Analytical Methods

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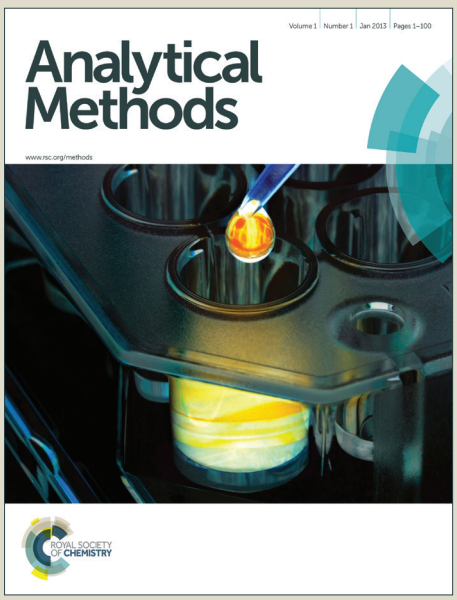

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Determination of rhodamine B in lipsticks by high performance liquid chromatography after extraction with AOT reversed micelles

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Abstract

In this study, we investigated the possibility of reverse micelles which were used for the determination of rhodamine B in lipsticks followed by high performance liquid chromatography (HPLC). The reverse micelle of surfactant sodium 1,4-bis (2-ethylhexoxy)-1,4-dioxobutane-2-sulfonate (AOT) was formed in n-hexane. Firstly, the samples were preprocessed by reverse micelle extraction, and then rhodamine B was extracted from the mixture with methanol by back-extraction. The effects of different parameters including concentration of surfactant, pH, amount of potassium chloride, ultrasonic time and type of organic solvents were evaluated and optimized during reverse micelles formation. The limit of detection of rhodamine B was 0.01 μg g^{-1}. The intra-day and inter-day precisions expressed as relative standard deviations were 3.6% and 4.5%, respectively. The recovery of rhodamine B obtained was 95.0%. The reverse micelles method proposed for the determination of rhodamine B in real samples of lipsticks has the advantages of simplicity, speed, high precision, low detection limit and the use of inexpensive equipment.

Keywords Rhodamine B; Lipstick; Reverse micelles; High performance liquid chromatography

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1. Introduction

Rhodamine B, 9-(2carboxyphenyl)-3,6-bis (diethylamino) xanthylium chloride, is broadly applied as a fabric dye and a pigment in drug and cosmetic preparations.[1] Rhodamine B with the chemical structure shown in Fig. 1, belongs to the class of xanthenes dyes, a basic red cationic dye which is highly soluble in water, methanol and ethanol. It is an analytical reagent for metals, a dyeing reagent in the cell fluorescence,[2] a tracing agent in water pollution studies and a color marker in herbicide sprays, colored glass, dyeing silk, wool, jute, leather and cotton.[3, 4] Rhodamine B is harmful if swallowed by human beings and animals, and causes irritation to the skin, eyes and respiratory tract.[5] As a result of its multiple effects on the human health, some countries had legislated and forbidden its applications.[6] In USA, the ‘‘Colours in Food Regulations’’ has classified rhodamine B as illegal dye. Thus, due to the hazardous nature of rhodamine B, it was considered worthwhile to make efforts to develop a simple method for the determination of rhodamine B in different samples.

Cosmetics are widely used as beauty products or for protecting skin from outer stimuli. In particular, cosmetic products directly contacting the skin could affect, not only skin, but also human health.[7] The emphasis on beauty in media and magazines promotes the use of cosmetics by adolescents and women.[8] Many studies reported an association between some ingredients of cosmetics and various health problems.[9] It is reasonable to assume that when a woman licks her lips, eats and/or drinks while wearing lipstick, she would ingest toxic substances from the lipstick. When these substances accumulate in the body over time, it may even cause cancer. The Food and Drug Administration (FDA) has now regulated the use of rhodamine B in the cosmetic industries, because of its carcinogenesis.[10]

Analytical methods for rhodamine B determination involve high performance liquid

chromatography (HPLC)[11] and spectrophotometry.[12, 13] The sample preparation methods include solid phase extraction (SPE),[14] dispersive liquid-liquid microextraction (DLLME)[15] and sol–gel-based immunoaffinity chromatography (IAC).[16] However, all of these methods are time-consuming and need a large sample volume. Therefore, the development of a simple, rapid, inexpensive and sensitive analytical method for analyzing rhodamine B in different matrixes, such as lipstick was necessary.

Reverse micelles having a number of technological applications have attracted considerable attention in recent years. When surfactant dissolves in organic solvent and the concentration is above the critical micelle concentration (CMC), it can form micelles in the organic phase, known as reverse micelles.[17] Therefore, reverse micelles are aggregates of surfactant monomers formed in a non-polar solvent. The polar head groups of the surfactants point inward and the hydrocarbon chains point toward to the non-polar medium.[18, 19] A common surfactant used to form reverse micelles is AOT. The reverse micelles formed with this surfactant can solubilize a large quantity of water in a wide range of non-polar solvents.[20] In reverse micelles, the main driving forces responsible for the solute distribution between the organized assembly and the organic medium are considered to be hydrophobic effects, hydrogen bonding interactions and electrostatic interactions.[21] The polar solvent molecules in reverse micelles are confined to the nanometer-scale polar-solvent pools of the reverse micelles. It is suggested that such polar solvent molecules behave differently from the pure solutions as a result of the specific interactions and confined geometries.[22] Reverse micelles produce polar–nonpolar interfaces and nano-sized locations for the fluorophores that are soluble in polar solvents.[23] This kind of reverse micelles influences the properties of many dyes.

In the previous studies, few researches were reported for the determination of rhodamine B in lipstick. Soylak et al. described a method based on coupling of solid phase extraction with UV

visible spectrophotometry for analyzing rhodamine B in lipstick.[14] However, reverse micelles extraction has been not previously investigated for determination of rhodamine B in cosmetics. In present study, the lipstick samples were preprocessed by using surfactant AOT and n-hexane during reverse micelle extraction, and then rhodamine B was extracted from the mixture with methanol by back-extraction. The effect of extraction conditions has been studied.

2. Materials and methods

2.1. Materials

The lipstick samples were purchased from a cosmetics store (Shantou, China). Rhodamine B was purchased from Aladdin (Shanghai, China). The stock mixed standard solution of rhodamine B (100 μg mL^{-1}) was prepared by dissolving an appropriate amount of these compounds in distilled water. The solution was stored in a refrigerator. The working standard solutions at different concentrations were obtained by diluting the stock standard solution with water. Spiked samples were gained by adding 1.0 mL mixed standard solution of rhodamine B to 0.5 g lipstick samples, shaking to mix evenly and drying with nitrogen.

AOT (Sigma >99% purity) were purchased from Aladdin (Shanghai, China). It was kept in a refrigerator. Various concentrations (v/v) of surfactant solutions were prepared by weighing appropriate amounts of the surfactant and directly dissolving the surfactant in n-hexane. Isooctane, cyclohexane and n-hexane were obtained from Kermel (Tianjin, China). Potassium chloride, sodium hydroxide and hydrochloric acid were of analytical grade and purchased from Kermel (Tianjin, China). The chromatographic grade methanol was obtained from Fisher (Pittsburgh, PA, USA). High purity water was obtained from a Milli-Q Water System (Millipore, Billerica, MA, USA). All vessels used for trace analysis were washed with methanol and distilled water before use.

2.2 Apparatus

Chromatographic analysis was performed with a LC-15C high performance liquid chromatograph (Shimadzu, Kyoto, Japan) comprising a SPD15C UV detector. A Zorbax SB-C18 column (150 mm × 4.6 mm I.D., 5 μm) (Palo Alto, CA, USA) was used as analytical column. A KQ5200E ultrasonic apparatus (Kunshan Instrument, Kunshan, China) was used to extract rhodamine B from the samples. A SH-36 vortex mixer (Jintan, China) was used to mix the micellar solution. A SHA-B shaking table (Shengtang, Jintan, China) was used for the back-extraction procedure. A TG 16WS high-speed centrifugation (Changsha, China) was employed to accelerate the phase separation process.

2.3. Analytical procedures

2.3.1. Reverse micelle extraction procedure

The scheme of the whole extraction procedure is shown in Fig.2. Lipstick (0.5 g) was accurately weighed and placed in a 50 mL centrifuge tube, and 10 mL 4% (v/v) surfactant AOT solution dissolved with n-hexane was added. The mixture was blended adequately, and then placed in ultrasonic bath for 5 min to extract rhodamine B from the sample. Ten milliliter distilled water (pH=5) and 3g potassium chloride were added to the sample solution and dissolved with assistance of a vortex mixer for 5 min. After that, the mixture was centrifuged at 5000 rpm for 5 min to promote the phase separation. Thereafter, the solution was completely separated into two distinct phases, the upper phase was the volume of surfactant phase in non-polar solvent containing rhodamine B and the lower phase was the large volume of aqueous phase and lipstick matrix such as lanolin. The upper phase was sucked out using a syringe with long pinhead and was used for the next procedure.

2.3.2. Back-extraction procedure

Two milliliter methanol was added to the upper surfactant phase obtained by above procedure. After that, the mixture was shaken for 15 min at 35°C. The solution was allowed to stand for 2 min. Thereafter, the solution was completely separated into two distinct phases, the lower phase was the phase of enrichment. The phase of enrichment was sucked out using a syringe with long pinhead. After filtrated through a 0.45 μm membrane, 20 μL of the solution was injected into the HPLC system for analysis.

2.3.3. HPLC analysis

The determination of rhodamine B was carried out by HPLC-UV system. The mobile phase was methanol–water (75:25, v/v), and the flow rate was 1.0 mL min^{-1}. The detection wavelength was set at 550 nm and the column temperature was set at room temperature. The chromatograms of rhodamin B standard and the spiked lipstick sample are shown in Fig. 3.

3. Results and discussion

3.1 Optimization of the reverse micelle procedure conditions

In this study, rhodamine B was extracted from lipsticks by reverse micelle. Electrostatic, steric, and hydrophobic interactions between rhodamine B and reverse micelles are considered to be the driving forces for the diffusion of rhodamine B into the reverse micellar core.[24] A number of experiments under different conditions were performed. The main variables which affect the reverse micelle process, such as the concentration of surfactant solution, pH, amount of potassium chloride, ultrasonic time and type of organic solvents were studied. When a condition was changed, the other conditions were set at the optimum values.

3.1.1 Effect of surfactant concentration

In reverse micelle extraction, the most popular surfactant used thus far is AOT, because no

cosurfactant is required to form stable entities with a minimum interfacial tension between water and organic phase. The surfactant AOT is used to form reverse micelles and microemulsions because it has a great ability to solubilize large amount of water in various organic solvents.[25, 26] As the concentration of surfactant increases, the number of reverse micelle goes up, thereby increasing the solubility of rhodamine B. However, when the concentration of surfactant is getting too high, it is possibly to form more complex aggregates in solution, meanwhile increases the difficulty of the back-extraction process. Therefore, we should choose the optimum surfactant concentration to reach the maximum recovery of rhodamine B. The effect of surfactant concentration on the recovery was studied in the range of 1% − 6% (v/v). According to the results (Fig. 4a), AOT was found to have higher recovery when surfactant concentration reached 4% (v/v), and no significant difference was observed above 4%. Consequently, 4% (v/v) was chosen as the optimum surfactant concentration with obtaining recovery of 98.5%.

3.1.2 Effect of pH

The pH of solution is a significant factor which affects the extraction performance of the analytes with ionizable groups during reverse micelle process. The maximum extraction efficiency should be achieved when the ion pairs formed between the positively charged dyes and the negatively charged surfactant AOT. The pH is the factor which influences the ionic form of target analyte and surfactant. In order to locate the optimal pH range for the quantitative rhodamine B extraction, experiments were performed to vary this parameter. Each desired pH value was obtained by the addition of HCl and/or NaOH solution. The effect of pH on the extraction of rhodamine B in lipsticks was studied in the range of 1-12. As can be seen from Fig. 4b, it appears that the recovery was initially increased by rising pH up to 5 and after this, the recovery remained approximately constant in the pH range of 5-7. When the pH value is higher than 7, the zwitterionic form of

rhodamine B in water causes a decrease in the extraction of rhodamine B ions into the reverse micelle. Thus, pH 5 was chosen as the optimum value with obtaining recovery of 94.9% for subsequent experiments.

3.1.3 Effect of salt addition

Generally, addition of salts enhances the extraction of analytes, because the salting-out effect can reduce the solubility of the analytes in water and forces more analytes into the organic phase. Adding of salts with appropriate concentration has obvious effect on the surface activities and the critical micelle concentration (CMC) decreases, while the lowest surface tension of the aqueous solution increases.[27] But too much of salts will have the opposite effect. The influence of potassium chloride concentration on the extraction recovery was evaluated by varying the range from 5% to 30%, in order to study the comparative effect of different amount of salts on phase separation. Potassium chloride was chosen because the larger K^+ ions are capable to cause higher solubilisation as compared to ions with smaller sizes such as Na^+.[28] K^+ cations are chaotropes which can break water structure.[29] The results (Fig. 4c) showed that the recovery of rhodamine B increased with the increase of the potassium chloride concentration from 5% to 15%, and no significant difference between 15% and 25%. The recovery decreased when the salt concentration was higher than 25%. Thus, 15% (m/v) potassium chloride with obtaining recovery of 93.7% should be chosen for the satisfied recovery.

3.1.4 Effect of ultrasonic time

Ultrasonic apparatus was used to assist the extraction of rhodamine B in lipsticks. The effect of ultrasonic time on the recovery of rhodamine B was studied by varying the extraction time from 1 to 20 min. The results illustrated in Fig. 4d showed that the recovery of rhodamine B increased dramatically as the ultrasonic time increase from 1 to 5 min, and when the ultrasonic time was

longer than 5 min, the recovery essentially unchanged. Thus, 5 min was evidently selected as the optimal ultrasonic time with obtaining recovery of 99.6%.

3.1.5 Effect of type of organic solvent

As mentioned in the literature, in the reverse micelle extraction, the organic solvents in combination with the surfactant AOT mainly are cyclohexane, [30] n-hexane, [31] isooctane,[32] and n-hexanol. [33] In these solvents, n-hexanol is not suitable for the back-extraction procedure by the reason of its dissolution in methanol. Cyclohexane, n-hexane and isooctane were used for the reverse micelle extraction of rhodamine B from lipstick samples. The influence of type of organic solvent was shown in Fig. 4e. In this work, n-hexane with obtaining recovery of 96.3% was selected because it can yield satisfactory recovery.

3.2 Optimization of the back-extraction procedure conditions

The extraction of rhodamine B using reverse micelles in lipsticks includes two steps: the first step is to extract rhodamine B from lipsticks into surfactant phase; the second step is to back-extract rhodamine B from the surfactant phase with methanol for conveniently HPLC analyses. For the second procedure, we inspect two main parameters including equilibrium time and temperature of back-extraction.

3.2.1 Effect of equilibrium time

The influence of mixing time on recovery of rhodamine B is substantial because it directly affects the contact between the two phases.[24] The results presented in Fig. 5a indicated that the recovery of rhodamine B increased with the shaking table time increasing from 5 min to 15 min, then kept constant from 15 min to 30 min. As a result, 15 min with obtaining recovery of 92.9% was selected in further experiments.

3.2.1 Effect of equilibrium temperature

The recovery increases as the temperature increases. The extraction result is not satisfactory

when shaking table temperature gets too low. However, excessively high temperature may lead to the decomposition of analytes. Therefore the influence of temperature on the recovery of rhodamine B was also investigated. Fig. 5b showed that the recovery of rhodamine B increased with temperature rising from 20°C to 35°C, and then it increased slowly when the shaking table temperature changed from 35°C to 45°C. Considering the matter of energy cost, the equilibrium temperature was set at 35°C with obtaining recovery of 90.1%.

3.3 Analytical performance

Chromatographic peak area was used for the quantification of the extraction of rhodamine B from lipsticks. Calibration graphs were obtained by plotting the peak area (y) versus concentration (x). The linearity obtained was in the range of 0.01-10 μg mL^{-1}, and the regression equation and correlation coefficients were as follows: $y=1.08\times10^{5}x+2.88\times10^{3}$, $R^2= 0.9995$. The limit of detection (LOD), defined as the concentration that yielded an S/N ratio of higher than or equal to 3, and the limit of quantitation (LOQ), defined as the concentration that yielded an S/N ratio of higher than or equal to 10, were determined by the analysis of the blank lipstick sample. LOD value was 0.01 μg g^{-1} and LOQ value was 0.033 μg g^{-1}. The intra-day and inter-day precisions, as well as the accuracies, were evaluated. The intra-day precision was determined by analyzing rhodamine B under the optimal conditions six times on the same day. The inter-day precision was evaluated by determining rhodamine B under the optimal conditions once a day in six consecutive days. The intra-day and inter-day precisions expressed as relative standard deviations (RSDs) were 3.6% and 4.5%, respectively. The mean recovery of rhodamine B obtained was 95.0%.

The analytical results of the proposed method were compared with those of other methods used in the literatures for analyzing rhodamine B, and the results were summarized in Table 1. As it can be seen from the table, the LODs, recoveries and precisions obtained by the proposed method were similar to other methods.[11, 12, 14-16] The results indicated that the method of reverse micelles

extraction was feasible for the separation of rhodamine B in lipsticks. This is a method with good potential, but much work has yet to be done to demonstrate its advantages of simple, rapid, inexpensive and with good selectivity

4. Conclusions

A simple and selective reverse micelle extraction procedure for rhodamine B in cosmetics like lipsticks has been reported. The use of AOT and n-hexane reverse micellar system provided a simple method to extract rhodamine B in lipsticks. Moreover, the recovery of rhodamine B was very high. The recommended procedure can be shown the wide range linearity, with high sensitivity resulting adequate for the quality control and routine analysis of lipsticks. This methodology has demonstrated potentiality in its application, it could be applied to the determination of rhodamine B present in other cosmetics products such as eye shadows, rouge and other types of cosmetic samples. Likewise, we are working in the development of a new application for the determination of rhodamine B in foods and beverages.

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Figure captions:

Figure 1. Chemical structure of rhodamine B.

Figure 2. The scheme of the whole extraction procedure.

Figure 3. Chromatograms of rhodamine B: (a) standard, (b) spiked lipstick sample (5 μg g^{-1}.).

Figure 4. Effect of AOT concentration (a), pH (b), concentration of potassium chloride (c), ultrasonic time (d), and type of organic solvents (e) on the recoveries of rhodamine B. (n=3).

Figure 5. Effect of equilibrium time (a) and temperature (b) on the recoveries of rhodamine B. (n=3).

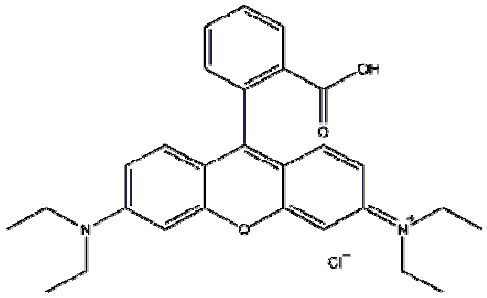

Figure 1

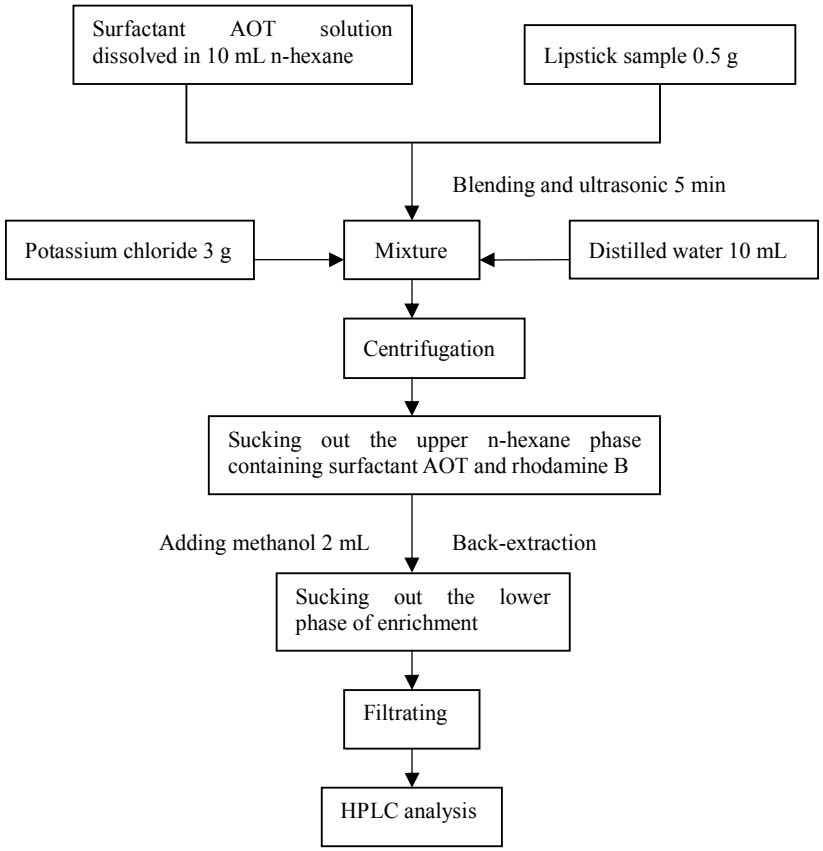

Figure 2

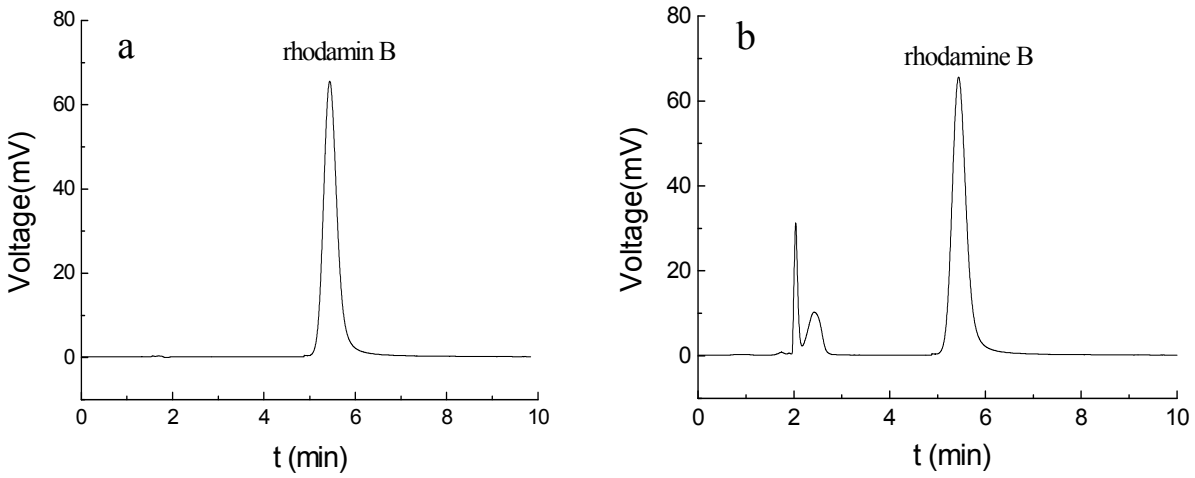

Figure 3

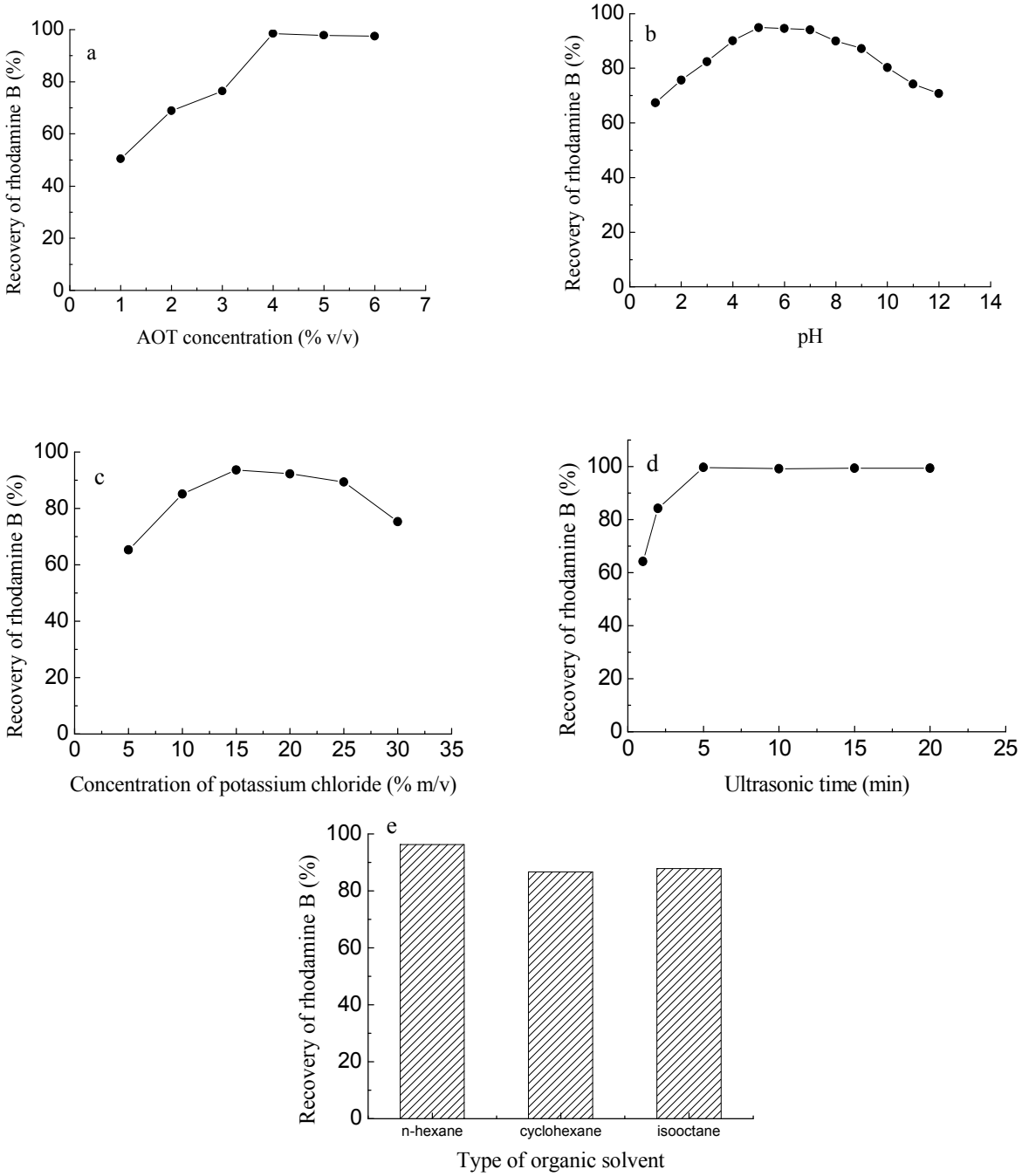

Figure 4

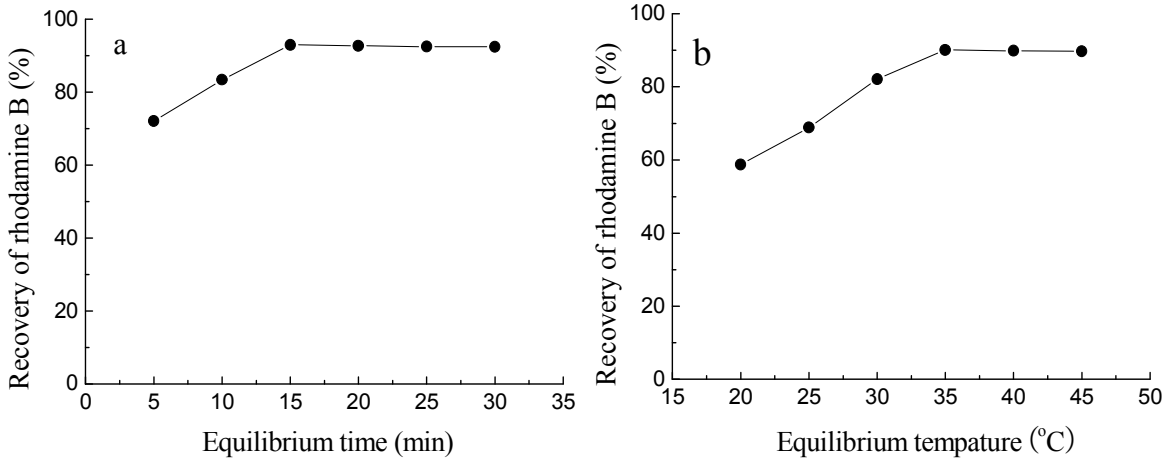

Figure 5

Table 1 Comparison of the proposed method with other methods used in the literatures

Detection technique	Sample preparation	Sample matrix	Linear range	LOD	RSD	Recovery	References
HPLC – FLD	Solid phase extraction	Water	0.5-1000 ng mL^{-1}	0.0015 ng mL^{-1}	6-10%	61-90%	[11]
Uv-visible-spectrophotometry	Ionic liquid-based dispersive liquid-liqud microextraction	Water	5-100 ng mL^{-1}	1.05 ng mL^{-1}	1.3%	-	[12]
Uv-visible-spectrophotometry	Solid phase extraction	Soft drink, waste water and lipstick samples	250-3000 ng mL^{-1}	3.14 ng mL^{-1}	5.0%	96-118%	[14]
Uv-visible-spectrophotometry	Dispersive liquid-liquid microextraction	Red wine and ice black tea	5-450 ng mL^{-1}	1.32-1.93 ng mL^{-1}	<4.5%	88.1-111.6%	[15]
HPLC–MS	Sol-gel-based immunoaffinity chromatography	Chilli powder	0.1-0.5 ng g^{-1}	1.0 ng g^{-1}	<9.5%	65.4-71.7%	[16]
HPLC-UV	Reverse micelle extraction	Lipsticks	0.01-10 μg mL^{-1}	0.01 μg g^{-1}	3.6-4.5%	95.0%	This work

"-" No reported; FLD, Fluorescence detection; MS, Mass spectrometry