Available online at www.sciencedirect.com
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

Li Qun Ouyang [a,b], Hai Long Wu [a,*], Ya Juan Liu [a], Jian Yue Wang [b], Yong Jie Yu [a], Hong Yan Zou [a], Ru Qin Yu [a]

[a] *State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, China*

[b] *Zhoushan Municipal Center for Disease Control & Prevention, Zhoushan 310062, China*

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)

* Corresponding author.
E-mail address: hlwu@hnu.cn (H.L. Wu).

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.

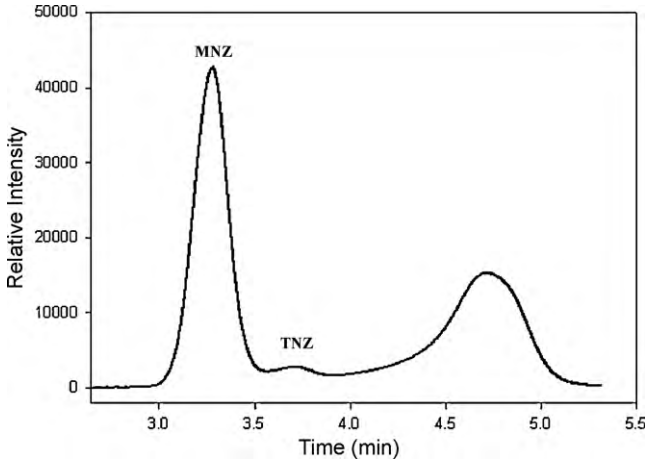

Fig. 1. Chromatographic profile of the sample S9 at 320 nm.

Table 1
Concentration of test samples P1–P8 and spiked plasma samples S1–S9 by using APTLD.

Sample	Test samples (μg/mL)		Plasma samples (μg/mL)	
	MNZ	TNZ	MNZ	TNZ
1	2.0(97.0)[a]	2.0(97.5)	1.6(106.9)[a]	8.8(94.3)
2	3.0(98.7)	3.0(93.7)	2.4(103.8)	7.2(104.6)
3	4.0(97.8)	4.0(96.8)	3.2(100.9)	6.4(105.2)
4	5.0(101.8)	5.0(99.0)	4.0(102.8)	5.6(102.5)
5	6.0(96.7)	6.0(93.5)	4.8(102.9)	4.8(105.6)
6	7.0(103.4)	7.0(100.1)	5.6(102.3)	4.0(105.2)
7	8.0(103.5)	8.0(98.4)	6.4(100.5)	3.2(102.5)
8	9.0(100.7)	9.0(97.0)	7.2(104.7)	2.4(102.9)
9			8.8(94.5)	1.6(102.5)
Average recovery (%)	100.0 ± 2.8[b]	97.0 ± 2.4[b]	102.1 ± 3.5[b]	102.8 ± 3.4[b]
T[c] (t-test)	$0.05 < t^7_{0.025}$	$3.59 > t^7_{0.025}$	$1.86 < t^8_{0.025}$	$2.45 > t^8_{0.025}$

[a] The recoveries of test and plasma samples.
[b] Standard deviation of recoveries.
[c] $T = (\bar{x} - \mu_0)/(S/\sqrt{n})$, $\bar{x}$ is the average recovery, μ_0 is 100%, n is degree of freedom and confidence level is 95%.

The linear correlation coefficients (r) for calibration samples of MNZ and TNZ are 0.9992 and 0.9997. The results of concentrations of test samples (P1–P8) were shown in Table 1. The recoveries of MNZ and TNZ are 96.7–103.5% and 93.5–100.1% as using APTLD, respectively. T-test was carried out in order to compare the recoveries with the ideal value of 100%. Though for TNZ $T = 3.59 > t^9_{0.025}$, the results for TNZ are acceptable and satisfactory as shown in Table 1. The results demonstrated that the method could give accurate results for test samples.

The estimated elution and spectral profiles for MNZ and TNZ of plasma samples were shown in Fig. 2a and b, respectively. The background factor in plasma samples was presented by the dotted line. As demonstrated in Fig. 2, the resolved elution time profiles and spectral profiles for MNZ (short dash) and TNZ (dash dotted dot) as using APTLD match quite well with the true signal of MNZ (solid) and TNZ (long dash) which was obtained by the HPLC-DAD analysis of a pure 8.0 μg/mL MNZ and TNZ solution, respectively. The recoveries of MNZ and TNZ for plasma samples summarized in Table 1, were 94.5–106.9% and 94.3–105.6%, respectively. T-test was performed to compare the recoveries with the ideal value of 100%. As shown in Table 1, for TNZ $T = 2.45 < t^8_{0.01}$, it suggests there is no statistically significant difference between the results when $\alpha = 0.02$ and the results are acceptable. It indicated that APTLD could provide accurate concentration prediction and good resolution of elution and spectral profiles for the analytes of interests in complicated system even in the presence of unknown and uncalibrated interferences, fully exploiting ''second-order advantage'' [13].

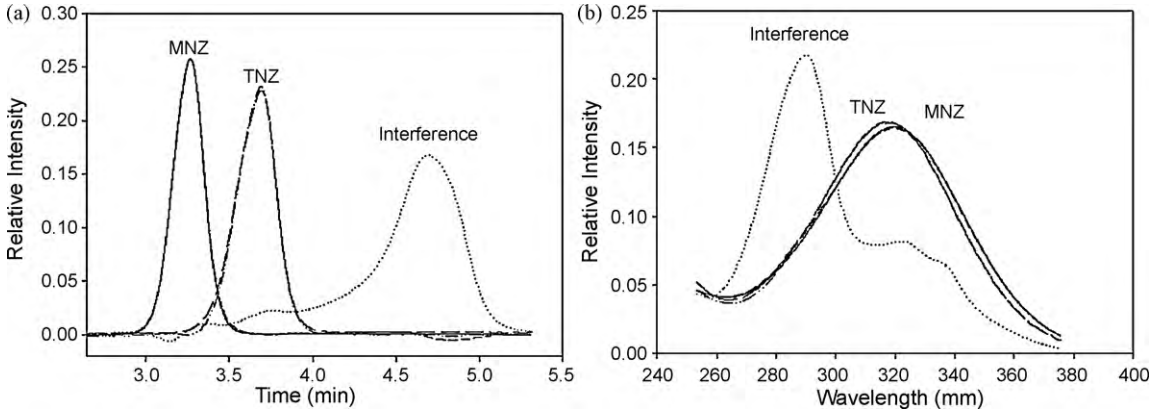

Fig. 2. (a) True elution profiles of MNZ (solid) and TNZ (long dash), and the APTLD-resolved elution profiles for the spiked plasma samples: MNZ (short dash), TNZ (dash dot dotted) and background factor (dotted). (b) True spectral profiles of MNZ (solid) and TNZ (long dash), and the APTLD-resolved spectral profiles for the spiked plasma samples: MNZ (short dash), TNZ (dash dot dotted) and background factor (dotted).

Table 2
Analytical figures of merit for determining MNZ and TNZ in plasma samples by APTLD.

	MNZ	TNZ
SEN[a] (mL/μg)	0.046	0.038
SEL[b]	0.47	0.62
S_0	0.027	0.028
LOD[c] (μg/mL)	0.089	0.092

[a] The sensentivity is determined as: $\mathrm{SEN}_n = k_n\{[(\mathbf{A}^T\mathbf{A})^{-1} \cdot (\mathbf{B}^T\mathbf{B})^{-1}]\}_{nn}^{-1/2}$, where *nn* designates the (n, n) element of a matrix and k_n is the total signal for component n at unit concentration, which is also a parameter for converting scores to concentrations.
[b] The selectivity can be simply obtained by dividing SEN by k_n.
[c] The limit of detection can be calculated as: $\mathrm{LOD} = 3.3S_0$, where S_0 is the standard deviation in the predicted concentration for three different background blank samples.

Table 2 summarized the analytical figures of merit such as sensitivity (SEN), selectivity (SEL) and limit of detection (LOD). The LOD for MNZ and TNZ in plasma samples were 0.089 and 0.092 μg/mL as using APTLD, respectively. The results from Table 2 including SEL, SEN and LOD are acceptable.

In conclusion, HPLC-DAD coupled with the second-order calibration method based on APTLD algorithm provides satisfactory concentration estimates for MNZ and TNZ of plasma samples with simple sample disposal and simple and green chromatographic condition in this paper. It showed that we can resolve partially overlapped peaks into their pure spectral profiles and concentration profiles even in the presence of unknown interferences as using APTLD method. Accordingly, our strategy is helpful for a chromatographic system.

Acknowledgments

This work was financially supported by The National Natural Science Foundation of China (No. 20775025) and The National Basic Research Program (No. 2007CB216404) as well as PCSIRT.

References

[1] G. Elizondo, M.E. Gonsebatt, A.M. Salazar, et al. Mutat. Res. 370 (1996) 75.
[2] H.B. Fung, T.L. Doan, Clin. Ther. 27 (2005) 1859.
[3] P. Nagaraja, K.R. Sunitha, R.A. Vasantha, et al. J. Pharm. Biomed. Anal. 28 (2002) 527.
[4] D.M. Joshi, A.P. Joshi, Ind. Drugs 33 (1996) 338.
[5] H.W. Sun, F.C. Wang, L.F. Ai, J. Chromatogr. B 857 (2007) 296.
[6] G.S. Sadhana, M.V. Gaonkar, Ind. Drugs 25 (1987) 121.
[7] M. Cronly, P. Behan, B. Foley, et al. J. Chromatogr. A 1216 (2009) 8101.
[8] A.L. Xia, H.L. Wu, D.M. Fang, et al. J. Chemometr. 19 (2005) 65.
[9] S.J. Zhao, J. Yuan, G.H. Sun, J. Med. Guangdong 23 (2002) 1126.
[10] L. Cheng, C.C. Zhen, Z.Y. He, et al. Acta Academ. Med. Zunyi 23 (2000) 284.
[11] R.A. Harshman, UCLA Working Papers in Phonetics, vol. 16, 1970, p. 1.
[12] J.D. Carroll, J.J. Chang, Psychometrika 35 (1970) 283.
[13] K.S. Booksh, B.R. Kowalski, Anal. Chem. 66 (1994) 782.