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A reversed-phase high performance liquid chromatography coupled with resonance Rayleigh scattering detection for the determination of four tetracycline antibiotics

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ABSTRACT

A new reversed-phase high performance liquid chromatography with resonance Rayleigh scattering detection (HPLC–RRS) was developed for simultaneous separation and determination of four tetracycline antibiotics (TCs). A good chromatographic separation among the compounds was achieved using a Synergi Fusion-RP column (150 mm × 4.6 mm; 4 μm) and a mobile phase consisting of methanol–acetonitrile–oxalic acid (5 mM) at the flow rate of 0.8 mL min^{-1}. Column temperature was 30 °C. The RRS signal was detected at $\lambda_{ex} = \lambda_{em} = 370$ nm. The recoveries of sample added standard ranged from 95.3% to 103.5%, and the relative standard deviation was below 2.79%. A detection limit of 2.12–5.12 μg mL^{-1} was reached and a linear range was found between peak height and concentration in the range of 10.36–518.0 μg mL^{-1} for oxytetracycline (OTC), 12.11–605.5 μg mL^{-1} for tetracycline (TC), 11.79–589.5 μg mL^{-1} for chlortetracycline (CTC) and 10.32–516.0 μg mL^{-1} for doxycycline (DC). The linear regression coefficients were all above 0.999. The method has been applied successfully to the determination of OTC, TC, CTC, DC in pharmaceutical formulations and in honey. The method was simple, rapid and showed a better linear relation and high repeatability.

1. Introduction

TCs have broad-spectrum antibacterial function and are active against both Gram-negative and Gram-positive bacteria. In recent years TCs have been widely used in pharmaceuticals and food additives. It is worth noting that a tremendous interest in the separation and analysis of TCs has occurred [1–3]. Nowadays, several methods have been employed to determine TCs in biological specimens and/or pharmaceutical formulations, such as microbiological assay [4], high performance liquid chromatography (HPLC) [5,6], high performance liquid chromatography–tandem mass (HPLC–MS) [7,8], RRS spectra [9,10], thin-layer chromatographic (TLC) [11], ultraviolet spectrophotometric (UV–vis) method [12,13], electrochemistry [14,15] and capillary electrophoresis [16,17] method. In Chinese Pharmacopoeia [5], HPLC has been accepted as a method for the determination of oxytetracycline (OTC), tetracycline (TC), chlortetracycline (CTC) and doxycycline (DC).

RRS is a special elastic scattering produced when the wavelength of Rayleigh scattering (RS) is located at or close to its molecular absorption band [18]. It forms new spectral characteristics and provides new information concerning molecular structure, size, form, charge distribution, state of combination and so on. In recent years, this technique has been increasingly applied to the determination of pharmaceuticals [9,10,19,20], biological macromolecules [21,22], inorganic ions

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[20,23,24], organic compounds [25] and some surfactants [26], because of its sensitivity, simplicity and speediness. However, some of the ion-associates were not stable in aqueous solution, and water was not a suitable solvent for some compounds. The intensity of RRS was affected by experimental factors such as the medium and temperature of the reaction, and so on. Especially, if several compounds existed simultaneously, RRS method could not determine them at the same time. As a result, the reproducibility, accuracy and selectivity of the method were sometimes poor.

HPLC has been applied more than 2/3 in Chinese Pharmacopoeia 2005 Edition, its development was the fastest in all analytical method because of its reproducibility, accuracy and selectivity performance. But its sensitivity could not meet the demand when trace component was determined. If HPLC can be coupled with RRS, using RRS as a new detection technique, integrating the merits of high sensitivity and selectivity will play an important role in developing HPLC. In this work, a new method was proposed by coupling HPLC with RRS, simultaneously determined four tetracycline antibiotics. Furthermore, its result could be compared with the result of the DAD detector. The method has been applied successfully to the determination of OTC tablet, TC tablet, DC tablet, CTC eye ointment and the residue in honey.

2. Experimental

2.1. Instrumentations

A liquid chromatography (Agilent 1100, USA) consisting of a G1322A online degasser, G1311A pump, G1316A column oven, G1321A fluorescence detector and G1315B DAD detector was used to separate, detect and record HPLC–RRS and HPLC-UV Chromatogram. A Hitachi F-2500 spectrofluorophotometer (Tokyo, Japan) was used to record the RRS spectra. A UV–vis 8500 spectrophotometer (Tianmei, China) was used to record absorption spectra. A TECNAI 10 transmission electron microscope (Philips, Holland) was used to observe the appearance and size of antibiotics.

2.2. Chemicals and reagents

OTC, TC, CTC and DC were purchased from National Institute for the Control Pharmaceutical and Biological Products (Beijing, China), HPLC-grade methanol and acetonitrile were purchased from Kermel Company (Tianjin, China). Analytical reagent grade oxalic acid, ethyl acetate and sodium acetate were purchased from Chemistry Reagent Factory (Chengdu, China). Double distilled water was used throughout. OTC tablets, TC tablets, DC tablets, CTC eye ointment and honey were purchased from shop.

2.3. Chromatography conditions

The chromatographic separation was accomplished with gradient elution on a Synergi Fusion-RP (150 mm × 4.6 mm; 4 μm) column (Phenomenex, CA, USA). The flow rate was 0.8 mL min^{-1}, and the column oven was 30 °C. The injection volume was 20 μL for the determination. The UV detection was monitored at 270 nm and the RRS detection was monitored at $\lambda_{ex} = \lambda_{em} = 370$ nm. The mobile phase consisted of methanol (A), acetonitrile (B) and oxalic acid (5 mM) (C). The gradient elution (linear gradient) performed was: 0–3 min hold at 9.5% A, 14.5% B, 76% C; 3–10 min from initial conditions to 18% A, 30% B, 52% C.

2.4. Preparation of standards

Individual standard solution of TCs (1 mg mL^{-1}) was prepared exactly, weighted and dissolved the corresponding compounds in mix solvents of methanol and hydrochloric acid (0.01 M) (20:80; v/v). The standard solutions were stored at 4 °C in darkness. Standard solutions with different concentration levels of the four TCs were prepared by serial dilutions of the stock solutions.

2.5. Preparation of samples

Individual sample solution of OTC tablets, TC tablets and DC tablets (1 mg mL^{-1}) was prepared [5] exactly weighting and dissolving the corresponding compounds in hydrochloric acid (0.01 M). Sample solutions of the three TCs were prepared by serial dilutions in mix solvents of methanol and hydrochloric acid (0.01 M) (20:80; v/v), respectively. The prepared sample solutions were filtered through 0.45 μm membrane filters and stored at 4 °C in a refrigerator.

The CTC eye ointment was dissolved into the diethyl ether, the ether solution was extracted by hydrochloric acid (0.01 M) (3× 10 mL). The extracted solutions were transferred into a 50-mL flask and diluted to the mark with water. The prepared sample solutions were filtered through 0.45 μm membrane filters and stored at 4 °C in a refrigerator.

A sample of honey (5 g) was dissolved in 15 mL of 0.1 M sodium acetate pH 4.5. The solution was stirred for 5 min using a vibratory stirrer at 1000 rpm. After filtration it was loaded on a Strata-X Solid Phase Extraction (SPE) (1 g, 20 mL) cartridge (Phenomenex, CA, USA) previously conditioned with 10 mL of methanol, 10 mL of water and 10 mL of 0.1-M sodium acetate solution. The SPE cartridge was then washed with 10 mL of water, and after 5 min of drying under vacuum, TCs were eluted with 5 mL of ethyl acetate directly in 25-mL round-bottomed flask. The eluate was evaporated to dryness under reduced pressure at 40 °C. The residue was dissolved in 0.25 mL mix solvents of methanol and hydrochloric acid (0.01 M) (20:80; v/v), stirred in vortex and filtered before being injected into the HPLC system.

3. Results and discussion

3.1. Optimization of chromatographic conditions

3.1.1. Selection of detection wavelength

The RRS spectra of four TCs, mixed solutions of different concentrations TCs and organic solvent were analyzed by scanning from 200 to 700 nm on the Hitachi F-2500 spectrofluorophotometer (Fig. 1). The results of RRS spectra showed that a trifle of organic solution could increase the detection signal, the maximal RRS wavelength of four TCs was at 411–440 nm.