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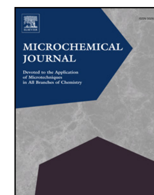

Study on simultaneous determination of three nitroimidazole residues in honey by high performance liquid chromatography–resonance Rayleigh scattering spectra

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ABSTRACT

A novel and high sensitivity method that high performance liquid chromatography combined with resonance Rayleigh scattering is established for the determination of nitroimidazole residues in honey, including Metronidazole (MNZ), Ornidazole (ONZ) and Tinidazole imidazole (TNZ). In pH 3.8 BR buffer solution, the three nitroimidazole drugs separated by HPLC, and then reacted with a post-column derivatization Phloxine B (PB) in the reaction tube to form 1:1 ion-association complex, which Leads to significant enhancement of the RRS signal. This paper optimized the experimental conditions of post-column derivation and discussed the mechanism of reaction for the enhancement of RRS signal. Under the optimal experimental conditions, the RRS signals of the three nitroimidazole drugs were enhanced with the increase of its concentration in the respective linear range. The detection limit (S/N) of MNZ, ONZ and TNZ was: $0.013\,\mu g{\cdot}mL^{-1}$, $0.049\,\mu g{\cdot}mL^{-1}$ and $0.27\,\mu g{\cdot}mL^{-1}$, respectively. The recovery rate was 97.4%–102.2%, and the relative standard deviation (RSD) does not exceed 5.3%, the reliability of this technique is verified, and the result of this method is satisfyingly.

1. Introduction

Metronidazole (MNZ), Ornidazole (ONZ), and Tinidazole (TNZ) are all nitroimidazole drugs, which is able to defend against the diseases such as amoebiasis, trichomoniasis, and anaerobic infections [1,2]. Their structure is shown in Fig. S1, MNZ is a nitroimidazole antibiotic that causes bacterial death when it is reduced in humans with effects on inhibiting bacterial DNA synthesis [3]. TNZ was first developed in the United States, it is a new generation of nitroimidazole drugs after MNZ [4] with higher efficacy, shorter duration of treatment, better tolerability. ONZ is the third-generation nitroimidazole drug widely used after MNZ and TNZ [5].

In recent years, some commonly used drugs such as chloramphenicol and MNZ have been strictly banned in agricultural production, Beekeepers have gradually used ONZ and TNZ as alternative drugs to control "Climbing bee disease" [6]. However, the nitroimidazole drugs have mutagenic, teratogenic and suspicious carcinogenic effects, so it is particularly important to investigate the quantitative determination methods of the three nitroimidazole drugs. Some methods have been reported in the literature, such as: (HPLC-UV) [7], GC [8], (CE) [9], ES [10], HPLC-MS [11], UV [12]. Although GC–MS, HPLC-MS and CE have high selectivity and sensitivity, but these methods usually require complicated equipment and sample pretreatment, HPLC-UV has the advantages of simplicity and high selectivity, but its sensitivity is not enough. Therefore, it is important to explore a simple and effective analytical method for the quantitative determination of nitroimidazole drugs.

HPLC is a simple and efficient method of analysis, the detection system plays a crucial role in its main components. The commonly used HPLC detectors are as follows: HPLC-DAD [13–15], HPLC-FLD [16], HPLC-MS [17]. However, RRS as a sensitively analytical method with sensitivity and fast analysis speed, which has been widely used in many fields such as protein [18], metalion [19], nucleic acid [20] and drugs [21]. A new method that HPLC coupled with RRS (HPLC-RRS) [22] provides a highly selective and highly sensitive method. Nowadays, the HPLC-RRS combination technology has been successfully applied to various fields, such as nucleic acids and proteins [23], antihistamines [24,25], Narcotics [26], psychotic drugs [27], Vitamin C [28], and antibiotics drug [29].

This article established a simple, highly selective and highly sensitive method to detect the three nitroimidazole drugs, this method is more innovative than previous post-column derivatization methods [30]. In this paper, the post-column derivatization experimental conditions were optimized, such as: the detection wavelength, the reaction

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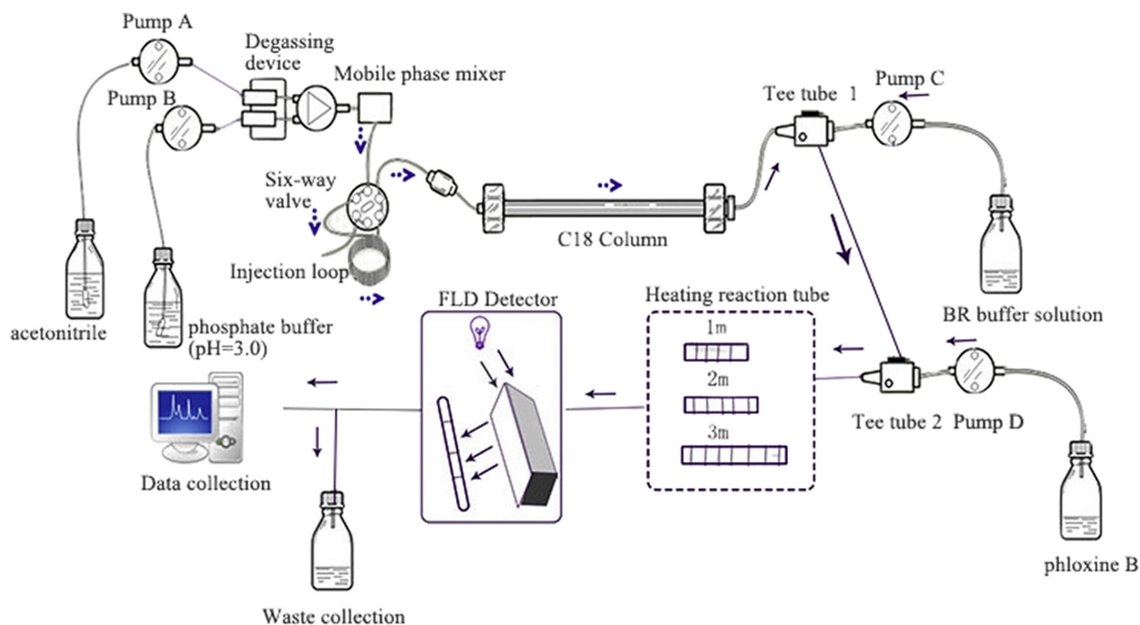

Fig. 1. The device diagram of HPLC-RRS for simultaneous determination of three nitroimidazole residues in honey.

tube length, the reaction temperature, etc. And the reaction mechanism and reasons for enhancement of the RRS signal were discussed by means of a series of methods, under the optimal experimental conditions, the RRS signals of the three nitroimidazole drugs were increased and proportional to the increase of drug concentration within the respective linear ranges, the detection limits and recovery rates were obtained. The results were satisfactory and verified the reliability of this combined technology.

2. Experimental

2.1. Instrumentations

A HPLC (Shimadzu, Japan) including two LC-20AD pumps, a DGU-20 A5R degassing unit and a RF-20A fluorescence detector. A PCX-BT post-column derivatization instrument was from Tian Mei Da Scientific Instruments Co. Ltd. (Shenyang, China). A F-2500 spectro-fluorophotometer (*Hitachi Company*, *Japan*) was used for recording the RRS and measuring the scattering intensities, a UV-2450 spectrophotometer (*Shimadzu*, *Japan*) was used to acquire absorption spectra. A pHS-3C meter (*Shanghai Scientific Instruments Company*, *China*) was used to measure the pH values of the solutions (Mettler-Toledo Instruments Co. Ltd, Shanghai, China). The surface of ion-association complexes were observed by scanning electron microscopy (SEM, FEI, Quanta 400 FEG, America) at an acceleration voltage of 25 kV.

2.2. Chemicals and reagents

MTZ and ONZ were purchased from Technical Sales Higher Biotech (Shanghai, China). TNZ was purchased from the National Institute for the Control Pharmaceutical and Biological Products (Beijing, China). Phloxine B was purchased from Chemical Reagent Stocking and Providing Station, (Shanghai, China). Britton-Robinson buffer solutions (pH 1.8–12) were prepared by mixing 0.2 mol·L^{-1} NaOH and 0.4 mol·L^{-1} H_3PO_4, HAc and H_3BO_3. They were purchased from the Chemistry Reagent Factory (Chongqing, China). Methanol and acetonitrile were purchased from Kermel Company (Tianjin, China). Double distilled water was used throughout. All reagents were filtered through a 0.2 mm pore size filter membrane (Millipore, Bedford, MA, USA).

2.3. Preparation of standard solutions

Standard solution: accurately weight 1 mg of MNZ, ONZ, TNZ into three small beakers and dissolve them with appropriate amount of acid, and transfer them to a 10 mL volumetric flask to make a stock solution of 1 mg·mL^{-1}. Protect them from light in a refrigerator of 0 °C–4 °C. Diluted with twice-distilled water to get different concentration of working fluid during the experiment, accurately weigh a certain amount of PB into a stock solution of 4×10^{-4} mol·L^{-1}. Different pH buffer solutions are obtained by mixing appropriate ratios of acid and base solutions.

Standard working solution: dilute the appropriate standard stock solution to the desired concentration with acetonitrile: phosphate buffer 30:70 (V/V), protect them from light at 4 °C, valid for 10 days.

2.4. Preparation of honey samples

The pretreatment of HPLC combined with solid phase extraction obtains good results [31–33] we accurately weigh 5.0 g of honey samples, which are self-produced honey, into a 50 mL beaker with 10 mL of purified water; and vortex for 2 min to dissolve, then add 10 mL of acetic acid ethyl ester with vortex for 2 min. The upper organic phase was drawn after centrifugation at 5000 r·min^{-1} for 10 min in a centrifuge, repeating the previous step with 6 mL acetic acid ethyl twice again. Combine all the organic phases and use a nitrogen blower to blow to near dryness at 30 °C. It was filtered with a 0.22 μm mixed phase needle filter.

2.5. The system of HPLC-RRS

The HPLC-RRS unit is shown in Fig. 1, the mobile phase of HPLC separation system was a volume ratio of acetonitrile to potassium dihydrogen phosphate buffer (pH = 3.0) of 30:70. The flow rate is 0.5 mL·min^{-1}; the injection volume is 20 μL, the separated column is a Kinetex® 5 μm C18 100 Å column (250 × 4.60 mm) and the column temperature is 35 °C. Firstly, the analytes separated from the HPLC were mixed with the BR buffer solution in the first three-way tube to be protonated, the mixture is then mixed with PB in a second tee, and flows into the heated reaction line for the associative reaction. Finally, the RRS signal is recorded by an FLD detector, and the detection wavelength λex = λem = 360 nm.

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