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ScienceDirect
Chinese Chemical Letters 21 (2010) 1223–1226
CHINESE CHEMICAL LETTERS
www.elsevier.com/locate/cclet

# Simultaneous determination of metronidazole and tinidazole in plasma by using HPLC-DAD coupled with second-order calibration

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Received 18 January 2010

**Abstract**

A method using HPLC-DAD coupled with second-order calibration was developed to simultaneously determine metronidazole and tinidazole in plasma samples in this paper. The second-order calibration method based on APTLD (alternating penalty trilinear decomposition) algorithm was proposed to analyze the three-way HPLC-DAD data from both standard and prediction samples, which makes it possible that calibration can be performed even in the presence of unknown interferences with a simple and green chromatographic condition and short analysis time. The results showed that good recoveries were obtained although the chromatographic and spectral profiles of the analytes of interest as well as background were partially overlapped with each other in plasma samples.



*Keywords:* HPLC-DAD; Second-order calibration; Alternating penalty trilinear decomposition (APTLD); Metronidazole; Tinidazole; Plasma

Metronidazole (MNZ) and tinidazole (TNZ) are the compounds of nitroimidazoles, widely used for the treatment of infections with *Giardia lambia*, *Tricomonas vaginalis*, *Entamoeba histolytica* and some anaerobic bacteria [1,2]. Several analytical methods have been reported for the determination of them, including spectrophotometry [3], polarographic [4], chromatography [5,6] and hyphenated chromatography techniques with mass spectrometry [7]. In this work, a method was proposed to simultaneously determine the contents of these compounds in plasma samples using HPLC-DAD coupled with second-order calibration method based on the alternating penalty trilinear decomposition (APTLD) algorithm [8]. Lomefloxacin (LMX) was added in plasma samples as the interference, due to its usefulness together with MNZ and TNZ to resist some pathogenic bacterium [9,10].

## 1. Experimental

MNZ, TNZ and LMX were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Changsha, China). Each stock solution of MNZ (1.00 mg/mL), TNZ (1.00 mg/mL) and LMX (0.1 mg/mL)

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1001-8417/$ – see front matter 
doi:10.1016/j.cclet.2010.04.016

was prepared in a 100 mL volumetric flask by dissolving with ethanol and then stored at 4 °C in the refrigerator. Appropriate solutions of different concentrations were prepared by diluting in methanol. Methanol used for HPLC-DAD measurements and solution preparation was of HPLC grade. The other chemicals were of analytical grade. Ultrapure water was prepared with a Milli-Q water purification system (Aquapro, China). Drug-free human plasma was obtained from the National Blood Center (Changsha, China).

Measurement has been performed in an LC-20AT liquid chromatographic system (Shimadzu Corporation, Japan), which consists of a degasser, four pumps, a manual injector provided with a 20 μL loop, a column oven and a SPD-M20A diode array detector (DAD). The separation was carried out in a WondaSil-C18 analytical column (150 mm × 4.6 mm, 5.0 μm; Shimadzu, Japan). In the extraction procedure, a centrifuge (Sigma, Germany) was used. The mobile phase was isocratic and consisted of methanol (35%, v/v) and water acidified with 1.0% acetic acid (65%, v/v), which was pumped at a flow rate of 1.0 mL/min with 20 μL injection volume. The column temperature was set at 30.0 ± 0.5 °C. Photometric detection was performed in the range of 200–380 nm, with a spectral interval of 1.5 nm. APTLD algorithm was programmed in Matlab environment.

A calibration set of nine samples was constructed. The levels corresponded to values in the range of 0.80–10.00 μg/mL for MNZ and TNZ. Also eight samples (P1–P8) as a test set were built with analyte concentrations within their corresponding calibration ranges to verify the accuracy of second-order calibration based on APTLD algorithm. Here LMX was added in test samples within the range of 2.0–9.0 μg/mL.

Nine of plasma samples (S1–S9), each 200 μL in volume, spiked with different amounts of MNZ, TNZ and LMX were diluted to 500 μL with methanol. The final concentrations of MNZ and TNZ were within the calibration concentration range. LMX was added within the range of 0–10.00 μg/mL. The mixture was vortex mixed for 30 s. After centrifugation at 12,000 rpm for 10 min in a centrifuge at 10 °C, 20 μL of supernatant was injected into the HPLC system.

## 2. Results and discussion

The APTLD-based second-order calibration method on a base of a trilinear component model [11,12], was recently proposed by Xia et al. [8]. It is performed by utilizing the alternating least-squares principle and the alternating penalty constraints to minimize three different alternating penalty (AP) errors simultaneously. The method has the property of being insensitive to the estimated component number and having fast convergence rate.

Chromatographic profiles for MNZ and TNZ of sample S9 were shown in Fig. 1. It could be seen, TNZ could not be effectively separated from MNZ and interferents in plasma sample. According to this situation, the second-order calibration method was used to solve the problem. The number of estimated components number was selected as 3. Then three-way data set (150 × 100 × 31) of samples was constructed and submitted for second-order calibration analysis, here the number 150 corresponds the number of elution time data points, 100 is the number of wavelengths and 31 is the number of samples.

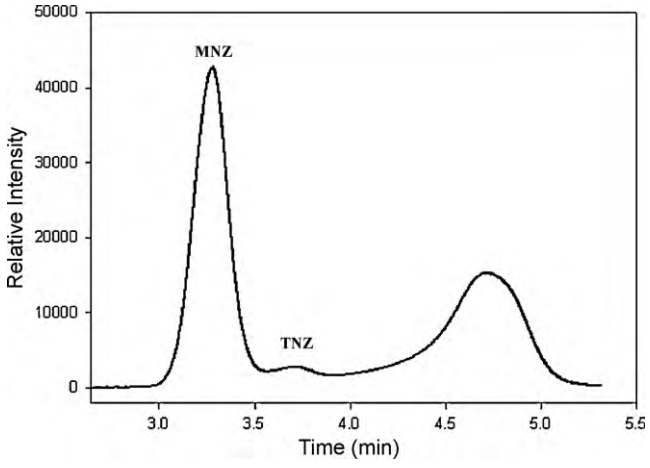


Fig. 1. Chromatographic profile of the sample S9 at 320 nm.