Simultaneous determination of ternary cephalosporin solutions by Raman spectroscopy

Chongyang Li (李重阳), Wang Lin (林 旺)*, Yangfan Shao (邵洋帆), and Yuanming Feng (冯远明)

Department of Biomedical Engineering, College of Precision Instrument and Optoelectronics Engineering, Tianjin Key Laboratory of Biomedical Detecting Techniques and Instruments, Tianjin University, Tianjin 300072, China

**Corresponding author: wlin@tju.edu.cn*

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Cephalosporins are widely used as veterinary and human antibiotics. However, cephalosporin abuse is harmful to human health and causes allergic reactions or antibiotic resistance. We investigate a method featuring Raman spectroscopy and chemometrics to quantify mixture solutions of three typical cephalosporins, namely, ceftriaxone, cefotaxime sodium, and cefazolin sodium. Partial least-squares regression models are built on spectral data that are preprocessed by various methods. With prediction relative errors within 5% and high correlation coefficients of 0.998, we demonstrate that Raman spectroscopy combined with multivariate analysis is feasible for use in the quantitative determination of cephalosporin solutions.

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Recent research has focused on the abuse of feed additives. Thousands of tons of antibiotics are used every year for therapeutic purposes or as growth promoters[1]. Cephalosporins represent a class of β-lactam antibiotics that work by inhibiting bacterial cell wall synthesis[2]. Considering the pharmacological importance of cephalosporin antibiotics and risks of developing side effects, rapid, simple, and non-destructive methods for the simultaneous determination of antibiotics in mixtures are an important necessity.

Determination of cephalosporins in solid binary mixtures has been conducted via infrared and Raman spectroscopy[3]. Cephalosporins in liquid binary mixtures, such as cephapirin and cefuroxime, have been assayed using first and second spectrophotometric methods[4,5]. Raman spectroscopy is an analytical technique based on the interaction of incident monochromatic radiation with the vibration energies of molecules. Compared to the other methods, Raman detection is relatively cheap, fast and easy for operation, non-destructive and requires little or no sample preparation. Since the band intensities caused by –OH stretching vibrations are weak in Raman spectra, water-rich samples may be directly analyzed by this technique[6]. In this case, Raman spectroscopy and some advanced techniques such as surface-enhanced and resonance Raman scattering, have been widely applied in modern analysis field[7–9]. We present a simple, rapid, and sensitive method for the simultaneous determination of aqueous ternary cephalosporin mixtures using Raman spectroscopy combined with multivariate analysis.

Three typical cephalosporins, namely, ceftriaxone (CRO), cefotaxime sodium (CTX), and cefazolin sodium (COL), were mixed together, and fifty-two samples were orthogonally designed to maximize the spectral information obtained. CRO, CTX, and COL concentrations ranged from 0.5 to 8 mg/mL. These concentrations are selected because they are far lower than the saturation concentrations reported and within the range expected in current production processes[10]. Raman spectra were measured by a QE65000-Raman scientific-grade spectrometer from Ocean Optics, Inc. The excitation laser featured a 785-nm wavelength and a 350-mW power. Excitation in this wavelength produces spectra with low background noise in aqueous biological systems[11]. All spectra were recorded from 200 to 2 000 cm^{-1} with a 120-s integration time. The spectra presented represent an average of three replicates.

Figure 1 shows the Raman spectra of the three solid analytes. Dominant bands attributed to cephalosporins appear from 1 585 to 1 645 cm^{-1}. The strong peak at 1 596 cm^{-1} in the COL spectrum can be assigned to the asymmetric stretching of COO-, besides the symmetric stretching occurs at 1 356 cm^{-1}. The peak for C-N-C stretch and CH_3 twisting is distinguishable at 997 cm^{-1}. The peak at 599 cm^{-1} can be assigned to N-H bending of amide. For the CRO solid sample, the lactam ring breathing vibration occurs at 757 cm^{-1}. And a shoulder at 1 236 cm^{-1} can be ascribed to the C=O stretching of unionized COOH. The peaks at 1 370 and 1 487 cm^{-1} are attributable to CH_2 bending and CH_3 deformation vibration, and the strong peak at 1 573 cm^{-1} is assignable to the C=N stretching vibration. The C=O stretching and the OH deformation of carboxylic acid show specific peaks at 1 403 and 1 356 cm^{-1} on the CTX spectrum, respectively. The C=C stretching of the cyclopentadiene ring is presented at 1 500 cm^{-1}, and the peak at 1 587 cm^{-1} is the C=C stretching of thiazine ring. The peak at 1 641 cm^{-1} in the CTX spectrum, as well as the peak at 1 627 cm^{-1} in the COL and CRO spectra, can be assigned to the C=O stretching vibrations of amide groups[3,12,13].

The initial calibration model was evaluated for outliers using principle components analysis (PCA). Over the spectral region studied, the first four principle components explained 98% of the variation in the data (PC1: 81%, PC2: 11%, PC3: 5%, and PC4: 1%). The first two PC scores among the 52 samples are plotted in Fig. 2, with two outliers marked. These outlier samples were removed from the dataset, and a partial least-squares

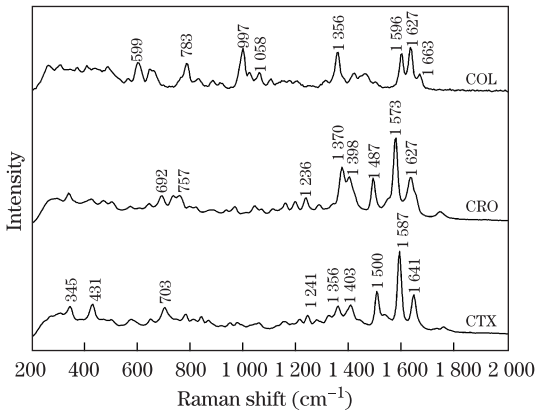

Fig. 1. Raman spectra of solid samples: COL, CRO, and CTX.

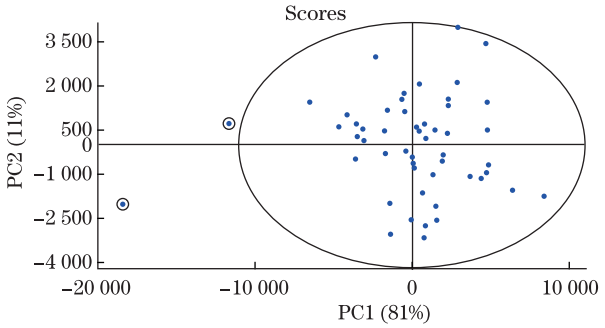

Fig. 2. PCA score plot of the Raman spectra of all of the samples.

(PLS) regression model was calculated accordingly.

Various preprocessing methods, including smoothing, first and second derivatives, multiplicative scatter correction (MSC)[14], standard normal variation (SNV)[15], baseline correction[16], and orthogonal signal correction (OSC)[17] were either applied alone or in combination. PLS factors, calibration and cross validation residual mean standard errors, and determination coefficients were used to describe the fitness of the models (Table 1). Models based on raw spectra resulted in good prediction coefficients and required only one or two more factors than the preprocessed data. Among the preprocessing methods tested, OSC preprocessing lowered the root-mean-square error of cross-validation (RMSECV) of the COL regression model by approximately 10%.

In the previous study, data preprocessing methods were analyzed to obtain optimal PLS regression models. The dataset was then split into calibration and prediction subsets containing 40 and 10 samples, respectively. Regression results for the determination of CRO, CTX, and COL concentrations in mixture solutions are summarized in Table 2. Figure 3 shows the predicted and reference concentration values of the three models studied. While results varied among the spectral data of different components, in general, high multivariate correlation coefficients between the reference and predicted concentrations and low prediction errors were obtained. The relative prediction errors were within 5%, which are well within the range of expected values. The models verify that excitation by a 785-nm laser could induce sufficiently strong Raman signals in the three analytes to enable accurate quantification in mixture solutions even at low concentrations.

Table 1. Coefficients of PLS Models with or without Preprocessing Methods

Preprocessing Method	Components	PLS Factors	RMSEC	RMSECV	R^2
No preprocessing	CRO	6	0.1682	0.2008	0.9936
	CTX	6	0.1485	0.1799	0.9952
	COL	6	0.2273	0.2744	0.9888
First derivative	CRO	3	0.3488	0.4108	0.9734
	CTX	3	0.1708	0.2277	0.9932
	COL	3	0.3326	0.4471	0.9702
MSC	CRO	5	0.2498	0.2947	0.9836
	CTX	5	0.2130	0.2476	0.9909
	COL	5	0.2907	0.3424	0.9825
SNV	CRO	5	0.2492	0.2936	0.9864
	CTX	5	0.2127	0.2471	0.9910
	COL	5	0.2900	0.3416	0.9826
Baseline	CRO	6	0.1690	0.2093	0.9931
	CTX	6	0.1800	0.2190	0.9929
	COL	6	0.2477	0.3151	0.9852
OSC	CRO	4	0.1614	0.1891	0.9943
	CTX	4	0.1429	0.1920	0.9945
	COL	4	0.2139	0.2477	0.9908

Table 2. Regression Results of the PLS Model

Component	PLS Factors	RMSECV	RMSEP	R	REP
CRO	3	0.2215	0.1246	0.9989	4.46%
CTX	3	0.1894	0.1491	0.9983	4.04%
COL	3	0.2736	0.1211	0.9987	3.98%

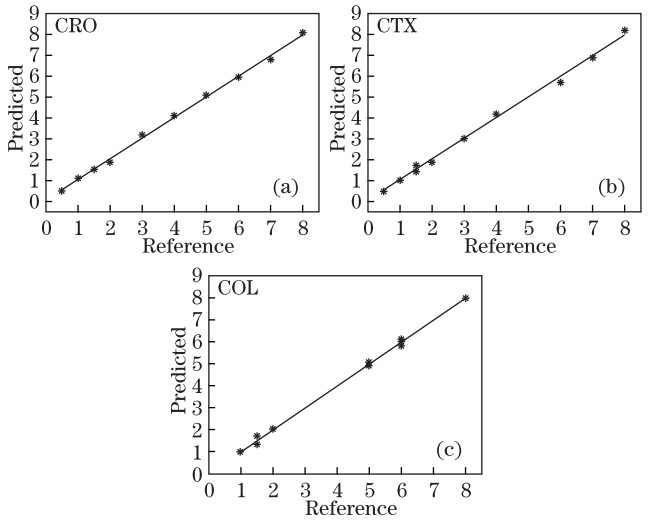

Fig. 3. Predicted and reference concentrations of ternary mixture solutions based on preprocessed Raman spectra.

In conclusion, different spectral preprocessing methods are applied to remove multiplicative effects, and simpler and more interpretable regression models are obtained. OSC-preprocessed PLS models show optimal performance for determining the concentrations of ternary cephalosporin mixture solutions. The results in this letter clearly demonstrate that Raman spectroscopy coupled with the PLS regression technique can precisely and rapidly determine the concentrations of components of cephalosporin mixtures without sample preprocessing.

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