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Sucrose as chiral selector for determining enantiomeric composition of phenylalanine by UV–vis spectroscopy and chemometrics

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Abstract

The determination of enantiomeric composition by partial least squares (PLS) modeling of UV–vis spectral data was investigated for samples of phenylalanine (phe) using sucrose as a chiral auxiliary. And a new data preprocess method, reference band normalization, was introduced to eliminate the spectral variations due to the changes of total concentration of phe. The determination coefficient (R^2) and the standard error of calibration set (SEC) of 13 standard samples are 0.9987 and 0.0128 respectively. The standard error of validation set (SECV) of 7 validation samples is 0.0049. The standard error of predict (SEP) of 6 blind samples for evaluating the robustness of the model is 0.0366. The regression model is robust to determine enantiomeric composition when total concentration varied. It is demonstrated that the reference band normalization is a convenient method of compensating for variations in total concentrations without knowing that in advance.

Keywords: Chiral analysis; Partial least squares; Sucrose; Enantiomer; Phenylalanine

The enantiomers often have distinctly different biologic activities. While one optical isomer of enantiomers may be beneficial to health, the other may be inactive or even toxic [1]. The determination of the enantiomeric composition of chiral compounds is a focus of chiral research [2]. Conventionally the analytical techniques used to determine enantiomeric composition of chiral compounds are high performance liquid chromatography [3], gas chromatography [4] and so on, which are time-consuming and laboursome. Whereas, UV–vis combined with PLS is a convenient and efficient way which we should take more concerned to.

As we all know, the UV–vis spectra of the enantiomers are the same in nonchiral media. But they may be different in chiral auxiliary due to the dissimilar interactions between enantiomer and chiral medium. Some researches on the determination of the enantiomeric composition were reported. Recent studies by Busch et al. have demonstrated that the molar fraction of four drug enantiomers can be determined due to guest–host. As a linear carbohydrate, sucrose is also a chiral compound. It is clearly demonstrated that sucrose can form complexes interaction [5] and produce diastereomeric interactions. The research on using sucrose as chiral selector to determine the enantiomeric compositions of amino acids by near-infrared spectrometry has been done by Tran [6]. The determination of

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enantiomeric compositions is also effective with the simple and inexpensive sucrose. Sucrose was used as a chiral selector to detect the molar fraction of R-metalaxyl and S-ibuprofen due to the UV spectrum [7].

In earlier works [8], the total concentration of all standard samples should be kept constant to eliminate the effect of variations when developing the multiple regression models. Reference band normalization is introduced to standardize the spectral of all samples. It is processed that the origin spectral at reference wavelength is divided by peak value of each spectrum. Specially, reference band is the highest peak of the whole spectrum of each sample.

The advantage of this normalization method is we need not know the total concentration of the sample and it can standardize the spectra to eliminate the concentration variation.

1. Experimental

Instrument: UV-vis spectrophotometer (Lambda 35, Perkin Elmer, USA), Quant+ V4.51 (Perkin Elmer, USA).
Sample: 6.0 mmol/L sucrose; 10.00 mmol/L L- and D-phe; D-phe with molar fraction from 0.00 to 1.00, 4.71 to 5.00 mmol/L; six blind samples 2.88, 3.41, 3.94, 5.77, 6.83, 7.88 mmol/L respectively.
Parameters: The wavelength range: 225–300 nm, quartz cell: 1.0 cm, spectral bandwidth: 1.0 nm.

2. Results and discussion

In this experiment, there are only three high purity components, D-phe, L-phe and sucrose compose the solution.

Since the background interference is tiny, we use 13 samples as calibration set, 7 as validation set and 6 as predict set when developing the model.

The absorbance of D-phe in sucrose solution is little higher than those in aqueous (Fig. 1a) since the stronger interaction formed between D-phe and sucrose. The stereoselective interactions of phe and sucrose are slightly different between two enantiomers (Fig. 1b). There may be a correlation between the spectra and the enantiomeric composition. However, it is difficult to use univariate calibration for analysis because of the small differences. This spectral discrepancy is the foundation of establishing multiple regression models. Accordingly, PLS method is used to reveal the relationship of the spectra and the molar fraction of D-phe.

The reference band normalization is a method to normalize the spectral data by the maximum value of each spectrum. It is processed that each individual spectrum divides its own maximum absorbance. The spectral data are initially normalized based on maximum peak height at 257.6 nm in our experiment. All samples are normalized in order to eliminate the variations of total concentrations. The differences of normalized spectra are expected to change only with the variations of molar fraction of D-phe isomer.

Fig. 2a is the spectra of standard samples after reference band normalization. It shows the 13 normalized calibration samples are corresponding to the molar fraction of D-isomer, that is, the spectra vary with enantiomeric composition of samples. The figure also indicates the sucrose can differentiate D-phe from L-phe and the different interactions with sucrose lead to the variation in spectra. After normalization the spectra are grouping and coincide with each other at

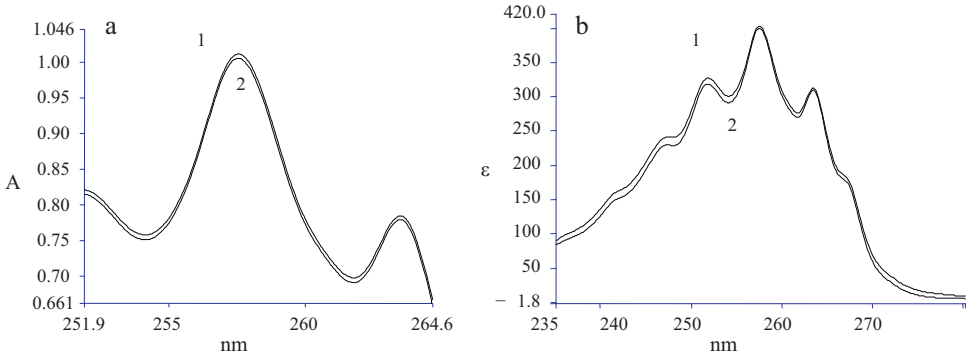

Fig. 1. The two spectra of D-phe in aqueous and sucrose solution with same concentration (a-1 sucrose; a-2 aqueous), concentration normalized UV spectra L- and D-phe in sucrose solution (b-1 D-phe; b-2 L-phe).