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Rapid communication

UV absorption method should not be applied for determining amoxycillin in acidic dissolution test medium

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Abstract

In order to clarify the suitability of assay method, the stability of amoxycillin (AMX) in the acidic solution at pH 1.2 was determined by two different methods, ultraviolet absorption (UV) and HPLC methods. The determination was simultaneously performed for the same sample solutions. The data from HPLC method indicated that AMX degraded and the concentration of AMX in the acidic solution was decreased. The apparent first-order rate constant (k_{obs}) for the degradation was 9.83×10^{-2} h^{-1}, and the half-life was calculated to be 7.04 h. On the other hand, the data obtained from UV method apparently represented that the concentration of AMX increased with time-course. This phenomena was brought about by the increase of UV absorption at 272 nm, which accompanied with the degradation of AMX. These results clearly indicate that UV method can not be used for determining the samples accompanying the degradation of AMX. In general, the dissolution test (drug release test) has been used for the evaluation of a sustained-release dosage form of AMX. It is impossible to avoid the degradation of AMX in the test under acidic conditions. The results indicated that the UV method should not be used for the determination of AMX in the test.

Keywords: Amoxycillin; Determination method; Stability; Ultraviolet absorption method; Dissolution test; Acidic solution

Amoxycillin (AMX) is an oral antibiotic which has a wide spectrum of antibacterial activity. AMX is usually administered three times a day due to its pharmacokinetic profiles with short blood half-life in human. In order to improve the pharmacokinetic profile by the pharmaceutical technique, a sustained-release dosage form for once- or twice-a-day administration was investigated (Hilton and Deasy, 1992). Recently, AMX is used for the treatment of *Helicobacter pylori* infection. Following this change of usage, a drug delivery system for the eradication of *H. pylori* has been reported (Patel and Amiji, 1996).

A dissolution test or a drug release test as in vitro evaluation method is essential for the studies on developing the sustained-release dosage forms and the drug delivery systems for eradication of

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H. pylori. In the studies previously reported, the buffer solutions around pH 1.2 have been used to evaluate functions of the dosage forms in stomach, and the buffer solutions at pH 6–7 have also been used to evaluate them in small intestine. AMX has been generally determined by ultraviolet absorption method (UV method) at around 272 nm in those experiments (Hilton and Deasy, 1992; Patel and Amiji, 1996; Whitehead et al., 2000).

On the other hand, the stability of AMX in aqueous solution was reported to be rather unstable, and the half-life of AMX in the acidic solution at pH 1 was 5.2 h (Erah et al., 1997). It was considered from this report that the evaluation of sustained-release dosage forms using the dissolution test or the drug release test was difficult under acidic conditions, for example, at pH 1. However, some reports described that the dissolution test and the release test of AMX had no trouble, and that their dosage forms tested were useful (Hilton and Deasy, 1992; Patel and Amiji, 1996; Whitehead et al., 2000).

We also tried to determine the dissolution profile of AMX in the buffer solutions at pH 1.2 from an intragastric buoyant sustained-release tablet. In this study, we found that the dissolution data at pH 1.2 obtained by UV method was quite indefinite. This determination method is of general, so we report our data as a rapid communication.

AMX and HPLC grade acetonitrile were purchased from Sigma Chemical Company (St. Louis, MO, USA) and Wako Pure Chemical Industries, Ltd (Osaka, Japan), respectively. Other reagents used were of special reagent grade.

AMX in sample solution was determined by following two different methods. (1) Ultraviolet absorption (UV) method: the absorbance of sample solutions was measured at 272 nm using a Hitachi 220A spectrophotometer (Tokyo, Japan) and the concentration of AMX was calculated by using a linear calibration curve. After a sample solution was taken out, the measurement of absorbance at 272 nm was performed as soon as possible. (2) HPLC method: the HPLC system consisted of a Shimadzu model LC-9A pump, SIL-6B auto-injector, SPD-6A UV photometrical detector, and CTO-6B column oven equipped with a Shimadzu model SCL-6B system controller and C-R4A chromatopac, all from Shimadzu (Kyoto, Japan). The chromatographic column was a YMC Pack AM312 ODS (150 × 6 mm, i.d.) obtained from YMC Co, Ltd (Kyoto, Japan). The values for the flow rate, the wavelength for determination, and the temperature of the column were 1 ml min^{-1}, 273 nm, and 40 °C, respectively. The mobile phase used for the determination of AMX was acetonitrile–water–$HClO_4$–$NaClO_4$ (128:872:1:5, v/v/v/w). The retention time of AMX was about 8 min. The concentration of AMX was calculated by using to a linear calibration curve. The injection sample for HPLC was prepared by following procedure; a 200 μl of sample solution was added to 200 μl of the second fluid of Japanese Pharmacopoeia XIV, and then well mixed. A 30 μl of the solution was injected onto the HPLC column.

For the stability study of AMX in acidic solution at pH 1.2, AMX was dissolved in the buffer solution at pH 1.2 that was the first fluid of the Japanese Pharmacopoeia XIV (JP XIV) to make an 1 mg ml^{-1} solution. A 90 ml aliquot of the buffer solution was pre-incubated for 30 min at 37 °C. The experiments were performed at 37.0 ± 0.2 °C and initiated by adding the AMX solution to produce a final concentration of 100 μg ml^{-1}. A 6 ml aliquot of the sample solution was taken out at appropriate intervals. The concentration of AMX in the sample solution was determined by UV and HPLC methods.

To prepare an intragastric buoyant sustained-release tablet, AMX (25 mg), 235 mg of hydroxypropylcellulose-H, 17.2 mg of citric acid (anhydride), and 22.8 mg of sodium bicarbonate were mixed and directly compressed under 120 kg cm^{-2} for 30 s using a Shimadzu hydraulic press.

Dissolution study was performed in duplicates using the JP XIV apparatus, and the paddle method of JP XIV at 100 rpm of paddle speed. The medium used was 900 ml of the first fluid of JP XIV, which was maintained at 37.0 ± 0.5 °C. Two tablets were used for each experiment. Samples were taken at appropriate time intervals and

Table 1
Stability of AMX in acidic solution at pH 1.2; difference of residual percent between UV and HPLC methods

Time (h)	Residual percent of AMX (%)	
	UV method	HPLC method
1	102.72 ± 1.00	94.42 ± 0.90
2	106.45 ± 0.80	85.40 ± 1.40
4	111.31 ± 0.71	70.35 ± 0.59
6	112.92 ± 0.69	57.65 ± 0.08

Each value represents the mean $\pm$ S.D. ($n = 3$). The initial concentration of AMX was 100 μl ml^{-1}.

assayed by UV and HPLC methods. Fresh media was added to replace the sample taken.

Table 1 shows the stability data of AMX in the acidic solution at pH 1.2, which were determined by two different methods, HPLC method and UV method. The data from HPLC method indicated that AMX degraded and the concentration in the solution was decreased. The apparent first-order rate constant (k_{obs}) for the degradation was 9.83×10^{-2} h^{-1}, and the half-life was calculated to be 7.04 h. This result was well agreement with the results of Erah et al. (1997). On the other hand, the data obtained from UV method apparently represented that the concentration of AMX was increased with time-course. This phenomena was brought about by the increase of UV absorption at 272 nm which accompanied with the degradation of AMX. These results clearly indicate that UV method can not be used for determining the samples accompanying degradation of AMX.

Table 2
Dissolution of AMX from the intragastric buoyant sustained-release tablet by using two different determination methods, UV and HPLC

Time (h)	% dissolved	
	UV method	HPLC method
1	18.4	14.9
2	28.4	19.7
4	41.8	26.0
6	54.3	28.4

Each value represents the mean of duplicates. The same sample solution was used for each determination method.

Table 2 shows the result of dissolution test for the intragastric buoyant sustained-release tablet at pH 1.2. We determined AMX both of UV and HPLC methods in this study. If the dissolution data were obtained from UV method only, the dissolution profile was evaluated to be favorable as a sustained-release tablet of AMX. However, the actual concentrations of AMX determined by HPLC method were rather lower values than the values of UV method. The difference increased with time-course.

The previously reported data that showed the dissolution and release profiles of AMX at around pH 1.2 are considered to be quite indefinite (Hilton and Deasy, 1992; Patel and Amiji, 1996; Whitehead et al., 2000). Furthermore, the reported characteristics of the new dosage forms is doubtful due to the usage of erroneous evaluation method. The data is not shown in this report, but the absorbance at 272 nm was increased with the degradation of AMX in the aqueous solutions at pH 4–7. In addition, the increase of absorbance was observed at not only 272 nm but also 250–300 nm.

As mentioned above, the generally used evaluation method for a sustained-release dosage form of AMX, which is a dissolution test or release test using around pH 1.2 buffer solution and employs UV method, is found to be no confidence. The problem of the determination method of AMX is solved by HPLC method. However, in order to examine the dissolution or release profile of AMX from a new dosage form under acidic conditions, at least the evaluation of the stability of AMX in the test medium used and in the dosage form should be required.

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