TRƯỜNG ĐẠI HỌC SƯ PHẠM TP HỒ CHÍ MINH
TẠP CHÍ KHOA HỌC
KHOA HỌC TỰ NHIÊN VÀ CÔNG NGHỆ
ISSN: 1859-3100 Tập 16, Số 6 (2019): 16-28

HO CHI MINH CITY UNIVERSITY OF EDUCATION
JOURNAL OF SCIENCE
NATURAL SCIENCES AND TECHNOLOGY
Vol. 16, No. 6 (2019): 16-28

Email: tapchikhoahoc@hcmue.edu.vn; Website: http://tckh.hcmue.edu.vn

DEVELOPMENT AND VALIDATION OF HPLC-PDA METHOD FOR THE SIMULTANEOUS DETERMINATION OF AURAMINE O AND RHODAMINE B IN FOODSTUFFS

Phan Thi Ngoc Trinh[1], Nguyen Thanh Thoi[1], Huynh Thi Nhan[1], Nguyen Ngoc Hung[1], Nguyen Thi Tuyet Nhung[1*], Le Thi Hong Van[2], Le Van Huan[2]

[1] Faculty of chemistry – Ho Chi Minh City University of Education

[2] Saigon Pharmaceutical Sciences and Technologies Center, University of Medicine and Pharmacy at Ho Chi Minh City

[*] Corresponding author: Nguyen Thi Tuyet Nhung – Email: nhungntt@hcmue.edu.vn

Received: 18/3/2019; Revised: 11/4/2019; Accepted:15/6/2019

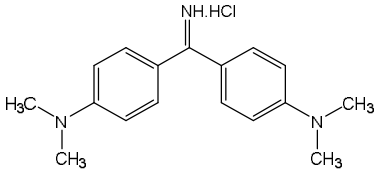
ABSTRACT

HPLC-PDA method was used to determine two basic dyes, Auramine O and Rhodamine B in food samples. The limits of detection and quantification of Auramine O (AO) and Rhodamine B (RB) were both 0.02 mg/L and 0.05 mg/L, respectively. The recoveries of AO and RB in matrices ranged from 83.00% to 105.91% with the concentration 0.5 µg/g, 1.0 µg/g and 1.5 µg/g. This is a simple and accurate method which can be applied to quantify of dyes in foodstuffs.

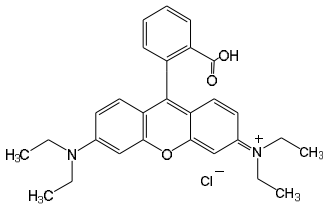
Keywords: Auramine O, Rhodamine B, HPLC-PDA, foodstuffs.

1. Introduction

Auramine O ($C_{17}H_{22}N_3Cl$, 303.84 gram/mol, yellow powder, pK_a 9.8, 10.7 (Sabnis R.W, 2010)) and Rhodamine B ($C_{28}H_{31}N_2O_3Cl$, 479.01 gram/mol, green powder, pK_a 3.7 (Peng Wang, Mingming Cheng, Zhonghai Zhang, 2014)) are synthetic azo–cationic dyes with the chemical structures shown in **Fig 1**. RB functions as a water tracer fluorescent and is commonly used as a colorant in textiles, paper industry (Adejumoke A. Inyinbor, Folahan A. Adekolab and Gabriel A. Olatunji, 2015) while AO is commonly used to dye wood products, leather, ink, and textile fabrics (Sabnis R.W, 2010). According to the International Agency of Research on Cancer, RB is associated with eye, skin, and respiratory tract irritation and AO is possibly carcinogenic to humans (Agents classified by the IARC Monographs, 2019). For that reason, the presence of AO and RB in foodstuffs may cause adverse effects on human's health. Ingestion of these dyes over time causes respiratory tract ailments as well as acute and chronic poisoning. Therefore, both of them are not listed as food additives allowed for use in Vietnam and other countries (Vietnamese Ministry of Health, 2001, 2018). However, because of their easy coloring, high stability, and low cost, AO and RB are still being used illegally in animal feed, food, and cosmetics as beauty color as well as to ensure the uniform appearance of foodstuffs.



AO



RB

Fig 1. *Chemical structures of AO and RB*

The color products have received the attention of many researchers in many countries in the world. The study of Dong-yang Chen, Hao Zhang, Jia-li Feng, Dong Zeng, Li Ding, Xian-jun Liu, Bang-rui Li (2017) has presented high performance liquid chromatography coupled with diode array detector to detect 10 industrial dyes (basic orange 2, basic orange 21, basic orange 22, acid orange II, auramine, basic rhodamine B, and Sudan I–IV) in a great variety of matrices such as meat products (smoked sausages, preserved ham, fresh meat, yellow-fin tuna), chili powder, and chili oil in China. While the limit of detection has been in the range of 0.007–0.01 mg/kg, high recoveries (80.6–104%) and good reproducibility (1.1–5.7%) have been obtained. Shruti Singh, Himani Shah, Ritika Shah, and Krishna Shah (2017) have separated and determined Sudan and Rhodamine B dye in chili and curry powder matrices by thin layer chromatography and spectrophotometry method. Brazeau J. (2018) has detected 27 color compounds (including RB) in food by liquid chromatography/ultraviolet visible. Using poly(sodium 4–styrenesulfonate) modified MIL–101(Cr)–NH_2 to enable effective adsorption and separation of AO, RB, and pararosaniline from foodstuffs prior to high performance liquid chromatography analysis, under optimized conditions, the recoveries of the three dyes in shrimp powder, chili powder, tofu sheets, and tomato sauce have been in the range of 86.8–119.3% (Xue Wang, Hui-Ling Duan, Shi-Yao Ma, Jun Wang, Han-Ying Zhan, & Zhi-Qi Zhang, 2018). Serum samples have been deproteinized with acetonitrile and separated by UHPLC on a reverse-phase C18, using a triple quadrupole tandem mass spectrometer in the selected reaction monitoring mode at $[M]^+$ ion m/z 443.39 → 399.28 for RB. Linear has resulted over a concentration range of 0.5–100 ng/mL, with a lower limit of quantitation of 0.5 ng/mL (Yung-Yi Cheng, Tung-Hu Tsai, 2016).

In Vietnam, some analytical studies have determined only AO or RB in matrices, such as cosmetics (Le Thi Huong Hoa, Nguyen Thi Hoang Lien, Thai Nguyen Hung Thu, Trinh Van Lau, 2011), chicken meat (Nguyen Thi Kim Thuong, Pham Tuan Anh, 2017). Differential pulse adsorptive stripping voltammetry, UV-Vis, HPLC, and UPLC are methods which have been commonly used. The standard TCVN 8670:2011 of the Vietnamese Ministry of Science and Technology (2011) mentions the method of

quantifying only RB in foodstuffs. Some analytical laboratories have identified AO and RB in food samples by liquid chromatography coupled mass spectrometry LC-MS/MS, which is a sensitive method but requires expensive analytical instruments and a complex sample preparation process. Therefore, the main purpose of this work is the simultaneous analysis of two synthetic colors in foods by HPLC-PDA, which is a suitable method for small and medium laboratories.

2. Materials and methods

2.1. Chemicals and reagents

AO (861030, purity ≥ 85%) and RB (R6626, purity ≥ 95%) were bought from Sigma. Methanol (99.9%, Lapscan, Thailand), formic acid (98-100%; Scharlau, Scharlab S.L - Spain), acetic acid (100%, Merck, Germany), and phosphoric acid (85%; Scharlau, Scharlab S.L - Spain) were used for mobile phase HPLC. Ammonia solution in water (25%, Merck, Germany), acetonitrile (99.9%, Merck, Germany), and sodium chloride (99.99%, Suprapur) were used to extract AO and RB from foodstuffs.

2.2. Instruments

Perkin-Elmer Lambda 25 was used for scanning the absorption spectra of AO and RB.

Waters 2996 HPLC-PDA, a column C18 (Zorbax Eclipse XDB - C18, 5 μm x 4.6 mm x 250 mm, Agilent), and a precolumn (Eclipse XDB - C18, Agilent) were used for chromatographic analysis. This system is a manual accessory for high-sensitivity determination of synthetic food colorants. The detection wavelengths of AO and RB were 435 nm and 555 nm, respectively.

Water Pro PS (Labconco) was used for preparing the aqueous mobile phase. The mobile phase comprised of (A) methanol and (B) 0.03% phosphoric acid in water. The gradient elution was conducted as follows: solvent A started at 30% and kept the balanced ratio in 5 minutes, then increased to 80% in 11 minutes, finally decreased to 30% in 4 minutes with the flow rate of 1.0 mL/min at 35^{o}C. The injection volume was 20 μL.

An ultrasonic assistance (Elmasonic S 60 (H) - Elma Schmidbauer) and a centrifuge machine (DNA concentrator - miVac, Genevac Ltd. Ipswich) were used for extraction of AO and RB in samples.

2.3. Sample collection and preparation

Nine different samples of three kinds of food: pickled collard greens, chicken meat, and bamboo shoots were purchased from Lotte Mart supermarket (District 10) and three local markets, namely Tan Phuoc (District 11), Do Dac (District 2) and Nhat Tao (District 10) in Ho Chi Minh City, Vietnam. All samples were minced and homogenized, then kept in clean, dry containers and stored in the refrigerator before being analyzed.

2.4. Preparation of standard solutions

Stock standard solutions of AO and RB were prepared by dissolving each standard compound in methanol in a volumetric flask at concentration of 1000 mg/L. The stock standard solutions were further diluted with methanol to give 100 mg/L standard solutions for the recovery tests. Calibration standards for AO and RB were prepared each day from the certified standard stock solution in the range from 0.05 to 2.0 mg/L. All the standard solutions were prepared in methanol.

2.5. Extraction procedure

Sample (0.5 g) was accurately weighed, added into a 50 mL centrifuge tube, then mixed with acetonitrile:NH_3 (0.5% v/v in water) at the ratio of 7:3. The sample was thoroughly mixed well by a vortex shaker for 10 minutes and extracted under ultrasonic assistance for 10 minutes at 40°C, then the mixture was centrifuged for 10 minutes and the solution was transferred into a volumetric flask. The same treatment was performed three times on the residual precipitates. All extraction solutions were combined and diluted to 25 mL with water, then filtered through a 0.22 µm filter of PTFE membrane before transferred into glass vials for HPLC analysis.

3. Results and discussion

3.1. Optimization of HPLC-PDA condition

Visible spectrums of AO and RB in two solvents, methanol and acetonitrile, were recorded in wavelength range of 400-600 nm on Perkin-Elmer Lambda 25. In **Fig 2**, maximum absorbance at respective wavelengths 430 ÷ 440 nm (AO) and 545 ÷ 555 nm (RB). With the same concentration of AO and RB in two solvents, the absorption signal of RB in methanol was higher than the figure in acetonitrile, which was the main reason for using methanol to dilute the stock standards. In this study, to achieve the best analytical results, PDA operated and monitored the absorbance at 435 nm for AO and 555 nm for RB.

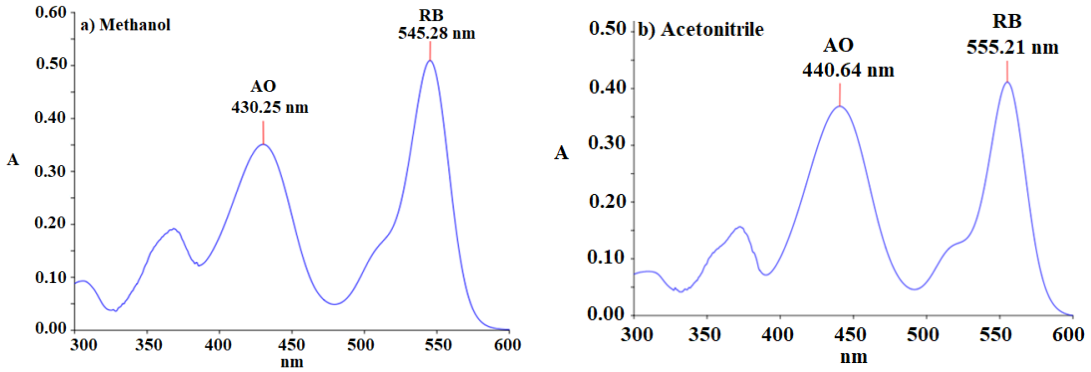
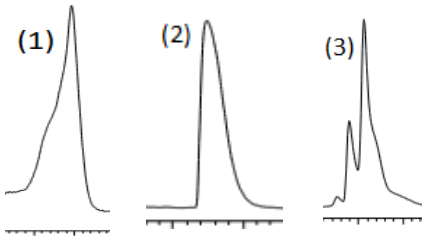
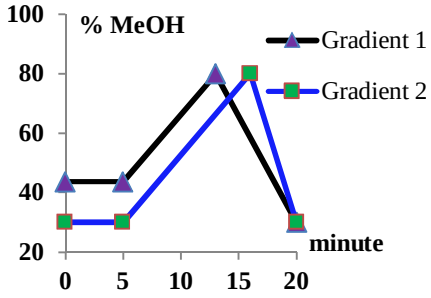


Fig 2. *Molecular absorption spectra of AO and RB in methanol (a) and acetonitrile (b)*

The mobile phase was optimized in these factors: pH, mobile phase composition, and elution program because of their significant effects on the chromatographic separation process.

Table 1. *Mobile phase conditions*

	Mobile phase conditions	Consequences
Organic solvent	(1) ACN - CH_3COOH (2) MeOH - CH_3COOH	(1) Better elution, lower signal, tailing peak (both AO, RB) (2) Better sensitivity but AO peak was tailed
pH	(1) MeOH - H_3PO_4 0,03% (pH 2.6) (2) MeOH - H_3PO_4 0,072% (pH 2.3)	Good separation peak, resolution (R_s) > 1.5, similarity of retention time, capacity, and peak asymmetry factor
Isocratic elution	(1) MeOH - HCOOH (pH = 3) 40:60 (2) ACN - HCOOH (pH = 3) 40:60 (3) ACN - acetate buffer (pH = 4,2) 80:20	 AO and RB weren't separated in these isocratic elution programs
Gradient elution (MeOH - H_3PO_4 0,03%)		- Gradient 1: peak fronting for AO chromatography, rising baseline and low AO intensity. - Gradient 2: narrow and symmetrical peaks. HPLC chromatogram was shown in **Figure 3**

Finally, the gradient mobile phase using methanol and 0.03% phosphoric acid aqueous solution was chosen as the preferred mobile phase in the following experiments due to its higher efficiency and reasonable analysis time.

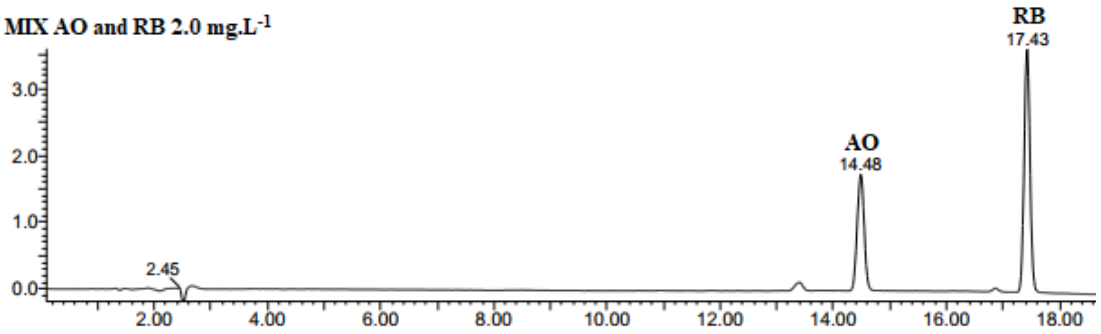


Fig 3. *HPLC chromatogram of the standard solution at AO (t_R 14.48 min) and RB (t_R 17.43 min) in 2.0 mg/L*

3.2. Evaluation of range linearity, limit of detection (LOD) and limit of quantification (LOQ)

From the data from the paper Ping Qi et al. (2016); Shruti Singh, Himani Shah, Ritika Shah, and Krishna Shah (2017) and the prediction of small concentration of AO and RB in food samples, the calibration curve was in range from 0.05 mg/L to 2.00 mg/L of each dye concentration.

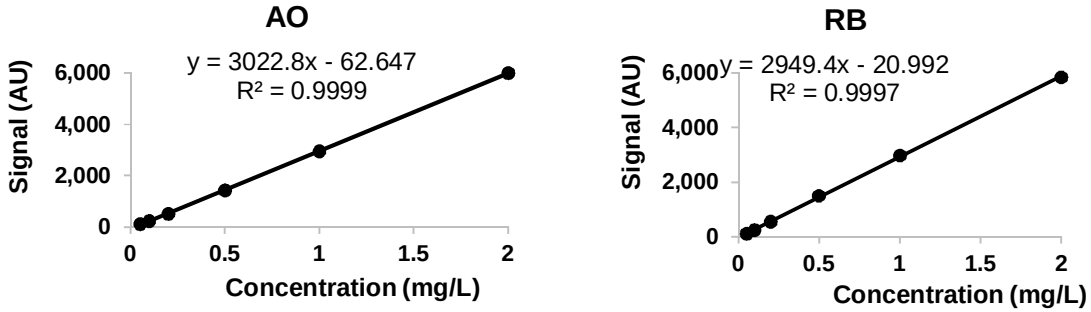


Fig 4. *Calibration curves for AO and RB*

Two calibration curves was linear in this range of concentration. The limits of detection (LOD) and quantification (LOQ) of AO and RB were estimated by the signal-to-noise S/N of each peak in the standard solutions. LOD and LOQ of these substances gained 0.02 mg/L and 0.05 mg/L, respectively.

3.3. Optimization extraction procedure

Accurately 5 g bamboo shoots sample was spiked 250 µL mixture standard 100 mg/L. The sample was kept in the refrigerator in 24 hours for optimizing the number of extraction.

Acetonitrile was used as an extraction solvent because AO and RB dissolved completely in this solvent, which adopted to effectively remove the matrix-matched components such as polar pigments, fatty acids, and protein in samples. The extraction process was as follows: added 10 mL acetonitrile: NH_3 (0.5% v/v in water) at the ratio of 7:3 to samples. The mixture was continued for 10 minutes in an ultrasonic bath, then was

centrifuged in 10 minutes. To optimize the sample preparation, the same treatment was repeated three more times by using 5 mL of the solvent acetonitrile: NH_3 each time. The each time extract was transferred to a volumetric flask, and diluted with water to 25 mL. The elution solution was filtered through a 0.22 μm filter of PTFE membrane and transferred into glass vials for HPLC-PDA analysis.

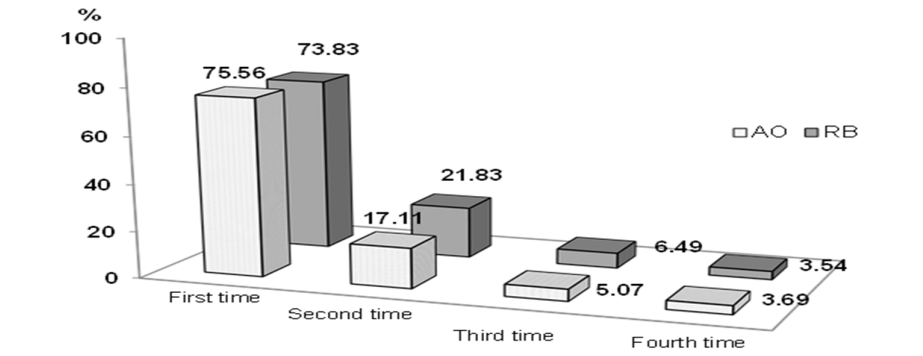


Fig 5. *Ratios of AO and RB in four times of extraction*

As shown in **Fig 5**, the recovery ratio of AO and RB in bamboo shoots sample was from 97.74% to 102.15% after three times of extraction. Therefore, the process extracted three times using total 20 mL acetonitrile: NH_3 (0.5% v/v in water), which was selected as an optimum condition of extraction.

3.4. Method validation

To survey the repeatability of the measurement, a mixture standard of 2.0 mg/L AO and 2.0 mg/L RB was measured 7 times by HPLC-PDA.

Table 2. *Repeatability of signals*

N	Retention time (min)		Peak area	
	AO	**RB**	**AO**	**RB**
1	14.467	17.400	5664.660	5588.101
2	14.450	17.383	5753.625	5646.242
3	14.450	17.383	5787.972	5693.114
4	14.450	17.383	5761.298	5711.542
5	14.450	17.383	5812.427	5704.386
6	14.450	17.383	5802.885	5776.700
7	14.433	17.367	5800.698	5748.406
Mean	$14.5 \pm 9.1 \times 10^{-3}$	$17.4 \pm 8.8 \times 10^{-3}$	5769 ± 47	5695 ± 58
RSD%	0.63%	0.051%	0.82%	1.0%

RSD of retention time and peak area were 0.63% and 0.82% (for AO); 0.051% and 1.0 % (for RB), respectively (n = 7). The method gained good precision. According to AOAC, with RSD value ≤ 1.8%, the result by HPLC-PDA had good precision.

Method validation and quality control were ensured using blanks and spiked samples.

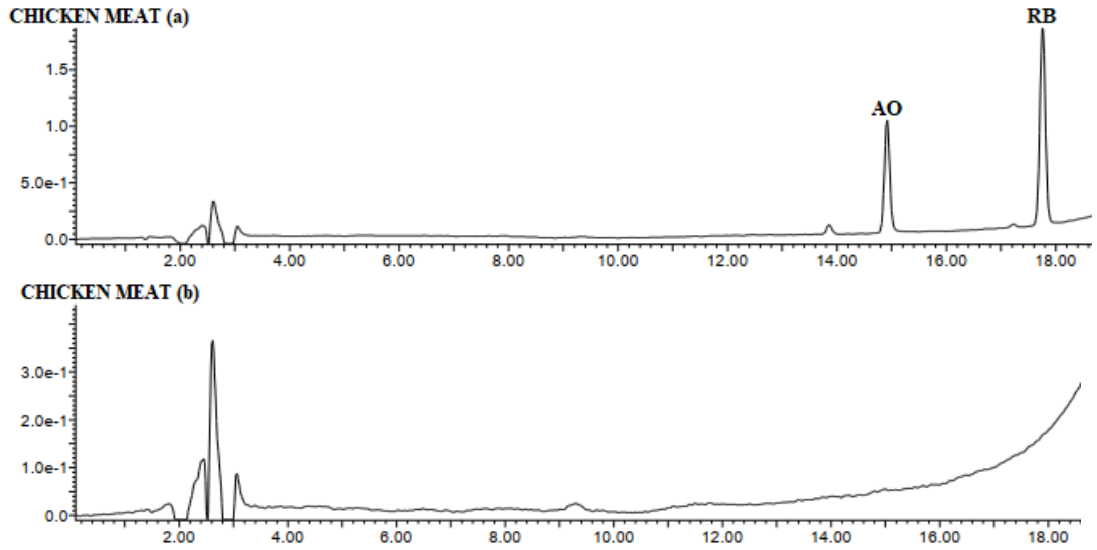


Fig 6. *HPLC chromatograms of spiked chicken meat sample (a) and chicken meat sample (b)*

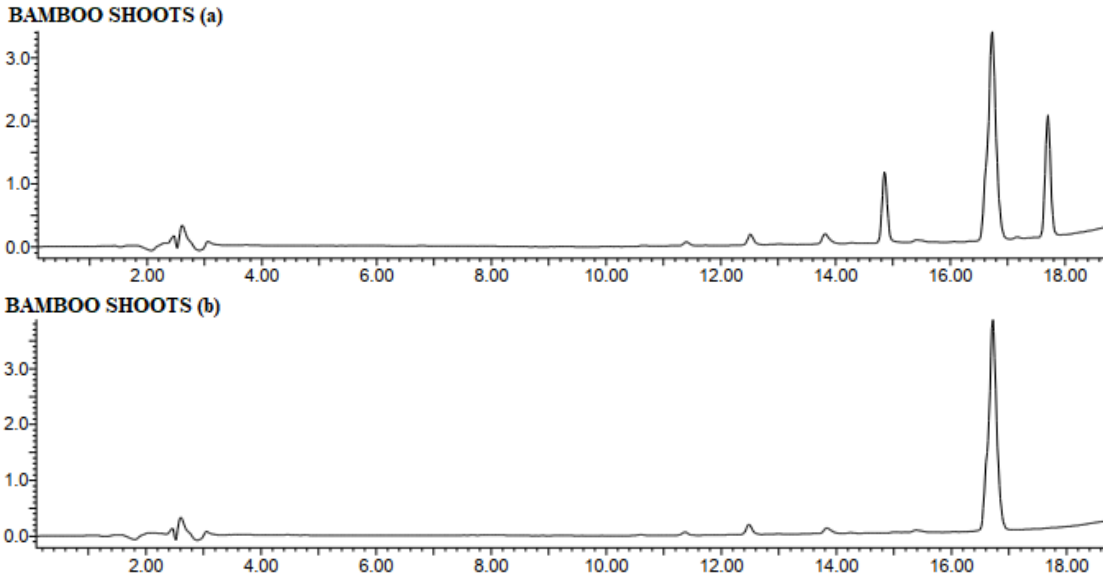


Fig 7. *HPLC chromatograms of spiked bamboo shoots sample (a) and bamboo shoots sample (b)*

There was a similarity in retention time of noise peaks in spiked samples and samples (**Fig 6a, Fig 6b, Fig 7a**, and **Fig 7b**). Meanwhile, using the peak purity test function of PDA detector, peaks of AO and RB in spiked samples were 100% purity **(Fig 6a and Fig 7a).** Consequently, the analytical results showed that AO and RB could be determined exactly in this method without the interferences of the food matrices.

Recoveries were determined as following: 5 g of a chicken meat sample spiked with mixture standard, then the sample was kept in the refrigerator in 24 hours until analysis.

Table 3. *Recoveries of AO and RB in the recovery samples*

Concentration (µg/g)	Peak area		Recovery (%)	
	AO	**RB**	**AO**	**RB**
0.5	888.191	1954.000	91.52	93.27
	900.504	1949.214	92.79	93.04
	868.357	1866.296	89.48	89.08
	891.151	1982.437	91.83	94.63
	883.218	1928.280	91.01	92.04
	885.340	1869.000	91.23	89.21
	857.136	1861.447	88.32	88.85
	Mean		90.9 ± 1.4	91.4 ± 2.2
1.0	1918.208	4345.709	102.54	100.80
	1894.076	4334.987	101.25	100.55
	1883.574	4292.576	100.68	99.57
	1981.334	4432.542	105.91	102.82
	1926.163	4384.205	102.96	101.70
	1903.961	4163.081	101.77	96.57
	1812.305	4251.641	96.87	98.62
	Mean		101.7 ± 2.5	100.1 ± 1.9
1.5	2656.685	5997.764	91.51	94.70
	2650.758	5788.187	91.31	91.39
	2409.565	5354.113	83.00	84.54
	2477.072	5661.422	85.33	89.39
	2496.536	5682.338	86.00	89.72
	2525.13	5983.245	86.98	94.47
	2716.479	6169.421	93.57	97.41
	Mean		88.2 ± 3.6	91.7 ± 4.0

The recoveries of AO and RB were determined by using this method and achieved in range from 83.00% to 105.91%. Therefore, this analytical method could be applied to determine AO and RB in foodstuffs.

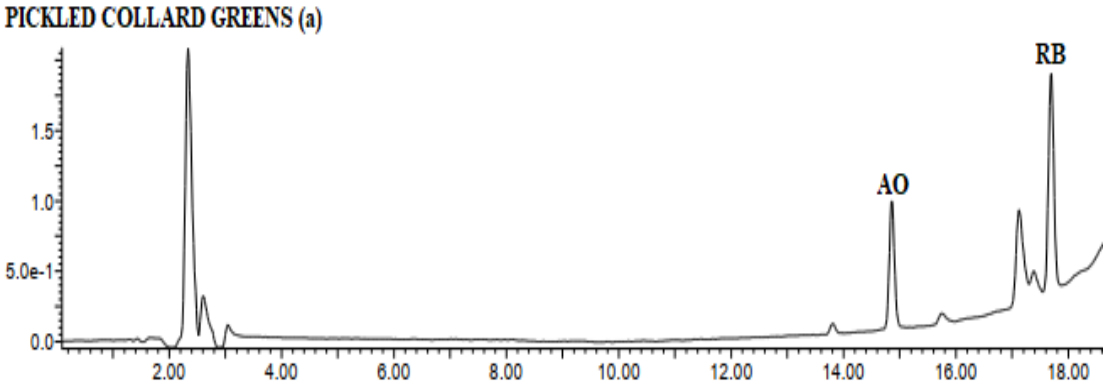
3.5. Some analytical results

This given method was applied to monitor AO and RB in nine food samples collected from some markets in Ho Chi Minh City.

Table 4. *Information of food samples*

Samples	Name	Location
Pickled collard greens	M1	Nhat Tao market
	M2	Tan Phuoc market
	M3	Do Dac market
Chicken meat	M4	Lotte Mart
	M5	Tan Phuoc market
	M6	Do Dac market
Bamboo shoots	M7	Nhat Tao market
	M8	Tan Phuoc market
	M9	Do Dac market

The results showed that both AO and RB were absence in nine samples. The sample M7 was sent to the analytical laboratory of Hoan Vu Scientific Technologies Company Limited for control analysis by LC-MS/MS method. LOD of Hoan Vu laboratory's method for AO and RB were 0.005 mg/L and 0.05 mg/L, respectively. The result of LC-MS/MS method was totally appropriate to the result in this study.



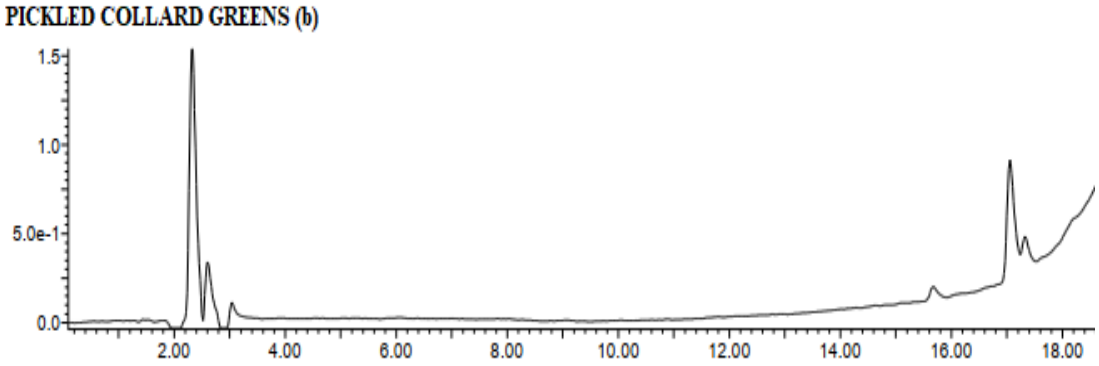


Fig 8. *HPLC chromatograms of spiked pickled collard greens sample (a) and pickled collard greens sample (b)*

4. Conclusion

In this study, HPLC-PDA method was developed and validated for simultaneous analysis of AO and RB in food samples. While the LOD and LOQ achieved by this method were 0.02 mg/L and 0.05 mg/L for two dyes, the recoveries ranged from 83.00% to 105.91%. The method was applied to determine two dyes in nine samples (pickled collard greens, chicken meat, and bamboo shoots) and the analytical results shows that these dyes were absent from all monitored samples. This study showed that HPLC-PDA is a reliable and fast tool for monitoring these dyes in foodstuffs in small and medium analytical laboratories.

❖ ***Conflict of Interest:*** *Authors have no conflict of interest to declare.*

REFERENCES

Adejumoke, A. Inyinbor, Folahan, A., Adekola,b & Gabriel A. Olatunji. (2015). Adsorption of Rhodamine B Dye from Aqueous Solution on Irvingia gabonensis Biomass: Kinetics and Thermodynamics Studies. *68*, 115-125.

Agents classified by the IARC Monographs. (2019). *International Agency for Research on Cancer, 1-123*.

Brazeau, J. (2018). Identification and Quantitation of Water-Soluble Synthetic Colors in Foods by Liquid Chromatography/Ultraviolet-Visible Method Development and Validation. *ACS Omega, 3*, 6577-6586.

Dong-yang Chen, Hao Zhang, Jia-li Feng, Dong Zeng, Li Ding, Xian-jun Liu, & Bang-rui Li. (2017). Research on the Determination of 10 Industrial Dyes in Foodstuffs. *Journal of Chromatographic Science, 55*(10), 1021-1025.

Le Thi Huong Hoa, Nguyen Thi Hoang Lien, Thai Nguyen Hung Thu, & Trinh Van Lau. (2011). Method development of determination of illegal dye Rhodamine B in cosmetics. *Vietnam Pharmaceutical Journal, 51*(10), 23-27.

Nguyen Thi Kim Thuong, Pham Tuan Anh. (2017, Sep-Oct). Study on determination Auramine O in food samples by differential pulse adsorptive stripping voltammetry. *Testing today - Journal of Vietnam Asscociation of Testing Laboratories*, (1), 6-10.

Peng Wang, Mingming Cheng, & Zhonghai Zhang. (2014). On different photodecomposition behaviors of rhodamine B on laponite and montmorillonite clay under visible light irradiation. *Journal of Saudi Chemical Society, 18*(4), 308-316.

Ping Qi, Zhi-an Liang, Yu wang, Jian Xiao, Jia Liu, Qing-qiong Zhou, Chun-hao Zheng, Li-ni Luo, Zi-hao Lin, Fang Zhu, & Xue-wu Zhang. (2016). Mixed hemimicelles solid-phase extraction based on sodium dodecyl sulfate-coated nano-magnets for selective adsorption and enrichment of illegal cationic dyes in food matrices prior to high-performance liquid chromatography-diode array detection detec. *Journal of Chromatography A, 1437*, 25-36.

Sabnis R.W. (2010). Synthesis and Industrial Applications. In *Handbook of biological dyes and stains* (pp. 27-29). John Wiley & Sons, Inc., Publication.

Shruti Singh, Himani Shah, Ritika Shah, & Krishna Shah. (2017). Identification and Estimation of Non-Permitted Food Colours (Sudan and Rhodamine-B Dye) In Chilli and Curry Powder by Rapid Colour Test, Thin Layer Chromatography and Spectrophotometry. *International Journal of Current Microbiology and Applied Sciences, 6*(7), 1970-1981.

Vietnamese Ministry of Health. (n.d.). Decision Issuing the "Regulation of the list of additives permitted for use in food". In *3742/2001/QĐ-BYT*.

Vietnamese Ministry of Heathy. (n.d.). The Circular issued the list of food, food additives, food processing support substances and tools, packaging materials, containing food to be identified as a commodity code according to the list of exported and imported goods to Vietnam. In *05/2018/TT-BYT*.

Vietnamese Ministry of Science and Technology. (n.d.). Foodstuffs - Determination of rhodamine B by high performance liquid chromatography (HPLC). In *TCVN 8670: 2011.*

Xue Wang, Hui-Ling Duan, Shi-Yao Ma, Jun Wang, Han-Ying Zhan & Zhi-Qi Zhang. (2018). Selective adsorption and separation of illegal cationic dyes from foodstuffs with anionic polyelectrolyte functionalization of metal-organic frameworks. *Food Additives & Contaminants: Part A, 35*(10), 1870-1880.

Yung-Yi Cheng, & Tung-Hu Tsai. (2016). A validated LC–MS/MS determination method for the illegal food additive rhodamine B: Applications of a pharmacokinetic study in rats. *Journal of Pharmaceutical and Biomedical Analysis, 125*, 394-399.

XÂY DỰNG VÀ ĐÁNH GIÁ PHƯƠNG PHÁP HPLC-PDA DÙNG ĐỂ XÁC ĐỊNH ĐỒNG THỜI AURAMINE O VÀ RHODAMINE B TRONG THỰC PHẨM

Phan Thị Ngọc Trinh[1], Nguyễn Thanh Thơi[1], Huỳnh Thị Nhàn[1], Nguyễn Ngọc Hưng[1], Nguyễn Thị Tuyết Nhung[1*], Lê Thị Hồng Vân[2], Lê Văn Huấn[2]

Khoa Hóa học – Trường Đại học Sư phạm Thành phố Hồ Chí Minh

Trung tâm Khoa học và Công nghệ Dược Sài Gòn – Trường Đại học Y Dược Thành phố Hồ Chí Minh

[*] Corresponding author: Nguyễn Thị Tuyết Nhung – Email: nhungntt@hcmue.edu.vn

Ngày nhận bài: 18-3-2019; ngày nhận bài sửa: 11-4-2019; ngày duyệt đăng: 15-6-2019

TÓM TẮT

Nghiên cứu này phát triển phương pháp HPLC-PDA với mục đích xác định hai thuốc nhuộm Auramine O và Rhodamine B trong các mẫu thực phẩm. Giới hạn phát hiện và giới hạn định lượng của Auramine O (AO) và Rhodamine B (RB) cùng có giá trị lần lượt là 0,02 mg/L và 0,05 mg/L. Hệ số thu hồi của AO và RB trong nền mẫu thực phẩm có giá trị trong khoảng 83,00% đến 105,91% ở các nồng độ chất phân tích 0,5 µg/g, 1,0 µg/g và 1,5 µg/g. Phương pháp phân tích được thực hiện đơn giản, có kết quả chính xác và phù hợp để định lượng các thuốc nhuộm này trong thực phẩm.

Từ khóa: Auramine O, Rhodamine B, HPLC-PDA, thực phẩm.