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Validation of UV-Visible Spectrophotometry for Measuring Rhodamine B Content in Crackers

Silva Oktriana, Suci Rizki Nurul Aeni*, Ira Prima Sari

Institut Kesehatan Rajawali, Rajawali Barat No.73 Street, Bandung 40184, West Java, Indonesia

** Corresponding Author. E-mail: sucirizkinurulaeni@gmail.com*

ABSTRACTS

Rhodamine B is a synthetic dye that is often misused as a food coloring in crackers. Rhodamine B consumption through food can irritate the digestive tract, impair liver function, and cancer. The purpose of the study was to determine the content of rhodamine B in crackers. This study used a quantitative method, and purposive sampling was employed as a sampling technique. Method validation was carried out against a UV-Visible spectrophotometer in testing Rhodamine B in crackers. Validation of the method produces a linear regression equation of y = 0.2901x + 0.0082, with a correlation coefficient value (r2) = 0.9985, and a Vxo value of 2.65%. The instrument validation shows a Limit of Detection (LOD) of 0.1582 ppm and a Limit of Quantification (LOQ) of 0.5274 ppm. The results from the accuracy-test showed an average %diff of 0.88%; 3.79%, and -6.88%, while the average percentages of recovery were 101%, 104, and 93% respectively. Precision testing results showed that the value of % RSD < 2/3 CV Horwitz. Qualitative measurements showed that all samples tested for Rhodamine B in this study showed negative results. This study suggested that UV-Visible spectrophotometry is a valid method that can be used to measure levels of Rhodamine B in food.

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1. Introduction

The development of production technology and the use of food additives have both positive and negative impacts. The addition of food additives aims to preserve food, make the appearance of food more attractive, and make food crispier and tastier. But on the other hand, is often found the use of prohibited food additives in food because the price is cheaper (Sucipto, 2015). Food coloring is one type of food additive that is used to make the color of the food more attractive. Rhodamine B is a dye that is often used to color paper, fabrics, or inks. This synthetic dye is a crystalline powder that is odorless, purple-red in color, and glows brilliant red in solution (fluorescent). Rhodamine B is commonly found in foods, such as rhodamine B-containing crackers in Manado (Dawile, Fatimawali, and Wehantouw, 2013).

Various techniques, like the UV-Visible Spectrophotometer, can be used to detect the presence of dyes. This method is known for having a high level of sensitivity and being very easy to use. The method of UV-Visible spectrophotometry is a widely used analytical approach. However, because the equipment's condition might affect linearity, accuracy, precision, detection limit, and quantity limit, it must be checked regularly. One element utilized to ensure accuracy and precision in the analysis is monitoring in the pre-analytic stage (MOH, 2012). Following technique validation, rhodamine B levels in cracker samples should be determined. The validation process was subjected to ensure that the UV-Visible spectrophotometric method can provide accurate results.

Validation of research methods can be used as a reference to assess the quality, level of trust, and consistency of the results of an analysis. Validation is a technique for demonstrating that each material, process, procedure, system activity, and piece of equipment utilized in a procedure will always provide the anticipated outcome (Yulianti et al., 2017). Analytical technique validation is an analytical approach used in research or to demonstrate the performance characteristics of methods that meet analytical standards and are appropriate for the assay (BPOM, 2001). Linearity, the limit of detection (LOD), the limit of quantitation (LOQ), precision, and accuracy were the validation test parameters employed in this work.

Rhodamine B is still being misused as a food additive by many food producers. The objective of this study is to use UV-Visible spectrophotometry to determine the amount of Rhodamine-B in cracker samples in Bandung. The UV-Vis spectrophotometric technique employed in this work is also being validated.

2. Literature Review

Rhodamine B is a dye that belongs to the Xhantenes. It is a synthetic dye that comes in the form of a crystalline powder that's green or reddish-purple, odorless, and fluoresces brilliant red in liquid form. Rhodamine B has the chemical formula $C_{28}H_{31}ClN_2O_3$ (EFSA, 2005), and it is soluble in water, alcohol, HCl, and NaOH, but not ether or alkaline solutions. It forms a compound with a pink antimony complex that is soluble in isopropyl ether when dissolved in a strong acid (Arfina, 2012).

There is a conjugate bond in the molecule Rhodamine-B that causes rhodamine B to be red (Cindayana, 2015). Chlorine, a halogen molecule, is known to form connections with Rhodamine B. The presence of halogen compounds in organic compounds is extremely hazardous and reactive. Halogens will form bonds with biological molecules to attain stability, producing harmful effects and inducing cancer in humans (Masthura, 2019).

A spectrophotometer is a tool that is widely used for the quantitative measurement of a

substance. Measurement of Rhodamine B using Spectrophotometry UV-Visible at a wavelength of 558 nm was performed in 16 red snacks obtained from the Cileungsi Market. Half of the snacks were positively tested for Rhodamine B (Patimah et al., 2020). A study in 2018 has validated the UV-Visible spectrophotometric method. This study validated the UV-Visible spectrophotometric method in determining the amount of Rhodamine B in sauce, using methanol and ethanol as solvents. Based on the results of statistical tests showed that there was no significant difference in the accuracy and precision of rhodamine B testing with methanol and ethanol solvents. The amount of Rhodamine B in samples analyzed using methanol and ethanol solvents was 2.4811 ± 0.3378 mg/kg and 2.4217 ± 0.4158 mg/kg, respectively (Rohyami et al., 2018).

3. Materials and Methods

Rhodamine B kit reagent was purchased from Labtest, and all chemicals used in this research were pro-analysis grade. Cracker samples were collected from traditional markets around Bandung. The spectrophotometer used in this research was from Genesys 10S. Laboratory glassware used in this research is test tube, beaker glass, volumetric flask, stirring rod, analytical balance, etc.

3.1. Preparation of Rhodamine B stock solution

A standard stock solution of 1000 ppm was prepared by dissolving 0.010 g of rhodamine B into a beaker glass and adding methanol to the mark. The solution was transferred to a 10 mL volumetric flask and the volume was adjusted and homogenized. Dilution from stock solution was performed to make rhodamine B with a concentration of 50 ppm. As much as 1.25 mL of 1000 ppm rhodamine B was put into a 25 mL volumetric flask and then the volume was adjusted with methanol to the mark and homogenized.

3.2. Measurement of the maximum wavelength of Rhodamine B

As much as 0.4 mL of 50 ppm rhodamine B was pipetted and then transferred into a 10 mL volumetric flask. The solution was diluted with methanol and then homogenized. The solution was measured in the wavelength range of 400-800 nm using a spectrophotometer UV-Visible. The maximum wavelength is determined based on the measurement result which shows the highest absorbance value.

3.3. Determination of calibration curve from Rhodamine B standard solution

A series of standard solutions were prepared with a concentration of 1; 1.5; 2; 2.5; and 3 ppm, using methanol as solvent. The absorbance of the solution was measured at a wavelength of 550 nm. The absorbance data of the standard solution was used to create a calibration curve, determine linearity, residual standard deviation, the limit of detection (LOD), and limit of quantification (LOQ).

4. Results and Discussion

4.1. Qualitative Analysis of Rhodamine B

Rhodamine B analysis has been carried out on 12 types of crackers obtained from several traditional markets in Bandung. The results of the Rhodamine B qualitative test on crackers using the Rhodamine B test kit were presented in Table 1.

Table 1. Qualitative Assay of Rhodamine B

No.	Sample	Colour	Results
1	Sample A	Orange	Negative
2	Sample B	Orange	Negative
3	Sample C	Orange	Negative
4	Sample D	Orange	Negative
5	Sample E	Orange	Negative
6	Sample F	Orange	Negative
7	Sample G	Orange	Negative
8	Sample H	Orange	Negative
9	Sample I	Orange	Negative
10	Sample J	Orange	Negative
11	Sample K	Orange	Negative
12	Sample L	Orange	Negative

The qualitative test of Rhodamine B begins with sample extraction by dissolving the sample with 4 M HCl and Methanol. Hydrochloric acid is added to keep the pH solution remain constant, and all compounds in the cracker ionized (Nafiq et al, 2020). Methanol was chosen as the solvent because it is semipolar and has a low boiling point, making it suitable for dissolving polar chemical compounds.

Qualitative testing of Rhodamine B was carried out using the Rhodamine B Rapid Test Kit. It consisted of two reagents, the first reagent containing a solution of $SbCl_5$ reagent (antimone pentachloride) in 5 N HCl and the second reagent containing a solution of toluene reagent (Andayani and Adisaputra, 2013). The principle of this assay is the reaction between the sample and the reagent that produces a color change (Masthura, 2019). The color change in the solution is caused by the formation of a brick-red complex compound from the sample containing rhodamine B with antimony salt which is soluble in organic solvents (Hardiyantari et al, 2020). Tests on samples were carried out by reacting the sample with the Rhodamine B Rapid Test Kit, followed by homogenization and observing the color changes that occurred. Samples containing Rhodamine B will have a purple color. All samples tested in this study showed negative results because there was no color change. Since all samples showed negative results, this indicates that Rhodamine B was no longer used as a food color in crackers. There are several types of red food coloring that are safe and permitted to be used in Indonesia, including erythrosine, red allura, Ponceau 4R, and carmoisin. The synthetic dye has a maximum concentration limit of 200 mg/kg in food. The research of Hevira et al, (2020) has tested Rhodamine B on 7 food samples in Payakumbuh and showed negative results. This shows the increasing awareness of food manufacturers to no longer use harmful synthetic dyes.

4.2. Measurement of the maximum wavelength of Rhodamine B

Validation of the method was carried out by determining the maximum wavelength on the measurement of Rhodamine B using a UV-Visible Spectrophotometer. Determination of the wavelength at the maximum absorption is carried out so that the measurement gives optimum results. The data from the wavelength measurement on the maximum absorption of rhodamine B in the wavelength range of 400-800 nm are shown in Figure 1. The test results show that the maximum absorption of rhodamine B is at a wavelength of 550 nm.

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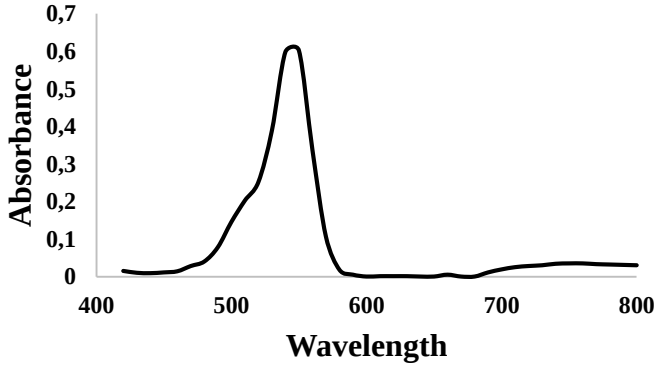

Figure 1. Measurement of Maximum Absorption of Rhodamine B

The process of validation of the Spectrophotometric method begins with selecting the maximum wavelength for Rhodamine B absorption. Each chemical compound has a different (specific) wavelength, therefore first testing the maximum wavelength of the compound (Kurnia et al, 2018). A compound will absorb the light spectrum more strongly at a certain wavelength (Winahyu et al, 2019). Measurement of absorbance of a colored solution will produce a peak absorption at visible light wavelengths.

In this study, the determination of the maximum wavelength of rhodamine B was carried out using a standard solution of 2 ppm which was measured in the wavelength range of 400-800 nm. The UV-Visible spectrophotometer was used because the compound rhodamine B has a chromophore group. The presence of a chromophore group in an organic compound such as a carboxyl group, an aromatic compound, and an ausochrome group, can absorb ultraviolet and visible light (Aminudin, 2014). Previous studies have shown that Rhodamine B has a spectrum range at a wavelength of 400-800 nm (Saputri et al, 2018). Based on the measurement results in Figure 4.1, it can be seen that the maximum absorption of the sample is at a wavelength of 550 nm. These results are in line with research by Rohyami et al (2018) where the maximum wavelength of rhodamine B is 550 nm.

4.3. Linearity Test of UV-Vis Spectrophotometric Method

Determination of the linearity of calibration curve of rhodamine B in methanol solvent with a concentration of 1; 1.5; 2; 2.5 and 3 ppm at a wavelength of 550 nm using methanol solution as a blank is presented in Table 2. The absorbances were plotted in a linear regression curve as seen in Figure 2.

Table 2. Linearity Test Results of UV-Vis Spectrophotometry Method

Concentration (ppm)	Absorbance
1	0.309
1.5	0.437
2	0.680
2.5	0.723
3	0.874
Vxo	2.65%

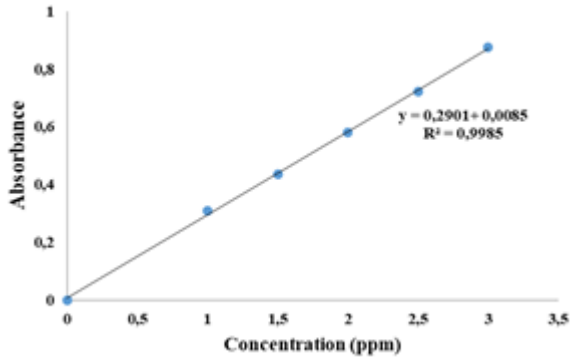

Figure 2. Calibration Curve of Rhodamine B

Based on the measurement results, the linear line equation is y = 0.2901x + 0.0082 with the correlation coefficient (r2) = 0.9985, and the Vxo value is 2.65%. The results of linearity test measurements revealed that the value of absorbance, correlation coefficient, and Vxo are valid. The value of the relation coefficient and Vxo obtained in this validation process meets the requirements for acceptance of validation (r2 > 0.99 and Vxo < 5%). These results indicate that the curve has fine linearity, thus there is a linear relationship between concentration and absorbance. Based on Lambert Beer's law, the higher the concentration, the higher the absorbance produced (Halimah et al, 2016).

4.4. Measurement of Limit of Detection and Limit of Quantification

The limit of detection and the limit of quantification from the validation process is presented in Table 3. Validation of the method showed that the value of LOD and LOQ has met the requirement for method validation. The detection limit is the smallest amount of analyte in the sample that can be detected and can still provide a significant value compared to the blank (Torowati et al, 2016). In this study, the detection limit or LOD value was 0.1582 ppm, meaning that the spectrophotometric method could still provide detection with a minimum rhodamine B level of 0.1582 ppm. While the quantitation limit (LOQ) can be interpreted as the smallest quantity of analyte in the sample that can still meet the criteria of accuracy and precision (Torowati et al, 2016). In this study, the detection limit or LOQ value was 0.5274 ppm, meaning that rhodamine B which can be analyzed quantitatively using this spectrophotometric method is ≥ 0.5274 ppm.

Table 3. Validation of Spectrophotometry UV-Visible Method

Parameter	Value
Slope	0.2901
Residual Standard Deviation (S y/x)	0.0153
Limit of detection (LOD)	0.1582 ppm
Limit of quantification (LOQ)	0.5274 ppm

4.5. Accuracy Test of UV-Vis Spectrophotometric Method

The accuracy of a method is determined by calculating the percentage of differentiation (% diff). A method is declared accurate if it has a percentage of differentiation <15% (Wibowo et al, 2019). In this study, accuracy was determined by the addition method to the simulation sample, namely by adding standard rhodamine B with concentrations of 1 ppm, 1.5 ppm, and 2 ppm which was carried out in triplo. Based on the calculation, the average percentage of differentiation is 0.88%, 3.79%, and -6.88% (Table 4.). All accuracy test results show a value less than 15%, so it is said that the method is valid (Wibowo et al, 2019).

Table 4. Accuracy of Spectrophotometry UV-Visible Method

Concentration (ppm)	Concentration from measurement (ppm)	Average of *diff* (%)	Average of recovery (%)
1	1.0089	0.88%	101%
1.5	1.5569	3.79%	104%
2	1.8625	-6.88%	93%

Accuracy can also be determined by calculating the percentage recovery (% recovery) of an analyte (Kurnia et al, 2018). The percentage recovery of the analytes was calculated and the average values were 101%, 104%, and 93% (Table 4.). Since the percent recovery was still in the range (80-110%), this proves that the method is valid (Rohyami et al, 2018). The percentage recovery value of more than 100% was due to the wet condition of the cuvette. To achieve high precision, several steps can be carried out during the experiment, such as calibrating the instrument, using high-quality solvents and reagents, regulating temperature, and carefully implementing and following standard operating procedures (SOP) (Harmita, 2003).

4.6. Precision Test of UV-Vis Spectrophotometric Method

The level of precision was carried out by re-testing a sample with known concentration several times; in this research, the measurement was done in triplo (Table 5).

Table 5. Precision of Spectrophotometry UV-Visible Method

Parameter	Day-1	Day-2	Day-3
Concentration (ppm)	0.8793	0.9221	0.8877
Deviation Standard	0.0082	0.01	0.0091
%RSD	0.933%	1.084%	1.025%
CV *Horwitz*	4.08%	4.05%	4.07%
2/3 CV *Horwitz*	2.72%	2.7%	2.71%

Precision is measured as standard deviation or relative standard deviation (coefficient of variation). Determination of the level of precision is conducted by measuring repeatedly under the same conditions in certain intervals of time (Utami, 2017). Precision measurements in this research showed that the Spectrophotometer UV-Visible had an RSD percentage of less than 2%. The % RSD value and Horwitz coefficient of variation from measurement should be less than 2%, thus the method can be stated as valid (Arikalang et al, 2018). In this research, results show that the spectroscopic method is valid.

5. Conclusions

Qualitative testing of the content of Rhodamine B using the Rapid Test Kit on cracker samples in this study showed negative results. The test results showed that the UV-Vis spectrophotometry method for determining the levels of rhodamine B met the requirements of good linearity, accuracy, precision, the limit of detection (LOD), and the limit of quantitation (LOQ). The linearity test resulted in a linear regression equation y = 0.2901x + 0.0082, the relation coefficient (r2) was 0.9985, the LOD value was 0.1542 ppm and the LOQ value was 0.5274 ppm, and the Vxo value was 2.65%. The precision test showed the percentage RSD values of 0.933%, 1.084%, and 1.025%, and Horwitz's 2/3 CV values of 2.72%, 2.7%, and 2.71%, respectively. Based on the accuracy-test, the average value (% diff) was 0.88%; 3.79%; -6.88% and recovery percentages of 101%, 104% and 93%, respectively.

6. References

Arikalang TG, Sudewi S, dan Rorong JA. Optimasi Dan Validasi Metode Analisis Dalam Penentuan Kandungan Total Fenolik Pada Ekstrak Daun Gedi Hijau (*Abelmoschus manihot L.*) Yang Diukur Dengan Spektrofotomter Uv-Vis. Jurnal Ilmiah Farmasi. 2018;14-21 Available from: https://ejournal.unsrat.ac.id/index.php/pharmacon/article/view/20102

Arfina. Analisis Kandungan Rhodamin B pada Kosmetik Perona Pipi yang beredar di Pasar Tradisional Kota Makassar. [serial online] 2012 Juli [cited 2020 Okt 09]; Available from: http://repositori.uin-alauddin.ac.id/3018/1/ARFINA.pdf

Cindaya, Nita Julita (2015) Analisis Kualitatif Penggunaan Rhodamin B Pada Terasi Di Pasar Tradisional Se-Kota Mataram. S1 thesis, Universitas Mataram.

Dawile S, Fatimawali F, Wehantouw F. 2013. Analisis Zat Pewarna Rhodamin B Pada Kerupuk Yang Beredar Di Kota Manado. Pharmacon Vol 2, No 3 DOI:

https://doi.org/10.35799/pha.2.2013.2386

[EFSA] European Food Safety Authority.Opinion of the scientific panel on foodadditives, flavourings, processing aids and materials in contact with food on request from the commission to review the toxicology of a number of dyesillegally present in food in the EU. EFSA 2005 J 263: 1-71.

Halimah N, Suryaningsih S, Mindara JY dan Hidayat S. Pengujian Kandungan Zat Pewarna Rhodamin B Pada Beberapa Jenis Makanan Dengan Mini Spektrofotometer Absorpsi Portabel. Prosidang Seminar Nasional Fisika. 2016; 5:25-28. Available from: URL: http://journal.unj.ac.id/unj/index.php/prosidingsnf/article/view/4154

Harmita. Petunjuk pelaksanaan Validasi Metoda dan Cara perhitungannya. Majalah Ilmu Kefarmasian. [Serial Online] 2004 Desember [cited 2020 Okt 25]; (1):119-122. Available from: URL:http://psr.ui.ac.id/index.php/journal/article/view/3375

Hevira. Analisis pewarna Rhodamin B pada kerupuk merah di Payukumbuh. [Serial Online] 2020 Mei [cited 2021 Juli 09]; (5):27-35. Available from: URL: https://www.google.com/url?sa=t&source=web&rct=j&url=https://www.researchgate.net/publication/341787466_Analisis_pewarna_Rhodamin_B_pada_kerupuk_merah_di_Payakumbuh&ved=2ahUKEwj6w4KypbDyAhXk_XMBHcxqASkQFnoECAMQAQ&usg=AOvVaw3EeZLvC66yu5XqNU_BVxfE

Kumalasari E. Identifikasi dan penetapan kadar Rhodamin B dalam kerupuk berwarna merah yang beredar di pasar Antasari kota Banjarmasin. [serial online] 2015 Mei [cited 2020 Sep 28]; 1(1):85-89. Available from: http://jurnal.akfarsam.ac.id/index.php/jim_akfarsam/article/download/17/16

Kurnia D, Yuliantini A, dan Faizal D. Pengembangan Metode Penentuan Kadar Neotam Dalam Sediaan Obat Dengan Spektrofotometer UV. Jurnal Kimia dan Pendidikan. 2018;3(1):66-76. Available from: http://ejournal.kemenperin.go.id/JTPII/article/view/2726/0

Masthura. Identifikasi Rhodamin B dan Methanyl Yellow pada manisan buah yang beredar di Kota Banda Aceh secara Kualitatif, AMINA. [serial online] 2019 [cited 2020 Okt 20]; 1(1) 39-44 Available from: https://journal.arraniry.ac.id/index.php/amina/article/view/1

Nugraheni B, Anggoro AB. Validasi Metode Analisis Ciproflaksin Menggunakan High Performance Liquid Chromatography. Inovasi Teknik Kimia 2016;1(1):06-08 .Available from: https://publikasiilmiah.unwahas.ac.id/index.php/inteka/article/download/1638/1715

Pangestu Ayu. Spektrofotometer UV Vis dan Refraktometer. [Online] 2011 [cited 2020 Des 29] Available from: URL:http://eprints.undip.ac.id/47907/3/BAB II.pdf

Prabowo MH, Wibowo A, Fauziyah L. Pengembangan dan Validasi Metode Analisis Rifampicin Isoniasid-Pirazinamid Dalam Fixed Dose Combination Dengan Metode Kromatografi Lapis Tipis Densitmetri. Jurnal Ilmiah Farmasi 2012 ;9(2):1-12. Available from: URL: https://journal.uii.ac.id/JIF/article/view/4208

Rahayu M dan Solihat MF 2018. Buku Ajar Teknologi Laboratorium Medik (TLM). Edisi 2018. Jakarta: Kementrian Kesehatan Republik Indonesia

Rohyami Y, Ratri HPI, Wihyarti. Validasi Metode Penentuan Rhodamin B dalam Contoh Saos secara Spektrofotometri UV-Vis dengan Dua Variasi Pelarut. Chem Anal 2018;1(1):20-28. Available from: https://ijca.uii.ac.id/media/282086-validasi-metode-penentuan-rhodamin-b-dal-dc545f44.pdf

Shintawati, Rina O, Zulkarnain I. Validasi Metode Analisis Piperin Dalam Lada Hitam Secara Spektrofotometri. Jurnal Teknologi Agro Industri 2018;10(2):53-58. Available from: http://ejournal.kemenperin.go.id/ tegi/article/download/4625/pdf_1

Sucipto C D. Keamanan Pangan untuk Kesehatan Manusia. [Online]. 2015 [cited2020 Nov 2]

Sukmawati, Sudewi S, dan Pontoh J. Optimasi Dan Validasi Metode Analisis Dalam Penentuan Kandungan Total Flavonoid Pada Ekstrak Daun Gedi Hijau (*Abelmoscus manihot L.*) Yang Diukur Menggunakan Spektrofotometer UV-Vis. Jurnal Ilmiah Farmasi. 2018;7(3):32-41. Available form: URL: https://ejournal.unsrat.ac.id/index.php/pharmacon/article/view/20117

UNODC. Guidance for the Validation of Analytical Methodology and Calibration of Equipment Used for Testing og Illicit Drugs in Seized Material and Biological Specimens. New York: United Nations. PP: 9-12; 2009

Utami AR. Verifikasi Metode Pengujian Sulfat Dalam Air dan Air Limbah Sesuai SNI 6989.20 : 2009. Jurnal Teknologi Proses dan Inovasi Industri. 2017;1(2):19-25. Available from: http://ejournal.kemenperin.go.id/JTPII/article/view/2726/0

Wibowo A, Maulidina DI, Fitri WS dan Dwi V. Validasi Metode Bioanalisis Vankomisin Dalam Spiked-Plasma Manusia Menggunakan Kromatografi Cair Kinerja Tinggi-detektor UV Untuk Aplikasi Pemantauan Kadar Obat Dalam Darah. Jurnal Ilmu MIPA. 2019;19(1):57-70. Available from: https://journal.uii.ac.id/Eksakta/article/download/11993/pdf

Winahyu DA, Retnaningsih dan Aprilia M. Penetapan Kadar Flavonoid Pada Kulit Batang Kayu Raru (*Cotylelobiummelanoxylonp*) Dengan Metode Spektrofotometri UV-Vis. Jurnal Analis Farmasi. 2019;4(1):29-36. Available from: http://ejurnalmalahayati.ac.id/index.php/analis farmasi/article/view/1304

Yohan, Astuti F, Wicaksana A. Pembuatan Spektrofotometer Edukasi Untuk Analisis Senyawa Pewarna Makanan. *Chemica et Natura Acta.* 2018;6(3):111-115. Available from: http://jurnal.unpad.ac.id/jcena /article/view/19099

Yulianti CH, Devianti VA, Fernanda MAHF. Validasi Metode Spektrofotometri Visible Untuk Penentuan Kadar Formaldehida Pada Pembalut Wanita Yang Beredar Di Pasaran. *Journal of Pharmacy and Science* 2017;2(1):9-16. Available from: http://www.ejournal.akfarsurabaya.ac.id/index.php/jps/article/view/60