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Monitoring of the hydrolysis process of bear bile powder using near infrared spectroscopy and chemometrics

Wenlong Li, Zhiwei Cheng, Haibin Qu *

Pharmaceutical Informatics Institute, Zhejiang University, Hangzhou 310058, China

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ABSTRACT

A near infrared (NIR) spectroscopy-based method was developed for monitoring the hydrolysis process of bear bile powder. During the hydrolysis process, samples were collected and measured using both NIR spectrometer and high performance liquid chromatography. The quantitative calibration models were established with the collected NIR spectra and the reference concentrations of tauroursodeoxycholic acid (TUDCA), taurochenodeoxycholic acid (TCDCA), ursodeoxycholic acid (UDCA), and chenodeoxycholic acid (CDCA) using partial least squares regression algorithm. After the models were established and validated, the samples of new batches can be determined rapidly, and the hydrolysis process of bear bile powder can be monitored quantitatively. Additionally, a moving block of standard deviation (MBSD) method was also developed for the endpoint determination of the hydrolysis process. The proposed methods have reduced the laborious workload of process sample analysis significantly, and the fast analytical results have contributed to the understanding and controlling of the bear bile powder hydrolysis process.

1. Introduction

Traditional Chinese medicine (TCM) is the precious historical and cultural heritage of the Chinese nation, and the modernization of TCM is an important step to maintain long-term sustainable developing and make access to the international mainstream of the pharmaceutical market. However, in the present, the overall level of the TCM production is still relatively backward. An outstanding problem is the shortage of monitoring and control means during the manufacturing of TCM. In most cases, the productions have to depend on the operating experience of the workers or strict specifications for the TCM production supervising, which may bring out risks for the TCM productions quality consistency due to that the inherent variability of raw materials is largely amongst different batches. U.S. Food and Drug Administration (USFDA) has initiated the Process Analytical Technology (PAT) strategy since 2004 to guide the process monitoring, control and optimization in the pharmaceutical producing enterprises, which also provides scientific ideas and practical tools for the process control in the TCM factories [1].

Near infrared spectroscopy (NIRS) is an effective process analysis tool characterized by its rapidity, accuracy, and simplicity, which make it a potentially appropriate technique for the TCM industries [2–6]. The technology has been widely used in recent years in the process monitoring of TCM, such as extraction process, alcohol precipitation, chromatography process, and liquid preparation process, and the purpose of the NIRS application includes process visualization and endpoint determination, which contributes to the understanding and control of the manufacturing process of TCM productions.

Bear gall powder is an expensive medicine in the TCM system. It has a long application history to cure

* Corresponding author. Tel./fax: +86 571 88208428.
E-mail address: quhb@zju.edu.cn (H. Qu).

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hyperpyrexia and skin ulcer in China, Japan and Korea. The main active ingredients are bounded bile acids, such as tauroursodeoxycholic acid (TUDCA) and taurochenodeoxycholic acid (TCDCA). In China, about one half of bear gall powder is used as the raw material to produce *Tanreqing* injection, which is a modern TCM preparation used mainly in treating infection of the upper respiratory tract and serious influenza, such as SARS (Serious Acute Respiration Symptom) and influenza A virus subtype H1N1 [7,8]. Hydrolysis is an essential step to increase the biological availability of bear gall powder, during which the TUDCA and the TCDCA will lose the taurine and converted into ursodeoxycholic acid (UDCA) and chenodeoxycholic acid (CDCA) under the condition of high temperature and strong basicity, which are shown in Fig. 1. The hydrolysis process takes a long time (21 h), however, the transformation regularity during the hydrolysis process is still not known due to the lack of the rapid analysis methods.

In this research, an NIRS based method was established for the rapid determination of the concentrations of TUDCA, TCDCA, UDCA and CDCA during the hydrolysis process, and on this basis, a moving block of standard deviation (MBSD) method for the endpoint determination was also proposed. To our best knowledge, this is the first report on the NIRS application in the hydrolysis process monitoring of animal origin TCMs, which is a common challenge in the Chinese pharmaceutical industry. And the presented method also provides a promising tool for solving the similar problems.

2. Materials and methods

2.1. Sampling method

The hydrolysis process of bear bile powder lasts for 21 h under the condition of strong basicity and high temperature. At intervals of one hour, 50 ml samples were collected during the whole process. A total of 176 samples were obtained from 8 batches of the hydrolysis process. After 1:25 (v/v) diluted with acetic acid–ammonium acetate buffer solution (pH = 4.5), the samples were rushed to the site analyzing cabin for NIR spectral collecting. Then the diluted samples were filtrated with 0.45 μm filtering film, and 8 μL filtrate was injected into the high performance liquid chromatography (HPLC) system for the reference values determination.

2.2. NIR spectra collection

The Antaris MX FT-NIR spectrophotometer (Thermo Electron Co., Madison, WI, USA) with an InGaAs detector and a handheld fiber-optics probe was used for the spectra collection after the samples were stabilized to 25 °C in a 500 ml beaker. The NIR spectra were collected in transflective mode with the optical length of 2 mm over the waveband of 10,000–4000 cm^{-1}. The spectral data interval was set at 4 cm^{-1} and each sample spectrum was obtained by averaging 128 scans with air as the background. The NIR instrument was controlled by a compatible PC, and a RESULT workflow-based software (Thermo Scientific, Madison, WI, USA) was used for data acquisition.

2.3. Determination of the reference values

The contents of TUDCA, TCDCA, UDCA and CDCA in the samples are determined using a validated HPLC-ELSD method as the reference method. For HPLC measurements, an Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA, USA) with a vacuum degasser, a quaternary pump, an autosampler, a thermostatic column compartment, and an evaporative light scattering detector (ELSD) were used. Separation was performed on an analytical chromatographic column Zorbax SB-C_{18} (i.d. 4.6 × 250 mm, 5 μm particle size) at 40 °C. The mobile phase consisted of (A) acetic acid–ammonium acetate buffer solution (pH = 4.5) and (B) acetonitrile. The gradient program was as follows: initial 70% (A), at 0–20 min, linear change from 70% to 25% (A). Re-equilibration duration was 10 min between individual runs. The flow rate of the mobile phase was 0.8 mL min^{-1}. For the ELSD detection, the drift tube temperature and the pressure of the carrier gas were set at 80 °C and 3.5 bar, respectively.

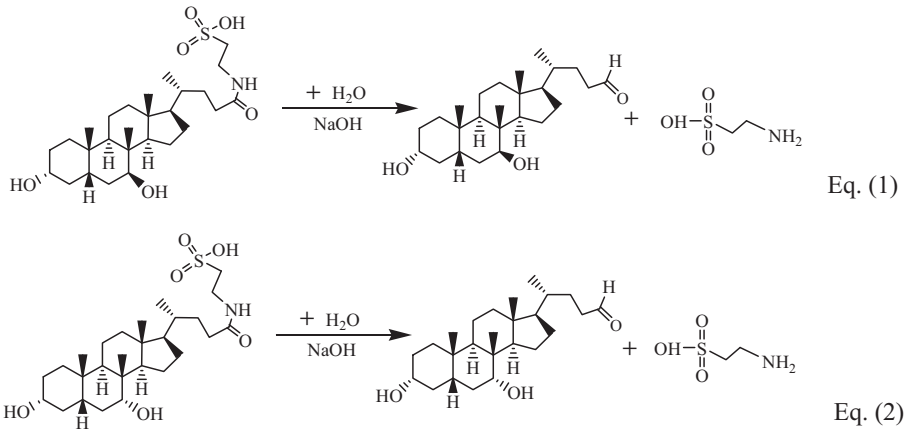

Fig. 1. The reaction equations of the hydrolysis of TUDCA (1) and TCDCA (2).