in Figure 1. The wavelength of 554 nm was used for detection in HPLC, because the measurement can be performed at the maximum absorbance wavelength of ± 2 nm in deviation (14).

Profile chromatogram used reverse phase system with a mobile phase of pH 3.50 phosphate buffer-acetonitrile (30:70) and a flow rate of 1.2 ml/min can be seen in Figure 2. The rhodamine-B is polar compound, so the retention time of rhodamine-B in reverse phase system may be more briefly than normal phase. The retention time of rhodamine-B was about 4.8 min and shorter than that of previous research with the mobile phase of acetonitrile-phosphate buffer pH 3.05-water (70: 7: 23). The influence of pH to the retention time of rhodamine-B may be caused by the cationic property of rhodamine-B. The affinity of rhodamine-B increased with mobile phase of pH 3.50 phosphate buffer-acetonitrile (30:70).

The standard curve equation was \( y = 84010.4 + 126371.8X \) with coefficient correlation of 0.999, while the result from the linearity test showed that the equation was \( y = 23818 + 8193.1X \) with coefficient correlation of 0.999. The decreased of sensitivity to determine rhodamine-B may be cause of the influence of sponge cake matrix. However, the relationship of the respond and concentration was linear according to Lambert Beer equation, so the method was applicable to analysis rhodamine-B.

Limit of detection and quantitation of method were 0.7 and 2.1 ppm, respectively. Comparing with the detection limits reported in literatures (10), the detection sensitivity was more smaller than 1000 times to LC-MS method. Because of the amount of rhodamine-B in food street was about range7,8-3200 ppm (15), this method was applicable to determine rhodamine-B.

Accuracy of the method can be showed by the value of recoveries of rhodamine-B (80, 100, and 120%) in white sponge cake (6). The experiments were performed three times repetitions for each analyte concentrations. Method was precise and accurate with the relative standard deviation (SBR) of ≤ 2% and percent recoveries of 90-110% (5) (Table 1).

**Table 1. The Percent Recovery and Relative Standard Deviation in Accuracy and Precise Test**

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*Figure 1. Absorption spectra of rhodamine B in methanol*

*Figure 2. Chromatogram of rhodamine B in the mobile phase pH 3.50 phosphate buffer-acetonitrile (30:70) with a flow rate of 1.2 ml/min*
This method has been successfully applied to screening of rhodamine-B in commercial sponge cakes, and it is valuable to ensure the safety of food. Analysis of rhodamine B in samples A and B obtained by the average levels of 15.31 and 17.64 ppm with a relative standard deviation respectively, i.e. 0.86 and 0.39%. Since Rhodamine-B was detected from samples and hence the sample size was lesser, it gives a scope for further research to explore the probability of detection of Rhodamine in such foods collected from street food corners considering more number of samples so that the prevalence of the use of Rhodamine in street foods in different regions in Indonesia could be assessed and also the types of food which are adulterated.

Conclusions

Reverse phase HPLC method with a mobile phase of pH 3.50 phosphate buffer-acetonitrile (30:70), flow rate of 1.2 ml/min, detector of 554 nm met the requirement of ICH for the analysis of rhodamine B in a sponge cake with a limit detection and quantitation limits were 0.7 and 2.1 ppm, respectively. Rhodamine B was founded in sponge cake samples at levels in the range of 15.31 and 17.64 ppm.

Acknowledgments

Thanks to Direktorat Jendral Pendidikan Tinggi Republik Indonesia, who have given the grant of this research.

References


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Table 2. Content of Rhodamine B in Sponge Cake Commercial

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