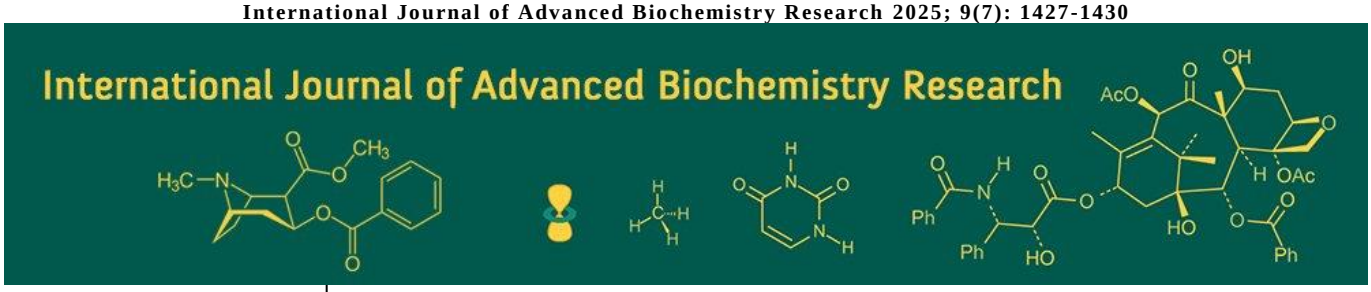

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; 9(7): 1427-1430
www.biochemjournal.com
Received: 07-04-2025
Accepted: 10-05-2025

Akashamrut M Patel
Food Safety and Quality Assurance Department, College of Food Processing Technology & Bioenergy, Anand Agricultural University, Anand, Gujarat, India

Kedar S Damle
Food Safety and Quality Assurance Department, College of Food Processing Technology & Bioenergy, Anand Agricultural University, Anand, Gujarat, India

Rashmin M Dhingani
Food Safety and Quality Assurance Department, College of Food Processing Technology & Bioenergy, Anand Agricultural University, Anand, Gujarat, India

Jafarali K Momin
Food Safety and Quality Assurance Department, College of Food Processing Technology & Bioenergy, Anand Agricultural University, Anand, Gujarat, India

Bhavesh H Joshi
Food Safety and Quality Assurance Department, College of Food Processing Technology & Bioenergy, Anand Agricultural University, Anand, Gujarat, India

Corresponding Author:
Akashamrut M Patel
Food Safety and Quality Assurance Department, College of Food Processing Technology & Bioenergy, Anand Agricultural University, Anand, Gujarat, India

Rhodamine detection in chili powder using TLC and direct fluorescence methods

Akashamrut M Patel, Kedar S Damle, Rashmin M Dhingani, Jafarali K Momin and Bhavesh H Joshi

DOI: https://www.doi.org/10.33545/26174693.2025.v9.i7r.4930

Abstract
Addition of unapproved colors in food products is constant threat. Rhodamine which is non permitted dye is commonly added to chili power. Various methods are approved in literature and most of them are very sensitive but at the same time very complex. In this study two simple methods i.e. thin layer chromatography and direct fluorescence are proposed for fast, easy and sensitive detection. It was found that TLC can detect up to 1 PPM and direct fluorescence method can dtect 0.1 PPM rhodamine in chili powder.

Keywords: Adulteration detection, Rhodamine, chili powder, TLC, direct fluorescence

Introduction
Colors are added in foods to increase attractiveness and addition non permitted dyes is food authenticity issue [1]. The synthetic dyes, compared to most natural dyes, have higher stability and lower production costs and thus non-permitted synthetic dyes are commonly used as spice adulterates [2]. As permitted dyes are considered safer, non-permitted dyes are more of concern.
Metanil yellow, Rhodamine B etc. might be illegally used in foods as a replacement for monitored Sudan dyes [1]. Rhodamine is reported in sweets and road side foods [3]. Artificial colorants are reported in parboiled chili powder and turmeric powder [4]. Study conducted in Allhabad, India for non-permitted food colors and found 10 out of 15 samples of turmeric powder, 9 out of 15 samples of chili powder and 7 out of 13 samples of Garam Masala were adulterated with metanil yellow, Sudan III and other artificial colors [5]. Survey in Lucknow urban and rural areas revealed that 30% samples have non permitted colors if we consider all foods. For turmeric samples metanil yellow adulteration and for chili powder Rhodamine B was adulterant in 5% samples. Para red, orange II, methyl yellow, and Rhodamine B are also consistently detected in foods [1].
Many methods are developed for detection of rhodamine B adulteration [6-13]. But most methods are complex. Here in this study comparison of two simple methods based on reported methods is presented.

Materials and Methods
Chili powder was made by grinding whole dried chilis obtained from market. Rhodamine used for spiking in chili powder was obtained from local supplier and was of Lobachemie brand having catalogue number 45170. It was in dry powdery form.
For spiked sample preparation, 10 g chili powder was taken into 100 ml glass beaker. Adulterant was spiked at desired level and then mixed in dry state. Then solvent was added and mixed with sample and then we went for extraction. Extraction of Rhodamine B was done using either using 70 ml water or 50 ml methanol. After throurh mixing for 5 minute the slurry is filter out using Whatman 41 filter paper. The filtrate was kept in air tight bottles for analysis. Negative control was extract of pure chili power without any extraction. Positive control were prepared by just adding rhodamine B in methanol directly at spiking levels. Serial dilution technique was used to achieve lower concentration of rhodamine when needed.

Three methods were compared in this study and that are spectrophotometric detection, direct fluorescence detection and TLC detection. For direct fluorescence detection samples were subjected to 254 and 365 nm UV lights and photographed. For TLC method reverse phase TLC was used with methanol added with 1% acetic acid as mobile phase.

Results and Discussion

Direct Fluorescent Detection

Spiking was started at 0.1% level and at this level adulteration was visible. This level corresponds to 1 g per kg or 1000 PPM. Light at 365 nm was most effective for this purpose and 254 nm light though theoretically better was not found effective. Reason why more fluorescence was expected at 254 nm was in fact that more UV light is absorbed by rhodamine at 254 nm and generally fluorescence follows absorption pattern [14]. In following images of direct fluorescence detection positive control is on left side, spiked sample in middle and negative control on right side.

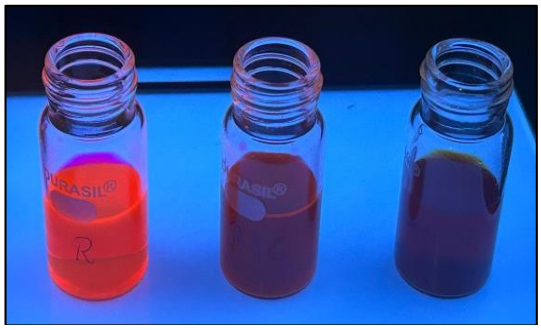

Fig 1: 1000 PPM samples under 254 nm light

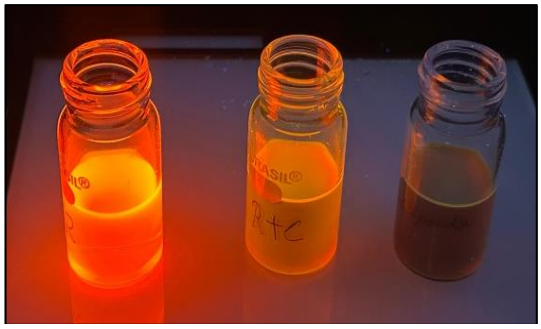

Fig 2: 1000 PPM samples under 365 nm light

It is clear from photographs at 1000 PPM spiking level that 365 nm is best suited wavelength for detection of rhodamine using direct fluorescent method. So all later on observations were taken at 365 nm.

Fig 3: 100 PPM samples under 365 nm light

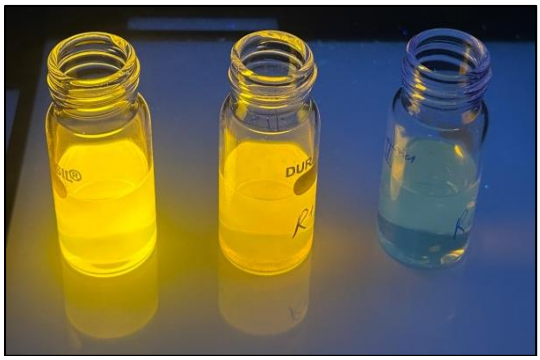

Fig 4: 10 PPM samples under 365 nm light

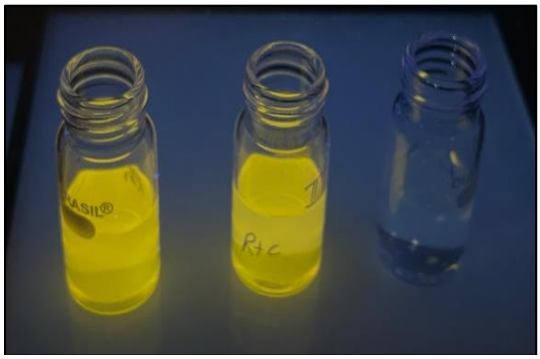

Fig 5: 1 PPM samples under 365 nm light

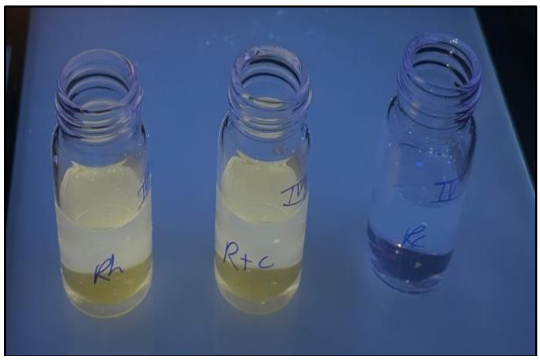

Fig 6: 0.1 PPM samples under 365 nm light

Fig 7: 0.01 PPM samples under 365 nm light

As we can notice decreasing spiking levels leads to diminishing signal. At 0.1 PPM it positive control sample is not showing any fluorescence. Thus it was considered as limit of detection for direct fluorescence method.

TLC Method

For TLC method all images shows negative control on left, spiked sample in middle and positive control on right.

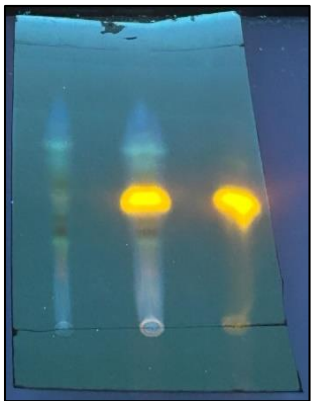

Fig 8: 1000 PPM Rhodamine TLC under 365 nm light for visualization

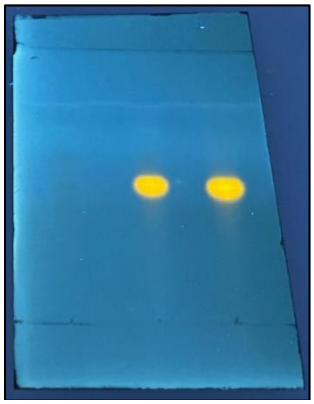

Fig 9: 100 PPM Rhodamine TLC under 365 nm light for visualization

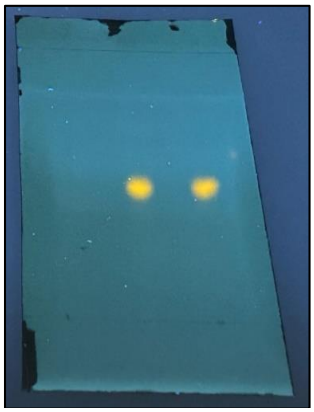

Fig 10: 10 PPM Rhodamine TLC under 365 nm light for visualization

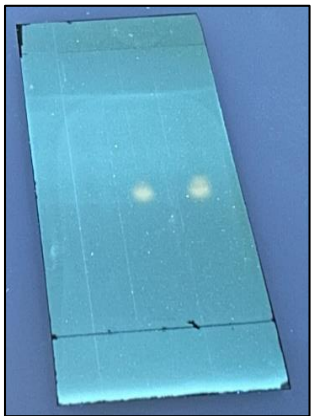

Fig 11: 1 PPM Rhodamine TLC under 365 nm light for visualization

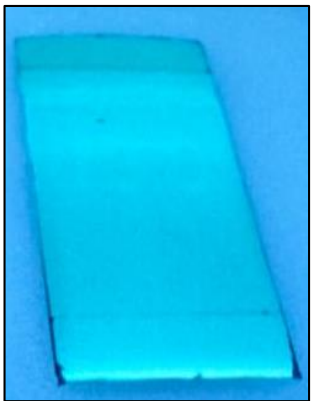

Fig 12: 0.1 PPM Rhodamine TLC under 365 nm light for visualization

For TLC reverse phase plates are must as rhodamine is hydrophilic and moves easily on non-polar surface. On normal phase it is difficult to move it as it sticks to stationary phase of TLC. It is clear from images that rhodamine cannot be detected either in control or spiked sample at 0.1 PPM. Thus we can consider this as limitation for detection of rhodamine.

Conclusions

It was found that TLC is not helpful when compared to direct fluorescence detection of rhodamine in chili powder. Direct fluorescence method was found 10 times more sensitive. TLC can detect 1 PPM rhodamine in chili powder while direct fluorescence based detection can to up to 0.1 PPM. Further direct fluorescence is easier method compared to TLC. Detection levels reported here are comparable to other more complex methods reported in literature [6-13].

References

1. Oplatowska-Stachowiak M, Elliott CT. Food colors: Existing and emerging food safety concerns. Crit Rev Food Sci Nutr [Internet]. 2017 Feb 11 [cited 2021 Dec 20];57(3):524-548. Available from: https://www.tandfonline.com/doi/full/10.1080/10408398.2014.889652
2. Hong E, Lee SY, Jeong JY, Park JM, Kim BH, Kwon K, *et al.* Modern analytical methods for the detection of food fraud and adulteration by food category. J Sci Food Agric [Internet]. 2017 Sep [cited 2024 Jan 17];97(12):3877-3896. Available from: https://onlinelibrary.wiley.com/doi/10.1002/jsfa.8364
3. Gahukar R. Food adulteration and contamination in India: occurrence, implication and safety measures. Int J Basic Appl Sci [Internet]. 2013 Dec 6 [cited 2024 Jan 17];3(1):47-54. Available from: http://www.sciencepubco.com/index.php/ijbas/article/view/1727
4. Harke S, Pawar A, Patil YY. Quality and Adulteration in Ethnic Spices and Food Ingredients in Local Market. Int J Food Agric Nat Resour [Internet]. 2023 Sep 30 [cited 2024 Jan 17];4(3):21-26. Available from: https://journal.fanres.org/index.php/IJFANRES/article/view/155
5. Jaiswal S, Yadav DS, Mishra MK, Gupta AK. Detection of Adulterants in Spices Through Chemical Method and Thin Layer Chromatography for Forensic Consideration. Int J Dev Res [Internet]. 2016 [cited 2024 Jan 17];6(8):8824-8827. Available from:

https://www.researchgate.net
6. Bai B. Spectrometric Determination of Rhodamine B in Chili Powder After Molecularly Imprinted Solid Phase Extraction. Bull Korean Chem Soc [Internet]. 2013 Nov 20 [cited 2024 Feb 1];34(11):3381-3386. Available from: https://doi.org/10.5012/BKCS.2013.34.11.3381
7. Fu DS, Wu PP, Zhong XD, Liu Q, Luo HD, Li YQ. A Simple Synchronous Fluorescence Approach for Rapid and Sensitive Determination of Rhodamine B in Chilli Products. Food Anal Methods [Internet]. 2015 Jan [cited 2024 Feb 1];8(1):189-194. Available from: http://link.springer.com/10.1007/s12161-014-9891-x
8. Huang Y, Wang D, Liu W, Zheng L, Wang Y, Liu X, *et al.* Rapid screening of rhodamine B in food by hydrogel solid-phase extraction coupled with direct fluorescence detection. Food Chem [Internet]. 2020 Jun [cited 2024 Feb 1];316:126378. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0308814620302363
9. Liang F, Jin D, Ma P, Wang D, Yang Q, Song D, *et al.* Rapid Determination of Rhodamine B in Chili Powder by Surface-Enhanced Raman Spectroscopy. Anal Lett [Internet]. 2015 Aug 13 [cited 2024 Feb 1];48(12):1918-1929. Available from: http://www.tandfonline.com/doi/full/10.1080/00032719.2014.1003428
10. Liu X, Bai B, Zhou Q, Yu Y, Ji S. Grafting of Molecularly Imprinted Polymers on to Carboxyl-Modified Multiwalled Carbon Nanotubes for the Extraction of Rhodamine B from Dried Chili Powder. Anal Lett [Internet]. 2013 Nov 2 [cited 2024 Feb 1];46(16):2583-2596. Available from: http://www.tandfonline.com/doi/abs/10.1080/00032719.2013.803252
11. Qi P, Lin Z, Li J, Wang C, Meng W, Hong H, *et al.* Development of a rapid, simple and sensitive HPLC-FLD method for determination of rhodamine B in chili-containing products. Food Chem [Internet]. 2014 Dec [cited 2024 Feb 1];164:98-103. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0308814614007407
12. Song S, Lin F, Liu L, Kuang H, Wang L, Xu C. Immunoaffinity removal and immunoassay for rhodamine B in chilli powder. Int J Food Sci Technol [Internet]. 2010 Dec [cited 2024 Feb 1];45(12):2589-2595. Available from: https://ifst.onlinelibrary.wiley.com/doi/10.1111/j.1365-2621.2010.02445.x
13. Wang M, Nie X, Tian L, Hu J, Yin D, Qiao H, *et al.* Rhodamine B in spices determined by a sensitive UPLC-MS/MS method. Food Addit Contam Part B [Internet]. 2019 Jan 2 [cited 2024 Feb 1];12(1):59-64. Available from: https://www.tandfonline.com/doi/full/10.1080/19393210.2018.1548504
14. Zhou W, Fang X, Qiao Q, Jiang W, Zhang Y, Xu Z. Quantitative assessment of rhodamine spectra. Chin Chem Lett [Internet]. 2021 Feb [cited 2025 Jul 1];32(2):943-946. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1001841721000577