

Short communication

Study on the compatibility of cefotaxime with tinidazole in glucose injection

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Abstract

An efficient HPLC method for the compatibility study of cefotaxime with tinidazole in glucose injection is described, which has been developed for the simultaneous determination of cefotaxime and tinidazole in glucose injection. The appearance and pH value of the mixed solution were investigated and the concentrations of cefotaxime and tinidazole were determined by RP-HPLC with an Agilent ZORBAX Eclipse XDB-C₈ column, gradient elution and dual wavelength detection on diode-array-detector (DAD) at room temperature (20 °C) within 24 h. It was found that the resulting appearance and pH value of the mixed solution showed slight changes, on the other hand, the quantity of cefotaxime decreased significantly. The results show that the mixed solution of cefotaxime with tinidazole in glucose injection must be used within 8 h in clinical due to the possible degradation of cefotaxime in tinidazole glucose injection. This study provides a convenient method for rational use of compatible drugs in clinical practice.

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1. Introduction

Cefotaxime, 7-[α -(2-aminothiazol-4-yl)- α -(z)-methoxy-iminoacetamido]-3-(1-methyl acetate)-meth-yl-3-cephem-4-carboxylate (Fig. 1), is the first third-generation of semisynthetic cephalosporin to clinical practice which shows the general concepts about the elimination of microbial activity against Gram-negative, Gram-positive and some anaerobic organisms while it also exhibits good stability against β -lactamase-overproducing bacteria [1,2]. Its characteristics of pharmacokinetics, dosage and clinic applications can be found in literatures [2,3]. Tinidazole, 1-[[2-(ethylsulphonyl)ethyl]-2-methyl]-5-nitroimidazole (Fig. 1), is widely used for its strong effectiveness against a broad spectrum of anaerobic bacteria [4]. However, the combination usage of different kinds of antimicrobial agents is widely applied in clinical treatment so

as to increase antimicrobial agent power against various organisms, cefotaxime is an antibiotic which undergoes degradation easily in aqueous solution [2] and as a consequence, it is very important to develop a method to monitor the compatibility of cefotaxime and tinidazole in clinical quality control (QC). Since the compatible usage of cefotaxime and tinidazole is widely used clinically in hospitals for treatment of mixed infection in China [5–7], it is very important to supervise the compatibility at the clinical dosage for the QC supervision in hospitals of China.

The simultaneous determination of cefotaxime and tinidazole by UV spectroscopy has been adopted by previous research works concerning the compatibility of cefotaxime and tinidazole [6–8]. The disadvantages of the reported methods are as follows, such as unsatisfied R.S.D.; time consuming due to changing different wavelengths for each component, complicated procedure for sample preparation and intricate data analysis. This study explored a simple and reliable HPLC method to supervise the compatibility of cefotaxime and tinidazole at the clinical dosage within 24 h so as to provide practical guidance for the clinic medication.

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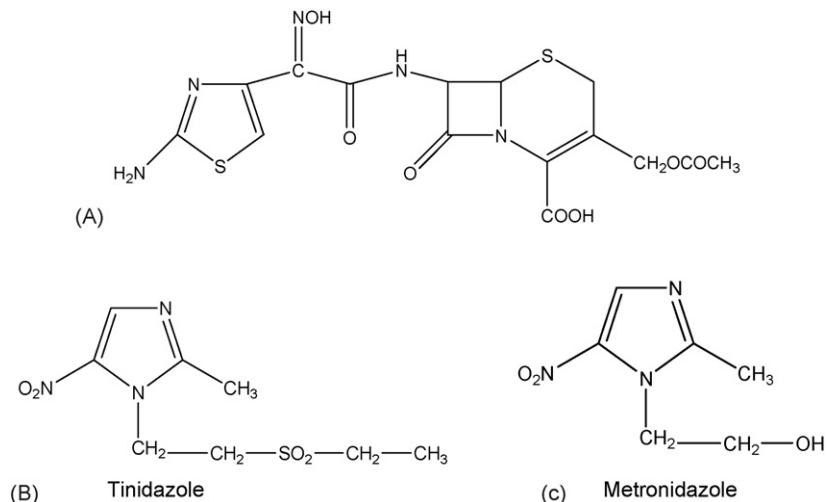


Fig. 1. Chemical structures of cefotaxime (A), tinidazole (B) and metronidazole (C).

2. Materials and methods

2.1. Chemicals and reagents

Cefotaxime powder for injection (cefotaxime 1.0 g, lot #040302) and cefotaxime (99.5%) were purchased from Guangzhou Baiyunshan Pharmaceutical Co. Ltd. (China) and used as received. Tinidazole injection (tinidazole 0.4 g and glucose 10.0 g in 200 ml, lot #041120) was donated by Shiyuan People's Hospital (Hubei, China). Tinidazole (99.8%) was obtained from Lizhu Scientific Pharmaceutical Co. Ltd. (China). Metronidazole (Fig. 1, internal standard, I.S., 99.8%) was provided by Wuhan Pharmaceutical Co. (Hubei, China). HPLC-grade methanol purchased from Bafang Co. (Agilent Technologies, Shanghai Branch, China) and ultrapure water obtained from a SG (SG Co., Germany) water purification system which was used throughout the experiments. All other chemicals were of the HPLC grade as received.

2.2. Instrumentation

An Agilent 1100 instrument system (Waldbronn, Germany) coupled with a G1311A quadruple high-pressure gradient pump, a G1315B diode-array-detector (DAD), a G1316A thermostat oven, a G1379A degasser and a six-way loading valve fitted with a 20 μ l loop was used for all the experiments. The gradient reversed-phase chromatography was carried out with an Agilent ZORBAX Eclipse XDB-C₈ (4.6 mm \times 150 mm, 5 μ m) column. All the chromatographic hardware control, data acquisition and data analysis were controlled by Pentium 4 computer with Agilent HPLC1100 Chemstation software.

Precision pH meter (Model PHS-3C, Leici Instrument Co., Shanghai, China), HH-S water-bath (Yingyuyuhua Instrument Co., Gongyi, China), KQ-100 ultrasonic cleaner (Ultrasonic Instrument Co. Ltd., Kunshan, China), ultrapure water system (SG Co.) served throughout the experiments, all experiments were conducted at 20 $^{\circ}$ C.

2.3. Chromatographic conditions

Mobile phase: methanol–water; flow rate: 1.0 ml/min; detection wavelength: UV, 238 and 318 nm; injecting volume: 20 μ l; column temperature: 30 $^{\circ}$ C.

2.3.1. Gradient program

Upon injection of the standard or test solution in the equilibrated chromatographic system using methanol–water (18:82, v/v) as mobile phase, the isocratic elution was kept for 2.0 min. Then the ratio of methanol was linearly increased to 40% in the time interval from 2.0 to 4.0 min. After isocratic elution of 40% methanol between 4.0 and 6.0 min, the ratio of methanol was linearly decreased to 18% in the time interval from 6.0 to 7.0 min and the system was equilibrated again with methanol–water (18:82, v/v) and the overall runtime was set to be 8.0 min.

2.4. Sample preparation

2.4.1. Standard stock solution

A nominal 250 mg portion of cefotaxime was accurately weighed, then transferred into a 100 ml of volumetric flask, dissolved and diluted to 100 ml with ultrapure water, degassed by ultrasonic and mixed well. A 2.0 ml of the resulting solution was transferred into a 50 ml of volumetric flask, diluted to 50 ml with ultrapure water, degassed by ultrasonic and mixed well. The resulting solution was stock solution A.

A nominal 100 mg portion of tinidazole was accurately weighed, transferred into a 100 ml of volumetric flask, dissolved and diluted to volume with ultrapure water, degassed by ultrasonic and mixed well. A 2.0 ml of the resulting solution was transferred into a 50 ml of volumetric flask, diluted to volume with ultrapure water, degassed by ultrasonic and mixed well. The resulting solution was stock solution B.

2.4.2. Internal standard solution

A nominal 5 mg portion of metronidazole was accurately weighed, transferred into a 100 ml of volumetric flask, dis-

solved and diluted to volume with ultrapure water, degassed by ultrasonic and mixed well. The resulting solution was internal standard solution C.

2.4.3. Stability test solution

An appropriate amount of cefotaxime powder equivalent to about 1.0 g of cefotaxime was accurately weighed, added to 200 ml tinidazole injection solution (tinidazole 0.4 g) and mixed well. The resulting solution was compatible solution D.

2.5. Compatibility test

2.5.1. Appearance and pH

An appropriate amount of compatible solution D was divided into 10 portions that were stored under the ordinary condition at the room temperature of 20 °C [2,3]. The pH value of each portion was measured while the appearance color and clarity were visually inspected after being stored 0, 1, 2, 4, 6, 8, 18, 20, 22 and 24 h, respectively.

2.5.2. Concentration determination

Another appropriate amount of compatible solution D was divided into 10 portions that were stored under the ordinary condition at the room temperature of 20 °C. A 2.0 ml of each portion was transferred into a 50 ml of volumetric flask after being stored 0, 1, 2, 4, 6, 8, 18, 20, 22 and 24 h, respectively, diluted to volume with ultrapure water, degassed by ultrasonic and mixed well. A 2.0 ml of the resulting solution was transferred into a 25 ml of volumetric flask, added with a 5.0 ml of solution C (I.S. solution), diluted to volume with ultrapure water, degassed by ultrasonic and mixed well. Each well-prepared portion was subjected to chromatographic determination immediately.

3. Results and discussion

3.1. HPLC analysis

3.1.1. Chromatographic parameters optimization

3.1.1.1. Optimal detection wavelengths. It was reported that cefotaxime and tinidazole interfered each other due to the UV spectrum overlap [7], so independent well-prepared solutions of the two components (the cefotaxime solution was about 25 µg/ml, the tinidazole solution was about 10 µg/ml) were prepared herein and subjected to the chromatographic investigation with the ultrapure water serving as the blank to optimize the detection wavelength. It was indicated by the 3D plot of the DAD detector signal that the maximal absorption of cefotaxime was at 238 nm and the absorption of tinidazole was near naught there while the maximal absorption of tinidazole was at 318 nm. A solution of metronidazole (about 10 µg/ml) also showed the maximal absorption at 318 nm. In order to overcome these disadvantages, a dual wavelengths detection strategy was adopted, 238 nm for cefotaxime and 318 nm for tinidazole and metronidazole.

3.1.1.2. Optimal gradient program. Gradient elution was employed due to the two considerations: better resolutions of

cefotaxime, tinidazole, metronidazole (I.S.) and interferents, such as the residuals in the production process of cefotaxime and tinidazole, shortened retention time for a more timesaving analysis. An optimal gradient program was chosen after repeated attempts as the description under Section 2.3.

3.1.1.3. The choosing of the internal standard. Metronidazole was supposed to be used only as the I.S. of tinidazole previously. Moreover, it was found that metronidazole could also be a favorable I.S. of cefotaxime in satisfied accuracy and precision of the method, which may be owed to the similarity of their chemical structures. Therefore, metronidazole was used as the I.S. of both tinidazole and cefotaxime in this study.

3.1.2. Selectivity and resolution

An accurate 5.0 ml of the solution A, an accurate 5.0 ml of the solution B and an accurate 5.0 ml of the solution C were transferred into a 25 ml of volumetric flask, diluted to volume with ultrapure water, mixed well and subjected to the analysis under the same conditions of Section 2.3. The typical chromatogram is shown in Fig. 2. Cefotaxime, metronidazole and tinidazole were completely separated from each other. The retention times were 2.254 for cefotaxime, 4.701 for metronidazole and 5.493 for tinidazole with the theoretical plates 5234, 15496 and 38708, respectively. The cefotaxime–metronidazole resolution was 17.72 and the metronidazole–tinidazole resolution 6.02. No any other impurity peaks were observed in pure solvent's chromatograms.

3.1.3. Linearity

An accurate series of 1.0, 2.0, 4.0, 5.0, 6.0, 8.0 and 10.0 ml of the solution A were transferred into seven 25 ml of volumetric flasks, then an accurate series of 1.0, 2.0, 4.0, 5.0, 6.0, 8.0 and 10.0 ml of the solution B were transferred into the above volumetric flasks accordingly, each containing an accurate 5.0 ml of the solution C, diluted to volume with ultrapure water, mixed well and subjected to the analysis under the conditions of Section 2.3.

The calibration curves were plotted by analyzing solutions containing 3.982–39.82 µg/ml of cefotaxime and 1.597–15.97 µg/ml of tinidazole, each sample containing the same amount of internal standard metronidazole. The area ratios of cefotaxime to I.S. were plotted versus the concentrations of cefotaxime. The calibration curve was linear with the regression coefficient of 0.9997 with intercept standard deviation 2.2371 and slope standard deviation 0.2452. The calibration equation was: $y = 0.05721x - 0.04836$, here y is the peak-area ratio of cefotaxime to metronidazole and x is the corresponding concentration of cefotaxime. Following the same way, the area ratios of tinidazole to I.S. were plotted versus the concentrations of tinidazole. The calibration curve was linear with the regression coefficient of 0.9998 with intercept standard deviation 1.8962 and slope standard deviation 0.2017. The equation was: $y = 0.06128x + 0.00410$, here y is the peak-area ratio of tinidazole to metronidazole and x is the corresponding concentration of tinidazole.

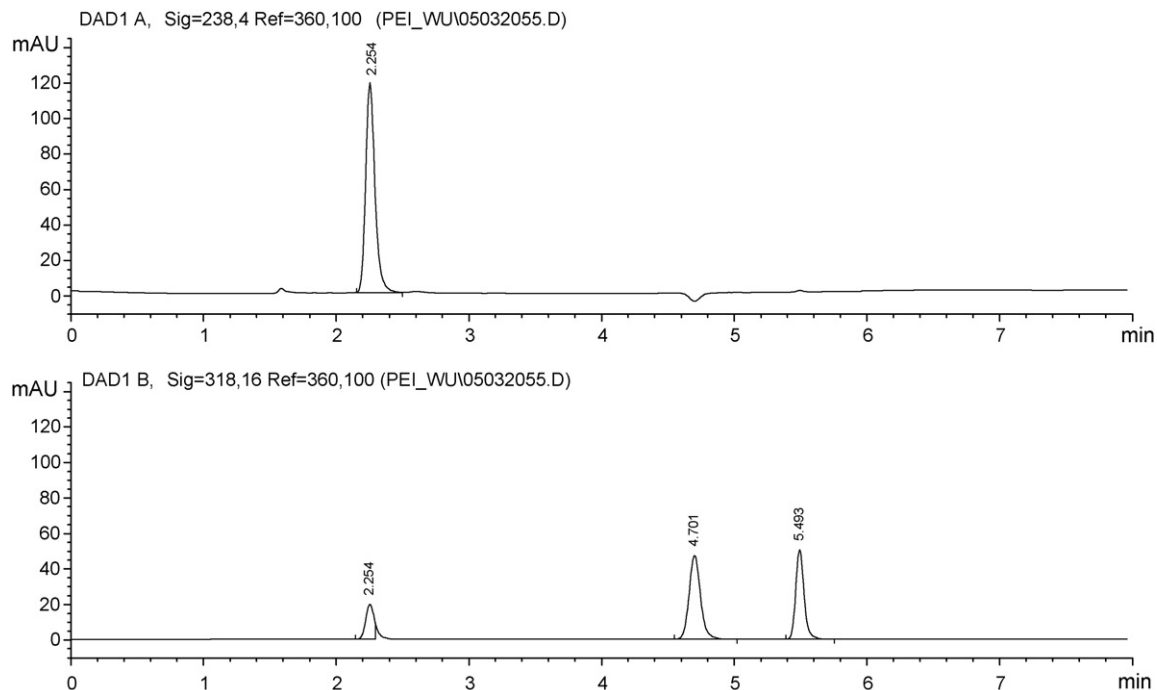


Fig. 2. HPLC chromatograms of compatible solution. Cefotaxime: 2.254 min, metronidazole (I.S.): 4.701 min, tinidazole: 5.493 min. Mobile phase: methanol–water, gradient elution: 0–2 min, 18% methanol; 2–4 min, 18% methanol to 40% methanol; 4–6 min, 40% methanol; 6–7 min, 40% methanol to 18% methanol; 7–8 min, 18% methanol. Flow rate 1.0 ml/min; detection: 238 and 318 nm; injection sample volume: 20 μ l.

3.1.4. Precision

An accurate 8.0 ml of the solution A, an accurate 8.0 ml of the solution B and an accurate 5.0 ml of the solution C were transferred into a 25 ml of volumetric flask, diluted to volume with ultrapure water, mixed well and subjected to the analysis under the identical conditions with Section 2.3. The R.S.D. for five replicate injections was 3.17% for the peak-area of cefotaxime and 0.29% for the peak-area ratio of cefotaxime to metronidazole, and the R.S.D. for five replicate injections was 2.95% for the peak-area of tinidazole and 0.16% for the peak-area ratio of tinidazole to metronidazole.

3.1.5. Accuracy

Three test solutions of low, moderate and high concentrations were prepared to obtain accuracy test results as following: (I) cefotaxime 7.96 μ g/ml + tinidazole 3.19 μ g/ml, (II) cefotaxime 19.91 μ g/ml + tinidazole 7.99 μ g/ml and (III) cefotaxime 31.86 μ g/ml + tinidazole 12.78 μ g/ml. These samples were prepared by mixing an accurate amount of the solution A, an accurate amount of the solution B and an accurate 5.0 ml of the solution C into a 25 ml of volumetric flask and diluted to volume with ultrapure water, respectively. Three injections were made for each solution under the identical conditions

with Section 2.3. The peak-area ratio of cefotaxime to metronidazole and tinidazole to metronidazole were recorded and calculated in the calibration curves. The results are shown in Tables 1 and 2.

3.2. Compatibility

3.2.1. Appearance and pH

Visual observations showed that the appearance of all the samples was transparent and a little yellow without deposit, turbidity or gas within 8 h of storage under ordinary conditions. Meanwhile, the pH value decreased slightly, the results are shown in Table 3. After storage of 18–24 h (overnight), the compatible solutions displayed a mild coloration variation and a slight decrease in transparency while the pH value showed continuous decrease.

3.2.2. Concentration

The peak-area ratio of cefotaxime to metronidazole and tinidazole to metronidazole were recorded and the concentrations of the two compositions in the compatible dosage were calculated by the calibration equations. The starting concentration of drug was designed as 100%; all subsequent

Table 1
The results of the accuracy of cefotaxime ($n=3$)

No.	Added C (μ g/ml)	Found C (μ g/ml)	Accuracy (%)	Mean accuracy (%)	S.D. (%)	R.S.D. (%)
I	7.96	7.99	100.38	100.05	0.41	0.41
II	19.91	19.84	99.65		0.62	0.61
III	31.86	31.90	100.13		0.45	0.43

Table 2
The results of the accuracy of tinidazole ($n = 3$)

No.	Added C ($\mu\text{g/ml}$)	Found C ($\mu\text{g/ml}$)	Accuracy (%)	Mean accuracy (%)	S.D. (%)	R.S.D. (%)
I	3.19	3.21	100.63		0.81	0.80
II	7.99	7.89	98.75	99.85	0.68	0.67
III	12.78	12.80	100.16		0.86	0.86

Table 3
Matrix pH and appearance vs. store time

Time (h)	pH	Appearance
0	6.55	Colorless
1	6.55	Colorless
2	6.54	Colorless
4	6.54	Colorless
6	6.54	Colorless
8	6.52	Colorless
18	6.50	Colorless
20	6.47	Pale yellow
22	6.44	Pale yellow
24	6.44	Pale yellow

Table 4
The ratio (%) of the amount of cefotaxime and tinidazole in the solutions to the original amount ($n = 3$)

Time (h)	Cefotaxime (%)	R.S.D. (%)	Tinidazole (%)	R.S.D. (%)
0	100		100	
1	100.69	0.48	100.11	0.56
2	99.85	0.62	100.06	0.63
4	99.03	0.29	100.30	0.34
6	98.67	0.44	100.01	0.38
8	98.12	0.37	99.27	0.59
18	95.98	0.60	99.31	0.34
20	94.71	0.35	100.54	0.27
22	93.42	0.59	99.98	0.50
24	91.05	0.27	99.73	0.62

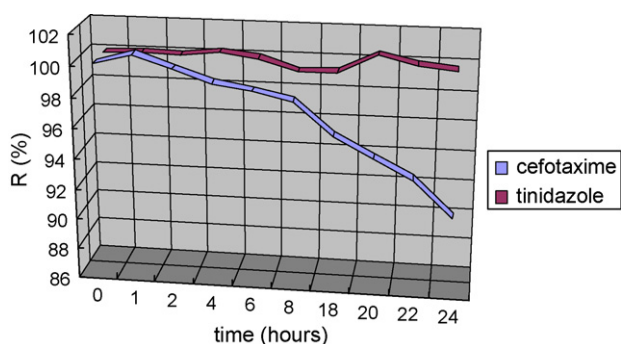


Fig. 3. Stability plot of cefotaxime and tinidazole (%) under room temperature vs. time.

concentrations were expressed as a percentage of the starting one. Stability was defined as 97–105% the starting concentration. Degradation data under storage conditions in terms of the ratio (%) of the amount of cefotaxime and tinidazole remaining in the compatible solutions to the initial amount (100%) are reported in Table 4 and plotted in Fig. 3. It indicated that cefotaxime and tinidazole were stable in these compatible solutions for 8 h at room temperature.

4. Conclusion

The compatibility of cefotaxime and blank glucose injection (blank compatible solution) was investigated in pretest which indicated that the ratio of cefotaxime decreased slightly to 98.53% after storage of 24 h under room temperature. It was concluded that there was little variation in the appearance, slight decrease in the pH value and no significant change in the amount of the two components within 8 h storage. Moreover, the results indicated that cefotaxime was unstable, and the concentration of tinidazole showed no significant change while that of cefotaxime decreased significantly with the ratio being 91.05% to 24 h, much lower than that of blank compatible solution, 98.53%, meanwhile the appearance and pH value showed variation accordingly. As a conclusion, the usage of the compatible solution consisting of cefotaxime and tinidazole glucose injection should be carried out within 8 h which complies with the accepted values for clinical guidelines. Whether the degradation mechanism of methyl acetate of cefotaxime is unclear in the compatible solution upon long time storage and the substantial causation needs further investigation.

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