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Quality of Taiwan Pharmaceuticals: Survey of Ampicillin Capsules

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ABSTRACT

In order to understand the quality of ampicillin capsules, fifty samples (46 domestic, 4 imported) were purchased from different counties and cities in Taiwan during 1991 fiscal year. All of the samples were investigated for appearance, mean weight, weight uniformity, identification of drug, potency, dissolution, content uniformity, and loss on drying. The results showed that 5 samples (10.0%) failed to meet the potency requirement of the official regulation (90-120%) and the contents of uniformity. Besides, there were three samples (6.0%) did not meet the specification of dissolution test.

Key words: **Ampicillin, quality, HPLC.**

INTRODUCTION

Ampicillin is a semisynthetic broad-spectrum penicillin and is acid stable but penicillinase sensitive. Its antimicrobial spectrum includes many gram-positive and gram-negative bacteria. It is effective in treating the infections of microorganisms such as *Escherichia coli*, *Haemophilius influenzae*, *Neisseria gonorrhea*, and *Proteus mirabilis* strains. Though the resistance of organisms to ampicillin is becoming more common[1-3], it is still one of the most frequently used antimicrobials in the treatment of respiratory disorders. In addition, ampicillin is well absorbed following oral administration[4].

Ampicillin, while intended for administration in liquid dosage forms, must be marked as the dry preparations and reconstituted before use[5] because of its relatively readily degraded nature in aqueous solution. For example, ampicillin in a concentration of 250 mg/mL at room temperature was found to lose 10% of its antimicrobial activity within one hour[6]. Anhydrous ampicillin preparation is considerably more stable to heat and humidity than its monohydrate derivatives. At 107 °C and 90% relative humidity, the decomposition tendency of the products of monohydrate ampicillin was estimated to be 79% while that of the anhydrous products was only 1%[7]. Conceivably this is due to the hydrolysis initiated by water molecule. It is therefore that the ampicillin preparations are always advised to store in the absence of moisture and at low temperature. Obviously, the hydroscopic nature of ampicillin

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might be the reason that the potency of its products is much easier to decrease than that of the other antibiotic products.

The present official assay method in British Pharmacopoeia(BP)[8] 1988 for the analysis of ampicillin bulk preparations is the imidazole-mercury coloring method. The United States Pharmacopeia(USP)[9] XXII described two official methods for the assay of ampicillin preparations: the high performance liquid chromatographic (HPLC) method for the bulk preparations and the iodometric assay for ampicillin final products. The Minimum Requirements for Antibiotic Products of Japan (MRAP)[10] 1986, stated three official methods for potency assay of ampicillin preparations: microbiological method, iodometric assay and spectrophotometric method. The regulations state that the results obtained from the microbiological method shall be conclusive. In this report, the ampicillin assay was first carried out by the HPLC method[9]. The failed samples were then re-tested by the microbiological method[10] and the optical method[10].

The primary responsibility for maintaining the quality of medicaments rests with the manufacturers, however, the Department of Health has a supervisory role. National Laboratories of Foods and Drugs (NLFD), the laboratory supporting division of Department of Health in Taiwan, has been conducting post-certification studies as well as pre-certification studies on commercial dosage forms of broad varieties of drugs. In order to understand the quality of ampicillin capsules, 50 samples (46 domestic, 4 imported) were purchased from drug stores in different counties and cities in Taiwan during 1991 fiscal year. All of the samples were investigated for appearance, mean weight, weight uniformity, identification of drug, potency, dissolution, content uniformity and loss on drying. The test specifications used in this survey are mainly from the United States Pharmacopeia XXII[9] and the Chinese Pharmacopeia[11] 1980 and, in some cases, from the manufacturers.

MATERIALS AND METHODS

I. *Reagents and Materials*

Acetonitrile (LC grade) was supplied by ALPS Chemical CO., Taipei, Taiwan. Glacial acetic acid (reagent grade) was supplied by E. Merck's Chemical CO., Darmstadt, Germany. Monobasic and dibasic potassium phosphate, citric acid and copper sulfate were purchased from Wako Pure Chemical Industries, Ltd, Japan. Ampicillin reference standard was obtained from United States Pharmacopeial Convention, Inc., Rockville, U.S.A. *Bacillus subtilis* was purchased from Culture Collection and Research Center, Hsinchu, Taiwan. The medium were purchased from Difco Co., Detroit, Michigan, U.S.A. Lot numbers of ampicillin capsule preparations were obtained from commercial sources.

II. *HPLC Method*

(Ⅰ) *Instruments*

A model 576 liquid chromatographic pump (Gasukuro Kogyo, Inc., Tokyo, Japan), a Gasukuro Kogyo model 502U spectrodetector, and a Gasukuro Kogyo model 12 chromatocorder were employed during the study. The mobile phase was pumped through a reversed-phase column (μBondapak C_{18}; 300 mm × 3.9 mm id.; 10μm; Waters P/N 27324) with an isocratic flow rate of 2.0 ml/min. The wavelength of UV detector was set at 254 nm, and chromatography was performed at room temperature. The injection volume was 20 μl for all the solutions.

(Ⅱ) *Mobile Phase*

The mobile phase was water - acetonitrile - 1M monobasic potassium phosphate - 1N acetic acid (909:80:10:1, v/v/v/v)[9]. It was filtered (0.45 μm, Millipore filter) and degased with an ultrasonic bath prior to use.

(Ⅲ) *Solutions of Standard and Samples*

Ampicillin standard or capsule contents were

accurately weighed and dissolved in water and mixed completely. The final concentration of these test solutions was 1.0 mg/ml and stocked for later analyses.

III. *Optical Method*

One ml of the standard and sample solution were pippetted and diluted with cupric sulfate test solution to 50 ml, respectively. These solutions were warmed in a water bath at 75 °C for 30 minutes and cooled rapidly to room temperature. The absorbance of each solution was determined by a Shimadzu UV-160A spectrophotometer at a wavelength of 320 nm[10].

IV. *Microbiological Assay*

Bacillus subtilis (ATCC 6633) was used in the bioassay. According to the cup plate method, standards and samples were prepared as 1.0 mg/ml (potency) concentrated solution with distilled water and then diluted to 5 and 1.25 μg/ml with 1% phosphate buffer solution (pH 6.0) on the day of analysis[10]. Five petri dishes with 9.0 cm inside diameter were used for each sample. After incubation at 37°C for 16 to 18 hours, the inhibition zone diameter was measured by a zone analyzer (ZA-F; Toyo, Tokyo, Japan).

RESULTS AND DISCUSSION

The results of the nature of non-complying of ampicillin samples are shown in Table 1. The appearance and loss on drying of all of the products were satisfactory. It is a requirement by Department of Health that a manufacturing date or batch number should be displayed on the label. One ampicillin sample did not comply with this criterion. Antibiotic (e.g. ampicillin) products are also required to display an expiration date. Two ampicillin samples did not comply with this criterion. A medicament has to contain the stated amount of the active ingredient. Five ampicillin capsules fell outside the specified content limits. Among these, one sample contained 67.8% of ampicillin and 15% of amoxicillin. It is considered a serious shortcoming because of non-complying with the active ingredient. The other four samples gave low assay results (68.3%, 80.7%, 85.9%, 88.8%). The uniformity of the content of active ingredient per dosage unit is an important

Table 1. Nature of non-compliances

	Non-complying Samples[a]	
Quality Aspect	Number	Percentage
Appearance	0	0%
Loss on Drying	0	0%
Labelling		
Lack of Expiry Date	1	2%
Lack of Manufacturing Date (or Batch Number)	2	4%
Unsatisfactory Identification	1	2%
Outside assay Limits	5	10%
Non-uniformity of Weight	1	2%
Non-uniformity of Content	5	10%
Dissolution	3	6%

[a]: Total member of samples was 50 (domestic 46, imported 4)

Table 2. Survey results for the potency of ampicillin capsules

Fiscal Year	Manufacturer	Potency		Number of Samples
		90-120%	<90%	
1986	Domestic	144 (71.6%)	57(28.4%)	201
	Imported	23 (95.8%)	1 (4.2%)	24
	Total	167 (74.2%)	58(25.8%)	225
1989	Domestic	34 (70.8%)	14(29.2%)	48
	Imported	2(100.0%)	0 (0%)	2
	Total	36 (72.0%)	14(28.0%)	50
1991	Domestic	42 (91.3%)	4 (8.7%)	46
	Imported	3 (75.0%)	1(25.0%)	4
	Total	45 (90.0%)	5(10.0%)	50

indication of the uniformity of the product throughout the batch. Five samples did not comply with the requirement.

The USP specification for dissolution of ampicillin capsules was used. No less than 75% of the labeled amount of ampicillin should be dissolved in 45 minutes. The test for dissolution is an indication of the bioavailability of the formulation. If a formulation does not meet the dissolution test, then it is presumed that the efficiency of this product will be impaired. Three samples failed to meet the specification. The assay values for these three samples were all less than 90% of the declaration.

Comparison of this survey results with previous data (28.0% failed to meet the requirement of the official regulation in 1989 fiscal year and 25.8% in 1986 fiscal year) suggested that the quality of ampicillin pharmaceuticals were indeed improved (Table 2). In the event of an unsatisfactory product being detected, it is the responsibility of the Taiwan Provincial Department of Health to take appropriate actions. Which normally includes penalty, and withdrawal of the defective batch from the market.

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臺灣地區市售安比西林膠囊製劑力價之調查

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摘 要

本計畫係於民國80年度，由全省各縣市抽購國產及進口安比西林(Ampicillin)口服膠囊製劑共50件，其中國產46件，進口4件，進行下列各分析項目之檢驗，其中包括外觀、平均重量、重量均一度、鑑別、力價、溶離度、含量均一度及水份含量。檢驗結果有5件檢體力價及含量均一度未達合格標準，不合格率為10.0%。另有3件檢體溶離度不符藥典規定，不合格率為6.0%。