

# Rapid and Sensitive Determination of Rhodamine B in Cosmetics

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## Key Words

Fluorescence Detection, Dye, HPLC, Acclaim 120 C18 Column

## Goal

To develop an efficient high-performance liquid chromatography (HPLC) method for the rapid and sensitive determination of rhodamine B in cosmetics

## Introduction

Rhodamine B, a brightly colored synthetic pigment (structure shown in Figure 1), has been extensively used as a dye in many industries, including those that produce paper, paints, textiles, leather, and porcelain. Long-term contact with rhodamine B may cause cancer and birth defects; thus, it has been prohibited as a food additive.<sup>1,2</sup> The addition of rhodamine B to cosmetics also may be linked to the potential threat of cancer and birth defects in humans, thereby creating the need to establish efficient methods for its sensitive and rapid determination in cosmetics.<sup>3</sup>

## Equipment, Software, and Consumables

- Thermo Scientific™ Dionex™ UltiMate™ 3000 Dual Rapid Separation LC (RSLC) system, including:
  - DGP-3600RS Dual-Gradient Pump (P/N 5040.0066)
  - SRD-3600 Integrated Solvent and Degasser Rack (P/N 5035.9230)
  - WPS-3000TRS Wellplate Sampler, Thermostatted (P/N 5840.0020), with a 25 µL sample loop (P/N 6820.2415) and a 25 µL syringe
  - TCC-3000RS Thermostatted Column Compartment (P/N 5730.0000)
  - FLD-3400RS Fluorescence Detector with Dual-PMT (without Flow Cell, P/N 5078.0025)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, version 7.1 or above
- Fisher Scientific™ CPXH Series Digital Ultrasonic Baths (P/N 15-337-410)
- Thermo Scientific™ Target2™ Nylon Syringe Filters, 0.45 µm, 30 mm (P/N F2500-1)

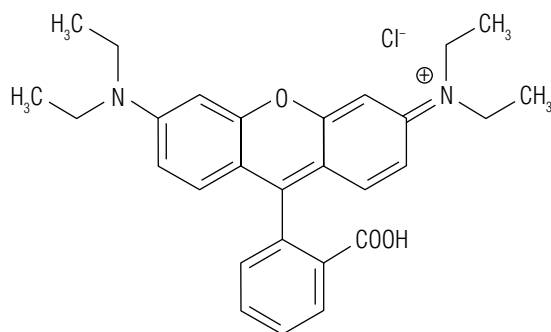


Figure 1. Structure of rhodamine B.



Table 1. Preparation of calibration standards.

Stock Standard of Rhodamine B for Calibration	Volume of Stock Standard of Rhodamine B for Calibration (mL)	Volume of DI Water (mL)	Final Volume of Calibration Standard (mL)	Final Concentration of Calibration Standard (µg/L)
Stock Standard 3 (1 µg/mL)	5	5	10	500
	2	8		200
	1	9		100
Stock Standard 4 (100 µg/L)	2.5	2.5	5	50
	1	4		20
	0.5	4.5		10
	0.25	4.75		5
	0.1	4.9		2
	0.05	4.95		1

### Reagents and Standards

- Deionized (DI) water, 18.2 M<sup>-1</sup>cm resistivity (generated by a Thermo Scientific™ Barnstead™ GenPure™ Pro water purification system, P/N 50131948)
- Methanol, 99.8%, HPLC Grade (Fisher Scientific P/N AC610090040)
- Acetonitrile, HPLC Grade (Fisher Scientific P/N AC610010040)
- Ammonium Acetate, HPLC Grade (Fisher Scientific P/N A639-500)
- Rhodamine B, ≥99%, Laser Grade (Fisher Scientific P/N AC41900-0010)

### Preparation of Standard Solutions

#### Stock Standard 1

Dissolve 0.01 g of rhodamine B standard in 10 mL of DI water. The concentration of Stock Standard 1 will be 1000 µg/mL.

#### Stock Standard 2

Dilute 200 µL of Stock Standard 1 to 10 mL with DI water. The concentration of Stock Standard 2 will be 20 µg/mL.

#### Stock Standard 3

Dilute 0.5 mL of Stock Standard 2 to 10 mL with DI water. The concentration of Stock Standard 3 will be 1 µg/mL.

#### Stock Standard 4

Dilute 1.0 mL of Stock Standard 3 to 10 mL with DI water. The concentration of Stock Standard 4 will be 100 µg/L.

### Standard Solutions for Calibration

For calibration, prepare nine working standard solutions with different concentrations by diluting the proper amount of the stock standard solutions with DI water. The volumes of each solution needed to make the calibration standards are shown in Table 1.

### Preparation of Samples

Three cosmetic samples—nail polish, pancake makeup, and lip gloss—were purchased from a beauty shop and a supermarket in Shanghai, People's Republic of China.

For pancake makeup and lip gloss, add 5 mL of DI water to 2 g of sample in a 10 mL conical flask with stopper. After extracting in an ultrasonic bath for 30 min, filter through 0.45 µm syringe filters prior to injection.

For nail polish, add 5 mL of DI water and 20 µL of 500 µg/L rhodamine B standard to 2 g of sample in a 10 mL conical flask with stopper. After extracting in an ultrasonic bath for 30 min, filter through 0.45 µm syringe filters prior to injection. The spiked concentration of rhodamine B in the nail polish sample will be 5 ng/g.

### Chromatographic Conditions

Column:	Thermo Scientific™ Acclaim™ 120 C18 Analytical, 3 µm, 3.0 × 150 mm (P/N 063691)
Mobile Phase:	Acetonitrile/100 mM ammonium acetate (dissolve 7.708 g of ammonium acetate in 1 L of DI water without pH adjustment), 40:60 (v/v)
Flow Rate:	0.5 mL/min
Injection Volume:	1 µL (partial-loop injection)
Temperature:	30 °C
Detection:	Fluorescence, excitation at 550 nm, emission at 580 nm

## Results and Discussion

### Optimization of Chromatographic Conditions

Different mobile phases—acetonitrile/water, methanol/water, acetonitrile/100 mM ammonium acetate, and methanol/100 mM ammonium acetate—were evaluated for the separation of rhodamine B using an Acclaim 120 C18 column. The rhodamine B peak tailed when water was paired with the organic solvent in the mobile phases. Peak symmetry was improved by substituting 100 mM ammonium acetate for water. Compared to methanol, acetonitrile yielded better peak symmetry and faster separation of rhodamine B. Therefore, an acetonitrile/100 mM ammonium acetate mobile phase was used in this work. In addition, fluorescence detection was used because of its high selectivity and response for rhodamine B.

### Reproducibility, Linearity, and Detection Limit

Short-term method reproducibility was estimated by making seven consecutive injections of a calibration standard with a concentration of 10  $\mu\text{g/L}$  rhodamine B. The retention time reproducibility RSD was 0.09 and the peak area reproducibility RSD was 0.75, demonstrating good short-term precision for this HPLC method.

Calibration linearity for fluorescence detection of rhodamine B was investigated by making three consecutive 1  $\mu\text{L}$  injections of a standard prepared at nine different concentrations (i.e., 27 total injections). Linearity (Figure 2) was observed from 2 to 1000  $\mu\text{g/L}$  when plotting concentration versus peak area. The linear regression equation was  $A = 288.02c - 2261$ , where  $A$  represents peak area,  $c$  represents concentration of analyte, and the coefficient of determination was 0.9995. This calibration curve was used to quantify rhodamine B in the cosmetic samples.

Seven replicate injections of a rhodamine B standard with a concentration of 2  $\mu\text{g/L}$  were used for estimating the method detection limit (MDL) using a signal-to-noise ratio = 3. The measured MDL of rhodamine B was 0.5  $\mu\text{g/L}$ .

### Analysis of Cosmetic Samples

Rhodamine B was not found in the pancake make-up and lip gloss samples, but 13.5 ng/g was detected in the nail polish sample. Figure 3 shows chromatograms of an unspiked nail polish sample and the same sample spiked with rhodamine B. To judge method accuracy, three injections of the nail polish sample spiked with a 5 ng/g of rhodamine B standard were made. The average recovery was 96%.

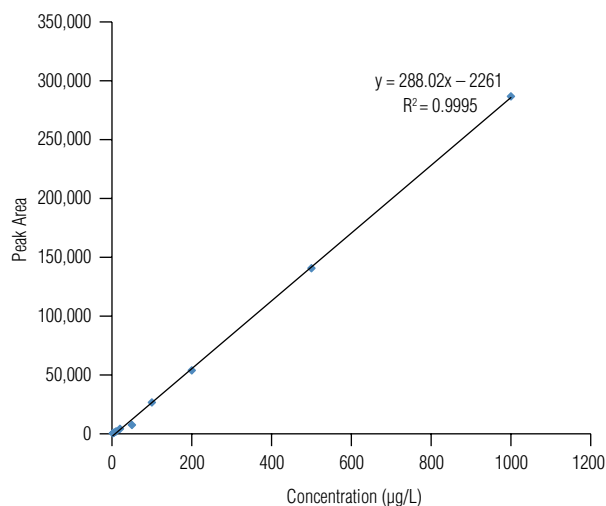


Figure 2. Calibration curve for rhodamine B.

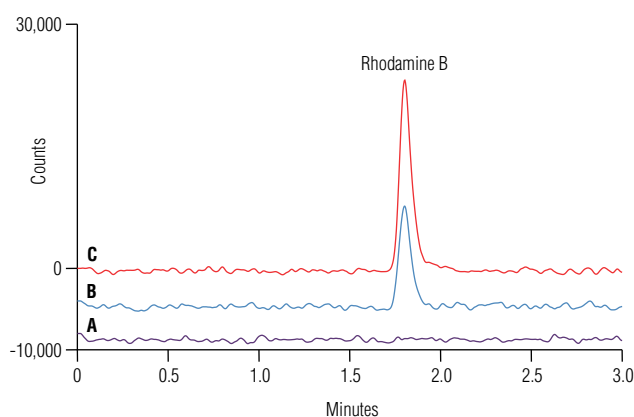


Figure 3. (A) A blank, (B) an unspiked nail polish sample, and (C) the same sample spiked with 5 ng/g of rhodamine B standard.

## Conclusion

This work describes an efficient HPLC method that uses fluorescence detection to achieve a rapid and sensitive determination of rhodamine B in cosmetics. The advantages of this approach include good method reproducibility and a wide linear calibration range.

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