ANALYSIS OF RHODAMIN B IN GROUND RED CHILI USING THIN LAYER CHROMATOGRAPHY-DENSITOMETRY

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Abstract

Rhodamin B is a textile and paper dye often used for coloring food to make it brighter. It is also harmful to health if consumed by human. One of the food that suspect containing rhodamin B is ground red chili. This research aimed to determine rhodamin B in ground red chili. Identification and analysis of rhodamin B in ground red chili is determined by Thin Layer Chromatography-Densitometry method. This method was carried out using the stationary phase of silica gel $F_{254,}$ eluated by a mixture of ethanol - amonia (19:1) and measured at maximum wavelength absorption of 550 nm. Thin Layer Chromatography-Densitometry method showed a good precision value with relative standard deviation of 0.37%, limit of detection and quantitation of 1.14 and 3.81 ppm respectively, and the recovery value of 99.8%. From eight samples of ground red chili analyzed using this method, only three samples C, D and G consists of rhodamin B with content of 19.30 µg/g, 51.28 µg/g, 68.30 µg/g respectively.

Keywords : *analysis, rhodamin B, red chili, TLC-Densitometry*

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INRODUCTION

Food safety is a requirement to be met by every food product that will be distributed or consumed by the public. to ensure that the food consumed does not contain biological, chemical and physical hazards that can be disturbing, harmful and dangerous to consumer health (1).

In practice many food manufacturers are prohibited to use food additives that are toxic or harmful to health such as synthetic dyes Rhodamin B which is used in coloring paper and textiles (2). Synthetic dyes Rhodamin B is a dye that is prohibited for food and declared as hazardous material according to Minister of Health of Republic of. 239/Menkes/Per/V/1985 and revised through the Regulation of the Minister of Health of Republic of. 722/Menkes/Per/IX/1988 of dye declared dangerous and banned in Indonesia. Rhodamin B are not allowed in food and drink, with a low dose of 0.117 mg / kg may inhibit growth, cause diarrhea that can lead to death (3). Lack of attention to this often resulted in negative impact of decreased consumer health, toxicity, eye irritation, respiratory tract irritation, irritation of the digestive organs, and cancer in the long term can lead to impaired liver function (4).

The dye is a complement to make food, beverages, and spices to be more attractive. The addition of dyes in food, beverages, and spices such as ground red chili pepper has a huge influence on taste and consumer appeal (3). Red pepper (Capsicum annum L.) is one type of vegetables that have high economic value.

In the processing of ground red pepper, carrots and garlic skin are added in order to add weight to the causes the color of red pepper to decrease or fade. Djarismawati 2004 has conducted a research on the knowledge and behavior of ground red pepper traders in using Rhodamin B in the traditional market in DKI Jakarta by chromatography on 90 samples of ground red pepper 3 traditional markets in Jakarta, 57 samples (63%) containing Rhodamin B (3). The use of Rhodamin B have been banned from use but there are manufacturers who deliberately adding Rhodamin B dye on the ground red pepper products as a red dye.

Rhodamin B dye analysis can be made by using visible spectrophotometry and thin layer chromatography-densitometry. Based on the findings, Rhodamin B dyes in ground red pepper can be analyzed by using thin-layer chromatography-densitometry.

METHOD

Materials. Ground red chili were obtained from traditional markets in Bekasi, which are Kranji Market, Bantar Gebang Market, and Jatiasih Market. From each market two samples are taken at randomly. There are 8 samples of ground red pepper taken (A, B, C, D, E, F, G, H).

Methods

Identification of standard reference Rhodamin B

Color reaction.

Supernatant was identified by color reaction. The results of the color reaction was then compared with the results of the color reaction of a comparable substance Rhodamin B. Coloring reagents used are (5):

a. H_2SO_4 (p) to produce yellow
b. HCl (p) to produce red orange
c. NaOH 10% to produce purple
d. NH_4OH 10% to produce violet

TLC and Rf values

Identification is done by dotting 5 microlitre test solution and standard reference solution Rhodamin B on silica gel F254 plates side by side, eluated chromatography with a mobile phase of ethanol - ammonia (19:1). Spots arising on plates were observed at 254 nm UV light and 366 nm. Rhodamin B identification is done by comparing the fluorescent spots contained in the sample with fluorescent spots of

the reference standards Rhodamin B, both color and its Rf value.

Assay of drying shrinkage

Samples obtained, 1-2 g weighed carefully inserted into the bottle stations. After the samples were dried in oven 105 ° C to obtain a fixed weight (6), drying shrinkage are calculated by using the formula:

$$\% \text{ drying shrinkage} = \frac{W1 - W2}{W1} \times 100\%$$

W1= weight of wet sample (g)
W2 = weight of dry sample (g)

Determination of optimum conditions

Selection of mobile phase (7)

Optimization of mobile phase carried out to obtain a good chromatogram chromatogram is rounded, not dilated and no tail. Optimization is done by dotting using the standard solution Rhodamin B on the plate as much as 5 ml and then eluated using several mobile phase. Mobile phase was attempted as follows:

1) Ethanol ¬ - ammonia - ethyl acetate (4: 11: 5)
2) Ethyl acetate - ammonia (10: 10)
3) Ethanol - ammonia (15: 5)
4) Ethanol - ammonia (18: 2)
5) Ethanol - ammonia (19: 1)

The mobile phase used was selected for further identification of Rhodamin B by thin layer chromatography, which is ethanol - ammonia (19:1).

Determination of the wavelength of maximum absorption

Carefully weighed 2.0 mg reference standard Rhodamin B, put in 10-mL volumetric flask (200 ppm). Then diluted with 70% ethanol to the marked line, shake to homogenize. A total of 5 ml Rhodamin B standard solution are dotted/spotted on silica gel plates F254 and the vessel chromatography are eluated with a mobile phase of ethanol - ammonia (19:1). Determination of the maximum absorption wavelength are performed using a densitometer at 300 nm to 600 nm.

Determination of standard curves

Carefully weigh 2.0 mg reference standard Rhodamin B, put in 25-mL volumetric flask, dissolved and diluted with 70% ethanol to the mark, shake to homogenize. Preparation of 7 series of solution are made from standard solution of Rhodamin B of 80 ppm (1.6 ppm, 3.2 ppm, 4.8 ppm, 6.4 ppm, 8 ppm, 9.6 ppm, 11.2 ppm).

A total of 5 ml of each solution concentration are dotted or spotted on TLC silica gel plates F254, then the chromatography vessel are eluated using a mobile phase of ethanol - ammonia (19:1). After eluated, the plate was dried at room temperature, then the spots detected under UV light at 254 and 366 nm. Spotting area measured at maximum absorption wavelength of 550 nm using a densitometer. There are a linear relationship between the concentration curve standard reference solution of Rhodamin B as the x-axis and the area as the y-axis. The regression line equation and the correlation coefficient (r) are calculated.

Validation of analytical methods

1. **Preecision** (accuracy) test. Carefully weighed 2.0 mg reference standard Rhodamin B was dissolved and diluted to a 10-mL (200 ppm). The next step are the same way as the determination of standard curves

2. **Accuracy test**. Test recoveries (accuracy) conducted to determine the accuracy of the assay method Rhodamin B in TLC-densitometry by adding a certain number of reference standards Rhodamin B into a matrix of ground red pepper (Spike placebo method) to determine the influence of the matrix. Reference standard

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components Rhodamin B was added 70% and 85%.

3. **Linearity test.** The experiment was conducted to determine the relationship between concentration and uptake. To test the linearity, a series of solutions with different concentrations are made.

   Carefully weigh 2.0 grams of ground red pepper samples which were extracted by cold maceration, soaked in 70% ethanol to 40 ml. Stirring was performed using stirer for approximately one hour and allowed to stand for 24 hours, then filtered with Whatman filter paper No. 40. Extraction is performed until the dye in samples of ground red pepper interested depleted (approximately 40 mL). Supernatant obtained was concentrated until the volume is less than 10 ml. Supernatant was put into 10-mL volumetric flask was diluted with 70% ethanol to the mark, shake homogeneous. (Solution A).

   Preparation of standard solution: carefully weighed 2.0 mg BP Rhodamin B, put in 25-ml volumetric flask, dissolved and diluted with 70% ethanol to the marked line, then shaken to homogenize (Solution B).

   Preparation for inearity test: each pipetted 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml, 0.6 ml, 0.7 ml of standard solution of Rhodamin B (solution B) are inserted into the 5-ml volumetric flask and then each was added 1.0 ml of solution A, diluted with 70% ethanol to the mark obtained levels (1.6 ppm, 3.2 ppm, 4.8 ppm, 6.4 ppm, 8 ppm, 9.6 ppm, 11.2 ppm). The next step the same as that of the test precision.

4. **Testing** the limits of detection. The determination limit of detection is based on data obtained from the linearity test.

5. **Testing** the limits **of quantitation.** The determination limit of quantitation is based on data obtained from the linearity test.

Analysis of rhodamin B on samples of ground red peppe

Preparation of standard solution:

Cooperation measures such as creating a standard solution of the linearity test.

Preparation of test solutions:

Cooperation measures such as creating a standard solution of the linearity test.
Cooperation measures such as those carried out on precision test

Levels = (X. Va) / Bs (µg / g)

Description:

Va = Final volume (ml)
Bs = Sample weight (g)

The results

Identification of reference standards Rhodamin B

Obtained samples C, D, and G has the same color reaction with the reference standard color reaction Rhodamin B. So stated sample C, D and G contain the dye Rhodamin B.

TLC and Rf calculations

From the calculation results of samples C, D and G have the same Rf value with the reference standard Rf Rhodamin B.

Determination of drying shrinkage

The results of drying shrinkage determination of ground red pepper 8 samples, obtained by different drying shrinkage

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percentage of each sample ranged between 70.49 to 83.16 percen

Determination of optimum conditions

Selection of mobile phase.

Obtained the best mobile phase is ethanol - ammonia (19:1), because it gave good chromatogram with good separation, rounded, not dilated and no tail.

Determination of the wavelength of maximum absorption

In the experiments, the maximum wavelength of Rhodamin B are obtained at 550 nm. As shown in FIG.

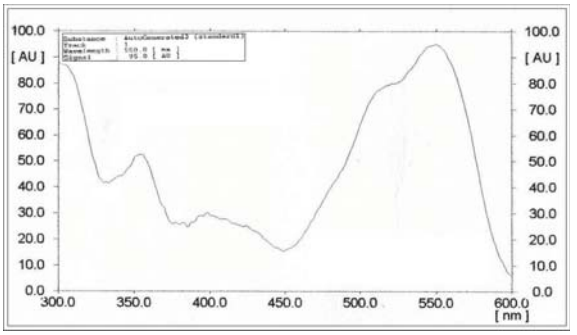

Figure 1. Rhodamin B reference standard spectrum at a wavelength of 300-600 nm.

Preparation Of Standard Curve

Six points of Rhodamin B concentration are obtained in linear (1.6: 3.2: 4, 8, 6, 4; 8; 9.6 ppm). This shows the force of the Beer Lambert law the intensity of light absorbed is proportional to the levels of substance solution. Equation of the curve above the regression line is y = 89.7839 + 0.0933 x with a correlation coefficient 0.9999. Equation is then used to calculate the levels of Rhodamin B.

Precision (Accuracy)

The precision of dotting/ spotting test results based on a comparison of standard solution 10 times as much as 5 ml Rhodamin B concentration of 200 ppm is 0.37%, the precision of the results obtained meet the requirements of less than 2% (8).

Linearity and Equal Test Regression Lines

In the experiments the regression equation of Y = 92.1467 + 160.9054 x are obtained with a correlation of (r) = 0.9936. The equation shows the linear relationship between concentration and the area indicated by the correlation coefficient (r) 0.9936.

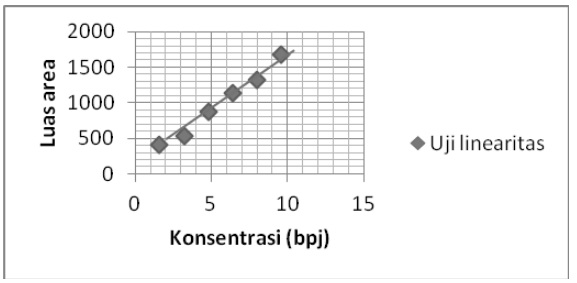

Figure2. The results of standard reference Rhodamin B linearity into

Detection test limits (Of detection limit, LOD)

Detection limits can be calculated based on the results of the linearity test with equation y = 92.1467 + 160.9054 x and the detection limit of 1.14 ppm obtained.

Quantitation test limits (Limit of quatitation, LOQ)

Limit of quantitation can be counted by linearity test results with the equation y = 92.1467 + 160.9054 x and the limit of quantitation of 3.81 ppm obtained.

Analysis rhodamin B in ground red chili with TLC-densitometry

The results of measurements of Rhodamin B from 3 samples of ground red chili containing Rhodamin B using TLC-densitometry method can be seen in Table I.

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Table I. The results of the TLC analysis of Rhodamin B-densitometry

Sample		sample weights	area	Levels of wet (µg/g)	Levels of dry (µg/g)	Average levels of wet (µg/g)	Average levels of dry (µg/g)
Code	Replication						
C	1	2,0013	356,6	19,8406	74,1771	19,30	72,14
	2	2,0012	342,2	19,0401	71,1806		
	3	2,0011	341,7	19,0135	71,1806		
D	1	2,0000	907,8	50,5495	202,2789	51,28	205,21
	2	2,0002	927,3	51,6303	206,6246		
	3	2,0004	928,0	51,6642	206,7393		
G	1	2,0004	1244,1	69,2641	249,1566	68,30	245,65
	2	2,0002	1226,3	68,2797	245,5907		
	3	2,0001	1209,2	67,3311	242,2104		

Table II. The results of the recovery test and t test

Sample weigt (g)	reference standards were added (µg)	concentrations of reference standards were added	Area	Recovery (µg)	Percent revovery	Percent average recoveries (%)
2,0020	71,86	70%	1563,2	71,44	99,4155	99,57
2,0015	71,85		1562,0	71,36	99,3180	
2,0004	71,81		1565,6	71,80	99,9861	
2,0016	87,27	85%	1704,8	87,26	99,9885	99,95
2,0014	87,24		1704,3	87,17	99,9198	
2,0011	87,22		1704,1	87,18	99,9541	

From eight samples of ground red pepper studied by using a densitometer obtained three samples containing Rhodamin B, the samples C, D and G, it is characterized by the emergence of the same peak as the reference standard Rhodamin B. The data showed that the mean levels of C sample is 19.30 ug/g, sample D is 51.28 ug/g, sample G is 68.30 ug / g.

THE RESULT OF THE RECOVERY TEST AND TEST T

In the table above shows that the average percentage recovery obtained Rhodamin B ground red pepper samples ranged from 99.57% - 99.95%. The average value of the percentage recovery of 99.8% are obtained. Values obtained meet the requirements for testing the recovery of 70% -120% (8).

CONCLUSION

From the results of research on ground red pepper, the following conclusion are obtained :

1. The optimum analytical conditions of Rhodamin B from ground red pepper using Thin Layer Chromatography method-densitometry are ethanol-ammonia (19:1) for

mobile phase and detected at maximum absorption wavelength of 550 nm.

2. Thin Layer Chromatography method-densitometry is a valid method for determining levels of Rhodamin B on the ground red pepper with 99.8% accuracy, precision with RSD 0.37%, 1.14 ppm detection limit and quantitation limit of 3.81 ppm.

3. The analysis of Rhodamin B at ground red pepper, 8 samples obtained 3 positive samples containing Rhodamin B is a sample C, D and G with an average grade of each sample of 19.30 mg / g, 51.28 g / g, 68, 30 ug / g.

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