Food Chemistry 170 (2015) 415–422

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Determination of tetracycline hydrochloride by terahertz spectroscopy with PLSR model

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ARTICLE INFO

Article history:
Received 6 November 2013
Received in revised form 22 April 2014
Accepted 11 August 2014
Available online 20 August 2014

Keywords:
Tetracycline hydrochloride
Terahertz spectroscopy
Quantitative detection
Partial least-squares regression

ABSTRACT

Antibiotic residues in agricultural and food products are of great concern to legislatures and consumers. Reliable techniques for rapid and sensitive detection of these residues are necessary to ensure food safety. In this study, tetracycline hydrochloride (TC-HCl) in powder and solution form was detected and quantified using terahertz (THz) spectroscopy. Partial least-squares regression (PLSR) was used to build calibration models. The results obtained in this study indicated that the PLSR model for powder samples was excellent and could be used for quality control. However, the PLSR model for solution samples was not robust and needed to be improved. Overall, THz spectroscopy combined with PLSR model had its potential for the rapid and non-destructive prediction of TC-HCl residue without sophisticated methods, although the accuracy was not high for solution samples which should be improved in future study.

1. Introduction

Since the discovery of the antibiotic action of antibiotics, they have been used for treatment and prevention of microbial infections in humans and animals. Most widely used antibiotic families are β-lactams, tetracyclines, macrolides and sulphonamides. The use of antibiotics is significant because they not only exhibit antibiotic activity against various bacteria and pathogenic microorganisms, but also significantly enhance growth when added to animal feed. However, the overuse of antibiotics has triggered the development of bacterial resistance, and leads to antibiotic residues in animal products. In recent years, antibiotic residues in food of animal origin are considered to be an important health risk because of increasing antimicrobial resistance (Butaye, Devriese, & Haesebrouck, 2001). As a result, maximal residue limits (MRLs) were established for different matrices by different countries. However, regardless of any legislation, abusing of antibiotics is becoming a serious problem. Therefore, there is increasing pressure to ensure food safety for the consumers.

Tetracycline (TC) is a broad-spectrum antibiotic, which has a broad antibacterial spectrum against both Gram (+) and Gram (−) microorganisms, including the species Spirochete, Actinomyces and Mycoplasma (Michalova, Novotna, & Schlegelova, 2004). In addition, TC is also used as a kind of feed additive to promote animal growth (Sczesny, Nau, & Hamscher, 2003), although European Union (EU) legislation has forbidden this practice. In order to protect consumer health and reduce antimicrobial resistance, several regulatory authorities have defined the MRLs for the TC in various foods. For example, EU has set the MRLs for TC as 0.3 mg/kg in liver, and 0.1 mg/kg in milk or muscle tissues (Regulation, 1990). The US Food and Drug Administration (FDA) has set the MRLs for the sum of TC residue as 2 mg/kg in muscle, 6 mg/kg in liver, and 0.4 mg/kg in milk (U.S. Code of Federal Regulations., 2003, chap. 1). So fast, the most widespread used analytical methods for separating and determining TC are microbiological assay (Pellinen, Bylund, Virta, Niemi, & Karp, 2002), chromatography (Fritz & Zuo, 2007), and capillary electrophoresis (CE) (Ma, Vickroy, Shien, & Chou, 2012) because of their superior sensitivity and selectivity. However, all these techniques are time-consuming, expensive, and require not only tedious sample-preparation procedures but also sophisticated laboratory facilities and specialist operators (Moreno-Bondi, Marazuela, Herranz, & Rodriguez, 2009). Other methodologies based on immunology assay including immunochemistry (Liu et al., 2009) and biosensors (Rebe Raz, Bremer, Haasnoot, & Norde, 2009) are time-saving, sensitive and highly selective, and can be carried out in situ. But these methods are very difficult to establish and usually yield false-positive results (Cháfer-Pericás, Maquieira, & Puchades, 2010). Thus, a simple, non-destructive, economic, and rapid technique is required for global use to detect TC residue.

Terahertz (THz) spectroscopy, being described from 0.1 to 10 THz (30 μm–3 mm) (Zhang & Xu, 2009), is a powerful tool for non-destructive detection (Qin, Ying, & Xie, 2013). THz

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http://dx.doi.org/10.1016/j.foodchem.2014.08.050
0308-8146/

spectroscopy is not only considered safe both for sample and operator due to its low-energy and non-ionizing but also can provide a direct measurement of the field amplitude and phase, which are related to the absorption coefficient and the permittivity of the sample (Zhang & Xu, 2009). Due to its superiority, THz technique has been used as an analysis tool in many fields such as physics (Ulbricht, Hendry, Shan, Heinz, & Bonn, 2011), pharmaceuticals (Zeitler et al., 2007), and biology (Parthasarathy, Globus, Khromova, Swami, & Woolard, 2005).

In recent years, THz time domain spectroscopy (THz-TDS) together with chemometrics is increasingly used in the fields of agricultural and food industry, mainly because of its low-cost, non-destruction and generally simple sample pretreatment. Two kinds of tartaric acid were successfully analysed through THz-TDS with multiple linear regression (MLR), partial least-squares (PLS) and quadratic PLS (QPLS), and the best analysis result was obtained by QPLS (Nishikiori et al., 2008). Main component concentration of edible oil samples was predicted using THz-TDS combined with support vector machine (SVR) and PLS (Li, Chen, & Li, 2010). Quantitatively analysis of pesticides using THz-TDS with chemometrics algorithms, including PLS, interval PLS (iPLS), and backward iPLS (biPLS) were reported (Ma, Wang, & Li, 2012), confirming that this technique was quite promising for pesticide residues detection in food safety control. Researches also showed that attenuated total reflection (ATR) THz-TDS with appropriate chemometrics had potential for rapid determination of vitamin C content (Ogawa, Kondo, & House, 2011). The ability of THz spectroscopy combined with PLS regression for quantitative analysis of several milk components, such as milk fat, total solids, and somatic cell counts had also been confirmed (Naito et al., 2011). Tetracycline hydrochloride (TC-HCl) is cheap, stable, easy to be absorbed and easy to decompose into TC. Therefore, it is widely used in veterinary medicine instead of TC. However, until very recently, no qualitative and quantitative detection of TC-HCl has been reported. Therefore, The main purpose of this article was to investigate the possibility of THz-TDS with chemometrics for the analysis of TC-HCl in form of powder and solution.

2. Materials and methods

2.1. Reagents and materials

TC-HCl was of USP grade and purchased from Aladdin (Shanghai, China). High-density polyethylene (HDPE) powder was purchased from Sigma–Aldrich (St. Louis, USA). Polyvinylidene fluoride (PVDF) membrane (GVWP04700, pore size: 0.22 μm, diameter: 47 mm) was purchased from Millipore (Billerica, USA), Deionised water was obtained from the Milli-Q SP Reagent Water System (18 MΩ, Millipore, Billerica, USA). All reagents were used without further purification.

The TC-HCl powder samples were crushed into small particles that were sufficiently smaller than the THz wavelength to reduce baseline offsets at higher frequencies (Ueno, Rungsawang, Tomita, & Ajito, 2006). These particles were then mixed carefully with HDPE powder at several different concentrations (from 0% to 50%, g/100 g), and three replicates were prepared for each concentration. HDPE powder was chosen because it was transparent to THz radiation and did not show any absorption feature. The mixture was then compressed into 13-mm-diameter pellets under pressure of 10 MPa with tablet press, and the mechanically determined pellet thickness ranged from 1 to 2 mm to provide a sufficient path length to eliminate the effect of the multiple reflection that occurred between the two surfaces of the pellet sample (etalon artifacts) in the spectra (Ueno et al., 2006).

For TC-HCl solution samples, PVDF membrane was used as a support. Such a membrane had a porous structure and the percentage of voids was 70%. Therefore, far infrared light such as THz waves can easily penetrate the membrane (Ogawa, Hayashi, Oikawa, Otani, & Kawase, 2008). More important, unlike sample holder such as cuvette, the PVDF membrane was thin enough so that the multiple reflections and Fabry–Perot effect could be ignored. In this study, A series of TC-HCl concentrations (from 0% to 2%, g/100 ml) were prepared by dissolving appropriate amounts of TC-HCl powder in deionised water, and three replicates were prepared for each concentration. Then, PVDF membrane was dipped into TC-HCl solution with different concentrations for 10 min. Finally, the PVDF membrane was air-dried for 12 h.

2.2. Spectral measurements

All THz measurements performed in this work were collected using a Z-3 THz-TDS system. The Z-3 with a frequency range of 0.1–3.5 THz was a commercially spectrometer manufactured by Zomega Terahertz Corporation. A large-aperture photoconductive (PC) antenna was used for THz generation and an electro-optic (EO) ZnTe crystal was used for THz detection. A FemtoFiber pro NIR laser (pulse width of <100 femtoseconds (fs) at a repetition rate of 80 MHz) at 780 nm was used as a pump source. More details about Z-3 THz-TDS system was described elsewhere (Lipscomb et al., 2012). Our experiment was carried out at room temperature, under the circumstance of a nitrogen purged container with the relative humidity of 0%. Three measurements were collected for each sample to reduce the random error. The reference waveform was collected when the THz pulses passed through a sample holder without sample mounted in it.

2.3. Parameters extraction

A conventional Fast Fourier Transform (FFT) was applied to obtain the spectral distribution of the THz pulse in the frequency domain (Zhang & Xu, 2009):

$$\tilde{E}(\omega) \equiv A(\omega)e^{-i\phi(\omega)} = \int dtE(t)e^{-i\omega t}$$

where $A(\omega)$ and $\phi(\omega)$ were the amplitude of the electric field and the phase of the electric field, respectively. $E(t)$ was the time domain waveform.

The absorption coefficient (α) and refractive index (n) of the sample could be extracted by comparing the sample spectrum with the reference spectrum (Zhang & Xu, 2009):

$$\alpha = \frac{1}{d}\ln\frac{A_R}{A_S}$$

$$n = 1 + \frac{(\phi_S(\omega) - \phi_R(\omega))c}{\omega d}$$

where, A_R and A_S were the amplitude of the reference and sample signal, respectively. $\phi_S(\omega)$ and $\phi_R(\omega)$ were the phase of the reference and sample signal, respectively. Variable ω was the frequency, C was the speed of light in a vacuum, and d was the thickness of the sample.

2.4. Chemometrics and data analysis

In this study, Matlab software (version 7.11.0.584) and Origin software (version 8.5.0 SR1, OriginLab Corp.) were used for spectra processing. TQ analyst software (version 8.4.259, Thermo Electron Corp.) was used for chemometrics analysis. Before the development of calibration models, outlier detection was applied (Aske, Kallevik, & Sjöblom, 2001). Spectrum outlier diagnostic was run