Simultaneous Determination of Salicylic Acid, Gentisic Acid and p-Aminobenzoic Acid by Excitation-Emission Matrix Fluorescence Spectra Using PARAFAC

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An excitation-emission matrix spectrofluorimetric method based on PARAFAC for simultaneous determination of salicylic acid, gentisic acid and p-aminobenzoic acid is described. Satisfactory recoveries show that the method can solve the difficult problem of simultaneous determination on the condition of close overlap of the spectra and realize the intention of substituting "mathematical separation" for "chemical separation".

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Salicylic acid (SA) derivatives, especially the acetylated form, such as aspirin, have a widespread and frequent use for a variety of medical conditions. Monitoring of salicylates has been a field of active investigation in past several decades. Salicylic acid and gentisic acid (GA) are the major metabolites of aspirin. They may co-exist with p-aminobenzoic acid (PABA) in biological fluids that serve as an active pharmaceutical ingredient. A large number of published methods for the determination of salicylic and gentisic acids or gentisic acid and p-aminobenzoic acid are indicative of the problems encountered with their determination with regard to specificity, sensitivity and simplicity in some degree.

Among these techniques, high performance liquid chromatography is preferred but only a few methods provide a complete analysis.1 Besides this, spectrofluorimetric method based on their intrinsic fluorescence is the probably best option because of high sensitivity. However, due to the close overlap of their fluorescence spectra, conventional spectrofluorimetric technique could not be performed for their simultaneous determination without a prior separation step. Presently, synchronous fluorescence techniques are widely used for simultaneous determination of these substances.2,3,5,7

In this paper, a trilinear decomposition method which boasts the significant characteristic that "chemical separation" could be substituted by "mathematical separation" in certain degree is employed to deal with three-way fluorescent data obtained by excitation-emission spectrofluorimetric method. At the same time, combined with the idea of second-order calibration, a successful simultaneous determination of these three components is obtained with satisfactory recoveries.

Theory

Second-order data

Second-order data are usually generated from modern hyphenated instruments including GC-MS, LC-DAD, LC-MS and fluorometric apparatus with two variable parameters such as excitation-emission fluorescence spectroscope. In an excitation-emission fluorescence spectrum, for example, the emission at each measured wavelength changes with the excitation efficiency of a sample as the exciting wavelengths are scanned. Such second-order data of a few samples can be collected in a three-way data array $X$ that should follow a trilinear model depicted in Equation (1):

$$
X_{ijk} = \sum_{n=1}^{N} a_{in} b_{jn} c_{kn} + e_{ijk}
$$

where $N$ denotes the number of factors, or the total number of existing species, including sought-for component(s) as well as unexpected interferent(s) usually accounted as a part of the analytical background; $x_{ijk}$ is the element $(i,j,k)$ of the three-way response array of $X$, $X$ is dimensioned by $J$ excitation wavelengths, $K$ emission wavelengths and $I$ samples; $a_{in}$ is the element $(i,n)$ of an $I \times N$ matrix $A$ with relative concentrations of the samples on the $N$ factors; $b_{jn}$ is the element $(j,n)$ of an $J \times N$ relative sensitivity coefficient matrix $B$ in excitation mode; $c_{kn}$ is the element $(k,n)$ of a $K \times N$ relative sensitivity coefficient matrix $C$ in emission mode; and $e_{ijk}$ is the element $(i,j,k)$ of an $I \times J \times K$ three-way residual array $E$. In subsequent discussion, the matrices $A$, $B$ and $C$ will be called the relative concentration matrix, the relative excitation matrix and the relative emission matrix respectively.

Second-order calibration based on PARAFAC

With second-order calibration based on PARAFAC,5,7 there are three major steps:

Step 0: Decompose three-way data array $X$ with PARAFAC.

In this step, the number of factors, $N$, is firstly determined. In order to start the iteration process, the matrices $B$ and $C$ are initialized by producing them randomly. In the main iterative loop, the current estimates of $B$ and $C$ are employed to calculate the least-squares estimate of $A$. Subsequently, a new least-squares estimate of $B$ is calculated from the current estimates of $C$ and $A$. Then, the new least-squares estimate of $C$ is determined from the current estimates of $A$ and $B$.
loop is repeated until a predesignated maximum number of iterative cycles or the stopping criterion is met, which is:

\[ \sigma^{(m)} / \sigma^{(m-1)} \leq z \]  

(2)

Where \( \sigma \) is the loss function:

\[ \sigma = \frac{1}{3} \sum_{j=1}^{3} \sum_{k=1}^{B} \sum_{i=1}^{A} (x_{ij} - \bar{x}_j - \bar{a}_k - \bar{v}_i)^2 \]  

(3)

\( z \) is some arbitrary small value, usually \( 10^{-6} \).

Step 1: Locate the columns of A, B and C corresponding to the analyte of interest.

Step 2: With known real concentrations of the calibration samples, process the corresponding column of A to obtain the concentration(s) of the analyte(s) of interest in the predicted samples.

**Experimental**

A Hitachi F-4500 spectrofluorimeter equipped with a xenon discharge lamp of 150W and two monochromators for excitation and emission was used to collect the spectra. This spectrofluorimeter is provided with F100 fluorescence software for acquiring and processing the spectral data. All measurements took place in a standard 10mm (pathlength) quartz cell, thermostatically controlled at 24.0±0.5°C. The data analysis was carried out in the Matlab (version 5.0).

All experiments were performed with analytical-reagent grade chemicals and pure solvents. Re-distilled water was used throughout. Stock solutions of salicylic acid (Beijing Chemicals), genistic acid (China Pharmaceuticals Co.) and p-amino-benzoic acid (Aldrich Chem. Co.) were prepared by dissolving appropriate each acid in water. Working standard solutions were prepared by dilution with water.

A 0.5mol/L buffer solution of pH 7.0 was prepared by mixing appropriate amounts of sodium dihydrogenphosphate with sodium hydroxide.

According to the Table 1, solution of certain volume of each acid was transferred into 25-mL calibrated flask and 5.0 mL of buffer solution were added to the flask. The solution was then diluted to the mark with water. The first five samples were regarded as calibration samples and the remaining samples were predicted samples. Before the determination, all solutions were thermostated for more than 40 min.

The range of excitation and emission wavelengths was 265–350 nm, 305–500 nm, respectively. The intervals were both 5 nm. All the spectra were obtained for excitation and emission slit widths of 5.0 nm. The scan speed was 1200 nm min⁻¹.
Results and Discussion

Wavelength range

Recording the fluorescent spectra of the three acids, SA has a maximum at approximately 260 nm of excitation wavelength and 400 nm of emission wavelength, GA is highly overlapping with SA, which has a maximum at approximately 320 nm and 450 nm. PABA has a maximum at approximately 260 nm and 320 nm. Considering aforementioned facts, the ranges of scanning were fixed from 265 nm to 350 nm for excitation and 305 nm to 500 nm for emission. Recorded excitation-emission spectra of the three acids are presented in Fig.1. There is a Rayleigh scattering in right part. To deal with the problem, there are two approaches. One makes the real number of factors add one; The other approach is to compensate each response matrix by subtracting the measurement blank from the sample measurement. Two kinds of results will be given in this paper.

Factors affecting fluorescence intensity

Instability of temperature is one of the major factors influencing accuracy of results. It is therefore recommended that a thermostat is used, choosing a measurement temperature of 24 °C.

Pulgarin et al. studied the effect of pH on the fluorescence of SA, GA and PABA\(^{[5]}\). Taking into consideration their experiments, pH of 7.0 is chosen as the optimum pH for the measurements.

Simultaneous determination of SA, GA and PABA

When nothing was done to eliminate the Rayleigh scattering and using \(N=4\), the resolved concentration, excitation and emission profiles are shown in Fig.2–4. Their correlation coefficients between true profiles are listed in Table 5. Analytical recoveries range from 98.10% to 102.07% (mean 100.42%) for SA, from 98.81% to 101.8% (mean 99.01%) for GA and from 95.20% to 99.62% (mean 97.72%) for PABA.

When effect of Rayleigh scattering was compensated by measuring several blanks and subtracting the average measurement from the sample measurement, the 3-factor PARAFAC gave better results. On this condition, analytical recoveries range from 98.64% to 101.90% (mean 100.38%) for SA and from 96.83% to 99.15% (mean 98.16%) for GA and from 97.01% to 100.50% (mean 98.09%) for PABA. If the number of factor \(N\) is further increased (e.g. \(N=4\)), degenerate solution would appear. In other words, no matter what measure is taken, satisfied recoveries can be obtained.

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