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Short communication

Simultaneous determination of Aloe-emodin and Rhein by synchronous fluorescence spectroscopy

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Abstract

A simple sensitive and quick assay to simultaneously determine Aloe-emodin (AE) and Rhein (RH) has been described based on their natural fluorescence. Due to their similar molecule structures, it is difficult to analysis and determine their contents by conventional fluorometry. Overlapping of fluorescence spectra was resolved by using synchronous fluorometry. The synchronous spectrum, maintaining a constant difference of $\Delta\lambda = 25$ nm between the emission and excitation wavelengths, has been selected as optimum to perform the determination. This method was applied to the simultaneous determination of AE and RH in simulated mixtures. Analytical recoveries range from 94.5 to 105.5% for AE and from 98.7 to 107.5% for RH. At the same time, the content of AE abstracted from *Cassitora* L. was successfully analyzed. The results were satisfactory.

Keywords: Aloe-emodin; Rhein; Synchronous fluorometry; *Cassitora* L.; Addictive principle

1. Introduction

Aloe-emodin (AE) and Rhein (RH) are the useful ingredients that extensively exist in plants such as Aloe, *Cassitora* L., Rhubarb and so on. Aloe, which is rich in AE, is widely used in cosmetic, medical, cosmetology, health care and food fields. They can be used as prescription items for a variety of medical conditions in clinic [1]. Their therapeutic action and toxicity are still the subject of considerable research and it is necessary to control the dose in biological fluid [2–4]. In literature [5–7], using phase solubility method, electroanalytical chemistry method and HPLC method to study their property and determining method, but applied synchronous fluorometry to determine the amount of RH and AE simultaneously have not been reported.

In fluorometric methods, high sensitivity and selectivity are generally expected. However, problems of selectivity can occur in multicomponent analysis because of the overlap of the broadband

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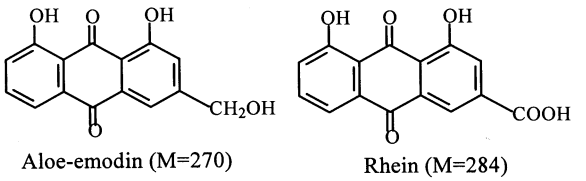

Fig. 1. The structures of AE and RH.

spectra observed. Specificity is a particular problem in the determination of fluorescent drugs. Synchronous fluorometry has been found to have several advantages such as simple spectra, high selectivity and low interference etc. It has attracted attention of many researchers [8–10] and developed rapidly since it was firstly proposed by Lloyd [11]. Synchronous fluorometry was classified constant-wavelength, variety-angle and constant-energy according to different scanning modes of monochromators. At present, constant-wavelength method is used most extensively, that is to maintain a constant difference wavelength between the emission and excitation wavelengths. Their structures can be seen from Fig. 1. Being anthraquinone derivatives, AE and RH have similar chemical properties. Their fluorescent spectra overlap considerably so that the conventional fluorometric method does not permit simultaneous determination of these compounds. This problem has been resolved by synchronous fluorescence spectroscopy (SFS) [16].

In the method described here, AE and RH in simulated mixture were determined directly and simultaneously by synchronous fluorometry and addictive principle. The developed method can obtain good precision and sensitivity and recovery. The content of AE distilled from *Cassitora* L. was successfully determined.

2. Experimental section

2.1. Apparatus

All fluorescent measurements were carried out on a RF-540 recording spectrofluorometer (Shimadzu, Kyoto, Japan), equipped with a xenon lamp source and 1.0 cm quartz cells. A UV-265 recording spectrophotometer (Shimadzu, Kyoto, Japan) equipped with 1.0 cm quartz cells was used for the UV spectrum scanning and determination experiments. All pH measurements were made with a pHs–3 digital pH–meter (Shang Hai Lei Ci Device Works, Shanghai, China) with a combined glass–calomel electrode. Data acquisition and data analysis were performed with the use of ORIGIN software version 5.0, running under WINDOW 98.

2.2. Reagents

Working standard solution of AE and RH were prepared by diluting in alcohol, containing 0.1 mg/ml. They were chemical comparison agents made in Biological Product Institution of Chinese Medicine. The sample solution of AE extracted from *Cassitora* L. was kept in alcohol. A solution of potassium chloride (0.5 M) and a buffer solution of Tris–(hydroxymetymethyl) aminol methane/hydrochloric acid (Tris–HCl) (pH 7.0; 0.2 M) were also used. All used chemicals were of analytical or higher grades. Doubly distilled and deionized water was used for the preparation of all solutions.

2.3. Procedure

A known volume of 0.1 mg/ml AE or RH solution 2.0 ml alcohol, 2.0 ml buffer solution of Tris–HCl (pH = 7.0; 0.2 M) and 2.0 ml KCl solution was placed into a 10.0 ml color comparison tube. The volume was increased to the mark with doubly distilled water and the solution was mixed and equilibrated at room temperature.

The conventional fluorescent spectra were scanned at fixed excitation wave 470 nm with emission wave 500–600 nm for AE and at fixed excitation 440 nm with emission wave 500–600 nm for RH. The synchronous fluorescence spectra was recorded by scanning both monochromators simultaneously at a 25 nm constant difference. The excitation monochromator was scanned from 445 to 545 nm. The emission monochromator was scanned from 470 to 570 nm. At the same time, the fluorescent intensity of AE and RH at 500 and 550 nm was recorded.

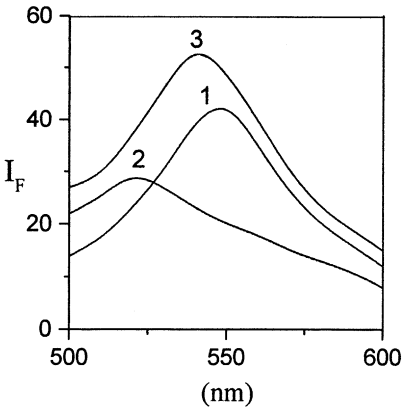

Fig. 2. Fluorescence spectra of AE and RH ($\lambda_{ex} = 470$ nm); 1, AE; 2, RH; 3, AE + RH.

3. Results and discussion

3.1. Spectra characteristic

Pure AE and RH solutions show maximum wavelengths at $\lambda_{ex/em} = 470/540$ nm and $\lambda_{ex/em} = 440/520$ nm, respectively. Fixed an excitation maximum at 470 nm, we scanned the fluorescence spectra of AE, RH and their mixture (see Fig. 2). The fluorescent spectra of AE and RH overlap considerably, this is because their structures are very similar. Fig. 3 shows synchronous spectra of AE, RH and a mixture of both compounds, maintaining a constant interval between the emission and excitation wavelengths $\Delta\lambda$, $\lambda_{em} - \lambda_{ex} = 25$ nm. Synchronous fluorometry made the spectral band of AE and RH narrow. The intensity values at wavelengths 500 and 550 nm are of adductive property.

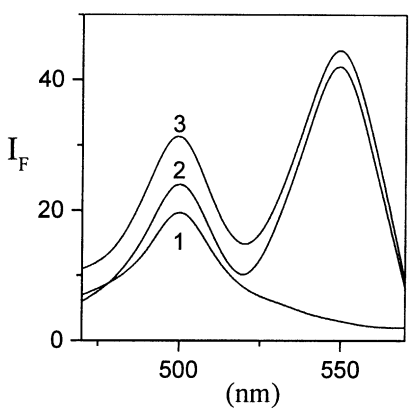

Fig. 3. Synchronous fluorescence spectra of AE and RH ($\Delta\lambda = 25$ nm); 1, AE; 2, RH; 3, AE + RH.

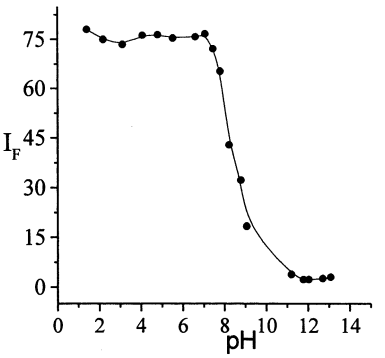

Fig. 4. Effect of acidity on the fluorescence intensity of AE.

3.2. Optimization of chemical variables

3.2.1. Effect of pH

Studies on the fluorescence intensity of pH of AE and RH in the pH range of 2.0–13.0 showed that the pH of the medium had a great effect on the fluorescence intensity (see Figs. 4 and 5). The results showed the optimum pH range for AE and RH was between 6.0 and 8.0.Therefore, a pH of 7.0 was fixed with the use of Tris–HCl buffer solution. As the volume of the buffer from 1.5 to 2.5 ml had little effect on the fluorescence intensity, 2.0 ml was adopted in subsequent experiments. The difference in the effect of the pH on

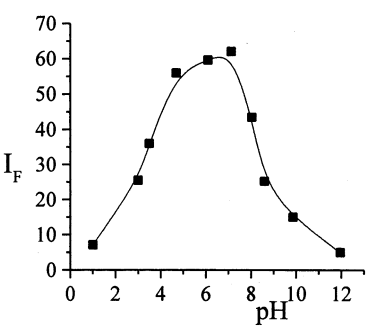

Fig. 5. Effect of acidity on the fluorescence intensity of RH.

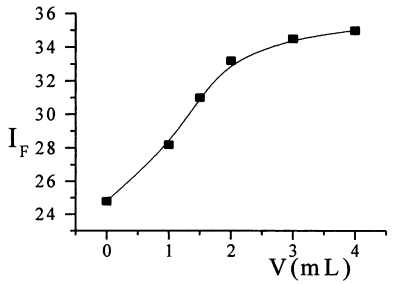

Fig. 6. Influence of ethanol concentration on the fluorescence intensity of AE.

the fluorescence of the RH and AE owe to their chemical structure (see Fig. 1). AE and RH show the property of monoacid and binary acid, respectively.

3.2.2. Effect of amount of alcohol

Since AE and RH are hydrophobic compounds, the amount of alcohol has great effect on their fluorescence intensity. The fluorescence intensity increased with an increase in the amount of alcohol upto 2.0 ml and then increased slowly thereafter (see Fig. 6). Thus, 2.0 ml was selected to ensure a sufficient solubility of the reagent throughout the experimental work.

3.2.3. Effect of ionic strength

The impact of ionic strength on the system was also studied by adding different amounts of 0.5 M KCl. The results showed that there was little effect on the fluorescence intensity of two drugs. Thus, 2.0 ml KCl was selected to maintain ionic strength 0.1 M.

3.2.4. Effect of time

The analysis solution was begun to be measured after 5 min. The fluorescent intensity remained constant for at least 4 h at room temperature.

3.2.5. Election of optimum $\Delta\lambda$

The optimum $\Delta\lambda$ value is very important in the process of synchronous fluorescence scanning. It can directly influence spectral shape, band width and signal value. For the selection of the optimum $\Delta\lambda$ value in the synchronous spectra for the resolution of the mixture, the following $\Delta\lambda$ values were taken into account 5, 10, 20, 25, 30, 40, 60 and 70 nm. When $\Delta\lambda$ is less than 10 nm, the spectra shape is irregular and the fluorescence intensity is very weak. When $\Delta\lambda$ is more than 40 nm, two peaks can not be separated completely. When $\Delta\lambda$ is between 10 and 40 nm, it appeared two distinct peaks with good shape. Then, maintaining $\Delta\lambda = 25$ nm, the synchronous fluorescence spectra were scanned at the conditions of $\lambda_{ex} =$ 445–545 nm and $\lambda_{em} = 470$–570 nm.

3.3. Determination of samples

3.3.1. Statistical parameters

Under the experimental conditions, there is a linear relationship between fluorescence intensity and two drugs' concentrations. The correlation coefficients and the regression equations were analyzed by using ORIGIN 5.0 software. On comparing the linear relation of fluorescence spectroscopy (FS) and SFS, SFS was found to be of better linear relationship and higher precision. It is feasible to analysis the amount of AE and RH.

The detection limits and the relative standard deviations as defined by IUPAC were obtained from series of ten blank solutions and standard solutions. All analytical parameters were summarized in Table 1.

3.3.2. Calculation of the constants of K^{AE}_{500}, K^{AE}_{550}, K^{RH}_{500}, K^{RH}_{550}

The method of SFS is the same as FS at low concentration. Its fluorescence intensity is expected to be linearly dependent upon the concentration of fluorophores. The following equation [12] is given:

$$F = 2.3\,Y_F I_0 \varepsilon b C \quad (1)$$

In this equation, F is the fluorescence intensity deducting the blank value, Y_F is the fluorescence quantum yield, I_0 is the intensity of emission light, ε is Moore absorbance index, b is the thickness of the cell. The above parameters are all constants, so the equation can be described as:

$$F = KC \quad (2)$$

Table 1
Analytical parameters

Parameters	AE	RH
The regression equations at wavelength 500 nm	$Y = 0.7357 + 34.9345X$	$Y = 0.4441 + 25.8992X$
The regression equations at wavelength 550 nm	$Y = 1.1407 + 121.147X$	$Y = 0.2467 + 2.6491X$
R_{FS}	0.9980	0.9577
R_{SFS}(500 nm)	0.9982	0.9994
R_{SFS}(550 nm)	0.9992	0.9978
R.S.D. (500 nm)	2.34%	1.56%
R.S.D. (550 nm)	1.87%	2.95%
Detection limit (500 nm) μg/ml	4.59×10^{-2}	$4.6.19 \times 10^{-2}$
Detection limit (550nm) μg/ml	1.33×10^{-2}	6.05×10^{-1}

According to the standard working curves, the K values of AE and RH at wavelengths 500 and 550 nm were obtained, respectively, as following values: $K_{500}^{AE} = 3.49 \times 10^3$ mg/ml, $K_{550}^{AE} = 1.21 \times 10^4$ mg/ml, $K_{500}^{RH} = 2.59 \times 10^3$ mg/ml, $K_{550}^{RH} = 2.65 \times 10^2$ mg/ml. For the mixture system of AE and RH, their fluorescence intensity at $\lambda = 500$, 550 nm is of linear relationship. The additive principle can be applied to this system.

$$F_{500} = K_{500}^{AE} C_{AE} + K_{500}^{RH} C_{RH} \quad (3)$$

$$F_{550} = K_{550}^{AE} C_{AE} + K_{550}^{RH} C_{RH} \quad (4)$$

The concentrations of C_{AE} and C_{RH} can be obtained from the simultaneous equations Eqs. (3) and (4). Their concentration unit is mg/ml.

3.3.3. Application of the method

The proposed method was applied to the determination of AE and RH in simulated solutions confected by a series of their standard solutions. The contents and the recoveries of AE and RH were obtained by using Eqs. (3) and (4). The results are illustrated in Table 2. About 100 g sample of *Cassia* L. was weighed and distilled according to the reference [13]. The final solution of AE was obtained and fixed up 100 ml with alcohol. The maximum of absorbance spectrum agrees with the standard solution. The content of AE determined according to the above process was 39.8 μg/g.

4. Conclusion

The simultaneous determination of AE and RH has been accomplished by synchronous fluorometry and its conditions optimized. The results obtained in this work allow us to conclude that the two components of the binary mixture are accurately determined by synchronous fluorometry. The narrowing of the emission band produced by the synchronous scanning considerably improved the resolution of the overlapping spectra. It was applied with good recovery rates.

Table 2
Determination results of recovery of AE and RH in simulated mixture ($n = 3$)

Number	Added (μg/ml)		Found (μg/ml)		Recovery (%)	
	AE	RH	AE	RH	AE	RH
1	8.0	80.0	7.62	84.4	95.3	105.5
2	12.0	100.0	12.9	98.7	107.5	98.7
3	20.0	60.0	18.9	62.8	94.5	104.6
4	40.0	120.0	38.4	121.6	96.0	101.3

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