High-Performance Liquid Chromatographic Method for the Determination of Blasticidin S in Formulated Products with Photodiode Array Detection

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An HPLC method with photodiode array detection is not only promising in distinguishing between the blasticidin S product and other antibiotic fungicide products that are falsely claimed to contain blasticidin S, but also for being capable of analyzing blasticidin S content in different formulations. The HPLC method detection limit was 0.05 μg/mL. The relative standard deviation (RSD) values of HPLC for the determination of blasticidin S in formulated products ranged from 0.70 to 2.83, and the RSD values for bioassay method ranged from 1.81 to 9.27%. This HPLC method may provide a useful monitoring technique for residue analysis.

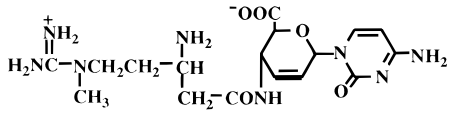
Keywords: *HPLC; bioassay; antibiotic fungicide; blasticidin S; molecular weight; detection limit; sensitivity; selectivity*

INTRODUCTION

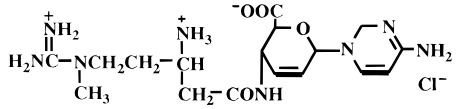
Blasticidine S [1-(4-amino-1,2-dihydro-2-oxopyrimidin-1-yl)]-4-[(*S*)-3-amino-5-(1-methylguanidino)valeramido]-1,2,3,4-tetradeoxy-β-D-*erythro*-hex-2-enopyranuronic acid (MW = 422.4; Figure 1, **I**) is a systemic antibiotic fungicide that controls the most destructive blast disease of rice plants caused by the fungus *Pyricularia oryzae* (Takeuchi et al., 1958). The minimum inhibitory concentration against the pathogen is 1−5 μg/mL (Yonehara, 1984). The free base of blasticidin S is a white, needle-like crystal that melts at 253−255 °C, with decomposition (Takeuchi et al., 1958), and its 4-(*N*-benzylamino)benzenesulfonate (Figure 1) is used in formulation products.

Official product analysis is conducted by a bioassay with *Bacillus cereus* IMA 1729 developed by Kaken Pharmaceutical Company, Japan. However, there are several problems associated with this bacteria bioassay. First, it is not easy to control the analysis quality. Second, it is not a selective method to distinguish a real product from a substitute product. Finally, it is a time-consuming method that usually takes ~3 days to analyze one sample. For example, a series of preliminary analyses showed that the response of inhibition zones sometimes decreased; the exact reason for the decrease is not clear. Aging of bacteria during the preparation for the bioassay, contamination by other less toxic organisms, such as *Aspergillus terreus*, that can transform the fungicide into a less toxic metabolite (Yamaguchi et al., 1972; Yamaguchi and Misato 1985), or development of blasticidin resistant strains of *Bacillus cereus* (Endo et al., 1987) might be the factors. Thus, an efficient and selective method without loss of activity is important.

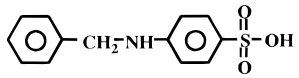
Gas chromatography (GC) is not a good method to analyze the blasticidin S because of the high melting point of the antibiotic compound. However, high-performance liquid chromatography (HPLC) could be used to detect the antibiotic compound because preliminary analysis with UV spectrometry indicated that blasticidin S absorbed UV light, with maximum absorption observed at 266 nm (0.1 N NaOH) or 274 nm (0.1 N HCl). Mierzwa et al. (1988) developed an HPLC method with photodiode array detection (220 nm) to analyze leucylblasticidin and mildiomycin. However, there is no published report on the use of this HPLC method for the analysis of blasticidin S in formulated products. We discuss here research to develop a more efficient and more selective HPLC method than the traditional bioassay method for the determination of blasticidin S in product formulations.



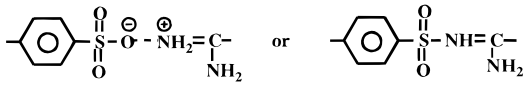
I. Possible actural structure of blasticidin S (MW=422.4)



II. Structure of blasticidin S monohydrochloride (MW=458.9)



III. 4-(*N*-benzylamino)benzensulphonic acid (BABS, MW=263)



IV. Structure of blasticidin S-BABS salt, and the linkage between blasticidin S and 4-(*N*-benzylamino)benzensulphonic acid by charge (MW=720.9, left), or by covalent bond (MW=703.9, right)

Figure 1. Structures of blasticidin S and its monohydrochoride and 4-(*N*-benzylamino)benzenesulfonic salt (BABS).

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MATERIALS AND METHODS

Standard and Samples. The blasticidin S hydrochloride working standard was purchased from Shinung Operation Company and the potency, defined as the weight of blasticidin S contained in the product, was 914 μg/mg. All formulated samples were purchased from 1991 to 1994.

Molecular Weight (MW) Determination. Because there are several kinds of blasticidin S involved [i.e., blasticidin S (MW = 422.4); blasticidin S monohydrochloride (MW = 458.9); blasticidin S-BABS salt (MW = 720.9 or 703.9; Figure 1), the MW of the standard from the manufacturer and the MW of the blasticidin S-BABS salt must be determined to obtain the proper concentration for HPLC calibration. Takeuchi et al. (1958) reported that the decomposition point of blasticidin S hydrochloride was 224−225 °C. Otake et al. (1965 and 1966) reported that the melting point for the monohydrochloride was 229−230 °C (decomposed), and that for dihydrochloride was 195−200 °C (decomposed). The MW of the manufacturer's standard was determined by analyzing the melting point and the decomposition temperature with a DSC 10 differential scanning calorimeter (DSC), a 951 thermogravimetric analyzer (TGA), and a thermal analyst 2000 (DuPont instruments). Temperatures were set from room temperature to 275 °C for DSC and to 680 °C for TGA. The heating rate was set at 10 °C/min and the nitrogen flow was set at 50 mL/min.

Fast-atom bombardment (FAB)-MS was used to identify the structure of the eluent peak from HPLC. Fast atom bombardment and linked scan (MS/MS in first field free region) at constant B/E (magnet/electric sector) were performed on a Jeol SX-102A double-focusing mass spectrometer of reversed geometry (JEOL, Japan). The FAB gun was operated at 6 kV, with xenon as the ionizing gas. One microliter of a sample solution was mixed with 1 μL of matrix (3-nitrobenzyl alcohol) on the FAB probe tip for subsequent analysis. Helium was used as the collision gas, and the pressure of the collision gas was adjusted to reduce the ion beam to 30% of its usual value. The B/E linked scan was acquired at a scan rate of 20 s/scan, and the mass scale in the negative linked scan mode was calibrated with a mixture of alkali halides.

Bioassay. The official method with bacteria was followed (Koken Pharmaceutical method). A stock solution of the blasticidin S hydrochloride working standard was prepared by weighing 0.0228 g of standard into a 25-mL volumetric flask and diluting with sterile 0.067 M phosphate buffer (pH 7.0) to 200 μg/mL (S_H) and 50 μg/mL (S_L). An aliquot of formulated blasticidin S was accurately weighed and diluted with the same sterile 0.067 M phosphate buffer to the definite volume to prepare a sample solution at estimated concentrations of 200 μg/mL (U_H) and 50 μg/mL (U_L). The test organisn was *Bacillus cereus* IMA 1729, and the media was nutrient agar (DIFCO 0001). The ratio (θ) of the potency (P_u) of test sample to that of the standard (P_s) is calculated as follows:

$$\theta = \frac{(\sum U_H + \sum U_L) - (\sum S_H + \sum S_L)}{(\sum U_H + \sum S_H) - (\sum U_L + \sum S_L)} \times \log 4 \qquad (1)$$

$$\theta = P_u/P_s \qquad (2)$$

HPLC System. A Beckman HPLC with a model 126 programmable solvent module, a model 168 diode array detector operated at 265 nm, a model 507 autosampler, and a sample injector valve with a 20-μL sample loop was used to analyze the blasticidin S and its formulations. Separations were achieved on Cosmosil C18AR column (150 × 4.6 mm i.d.) preceded by a guard column of similar packing (50 × 4.6 mm i.d.) at 40 °C. The mobile phase consisted of phosphate buffer (pH 6.0, 0.067 M) and methanol. A sample of 20 μL was injected, and the pump flow rate was set at 0.8 mL/min. The gradient conditions used for the best separation were the following: isocratic at 10% phosphate buffer (in methanol, by volume) for 3 min, linear gradient to 15% within 1 min, maintained 1 min, linear gradient to 50% within 10 min, followed by a hold at 50% for 5 min. The total run time to complete the chromatographic analysis of each sample was 20 min. Solvents used for HPLC were all HPLC grade and were filtered through a 0.45-μm nylon fiter (Phenomenex) when used. Phosphate buffer was prepared by mixing 0.067 M aqueous potassium dihydrogen phosphate (KH_2PO_4) solution and a 0.067 M aqueous disodium hydrogen phosphate (Na_2HPO_4) solution.

The reproducibility of retention time, peak area, linearity, and detection limit was used to evaluate the selectivity, sensitivity, and the reliability of the HPLC method.

HPLC Calibration Curve. Blasticidin S is most stable at pH 5.0−7.0, less stable at pH 8.0−9.0, and least stable at pH 4.0 (Tokeuchi et al., 1958). Therefore, a pH 6.0 phosphate buffer was selected to prepare the stock solution and a pH 7.0 phosphate buffer was used to prepare the working solution. A proper amount of blasticidin S hydrochloride standard (MW = 458.9) was weighed, diluted first with pH 6.0 phosphate buffer (0.067 M) for the stock solution, and then diluted to proper concentrations with pH 7.0 phosphate buffer (0.067 M) for the HPLC calibration curve. The final concentrations of blasticidin S were 0.05, 0.08, 0.1, 0.2, 0.6, 1.1, 2.2, 4.5, 8.9, 17.8, 28.6, 35.8, 44.7, and 107.5 μg/mL. Three replications were conducted, and a linear regression was used to analyze the suitability.

Limit of Detection. The method detection limit (MDL) was determined by adding 0.0028 g of blasticidin S hydrochloride standard (purity 96.0%, HPLC, area%) in a 25-mL volumetric flask and diluting with pH 7.0 buffer to make the final concentration of 107.5 μg/mL. The HPLC analysis was repeated seven times, the standard deviation (SD) was calculated, and the three SD values were used as the MDL. Precision expressed as relative SD (RSD) was used in judging the acceptability of the method.

Matrix Effects. There are three basic formulations for blasticidin S commercial products solution (S), emulsifiable concentrate (EC), and wettable powder (WP). The accurate composition in each formulation was not available, so the influence of the composition on the HPLC method was not known. Thus, a standard addition method (addition of different amounts of standard solution into five equal aliquots of the same formulated samples) was applied to analyze the blasticidin S content in each formulation. A plot of response versus concentration (blank included) extrapolated back would give the abscissa intercept to indicate the original concentration of sample. This extrapolated value was used to compare the matrix effect, and there will be no interference with the HPLC analysis if (*i*) the extrapolated concentration was close to the concentration calculated from the standard calibration curve, and (*ii*) the slope of addition curve was close to the slope of the standard calibration curve within the range ±15%.

RESULTS AND DISCUSSION

Determination of the Molecular Weight of the Blasticidin S Standard. The DSC diagram showed that the melting point of the manufacturer standard was 227.14 °C (decomposed; Figure 2), which was close to the reported melting point of blasticidin S monohydrochloride (mp = 229−230 °C, decomposed; Otake et al., 1965, 1966). These melting points indicate that the standard obtained from manufacturer was in the monohydrochloride form (Figure 1, **II**).

Swaminathan et al. (1981) reported that the crystal of blasticidin S hydrochloride purified from water was a highly hydrated form. This result implied that the blasticidin S monohydrochloride from the manufacturer might be in a hydrated form. However, the TGA diagram showed that there was no weight loss from 70.7 to 207.7 °C (Figure 3). This lack of weight loss indicated that the endothermic peak at 110.37 °C in the DSC profile was a crystal relaxation, and proved that the standard of blasticidin S monohydrochloride salt was in the anhydride form. Thus, a MW of 458.9 g/mol of blasticidin S monohydrochloride was used for calibration

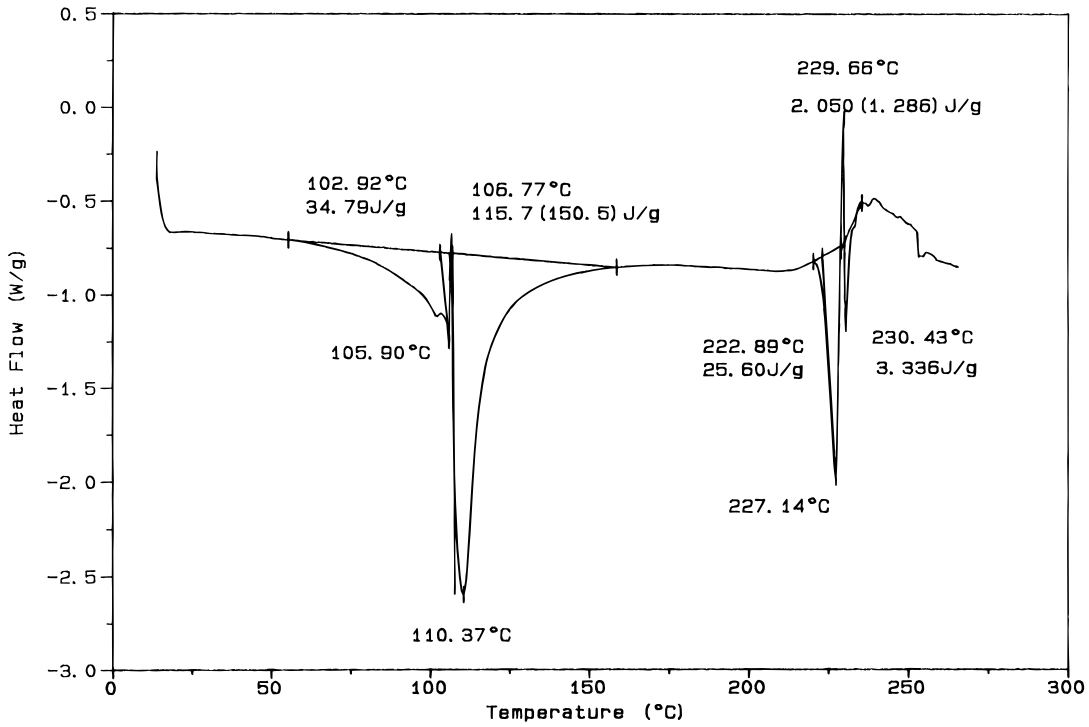

Figure 2. DSC profile of blasticidin S hydrochloride.

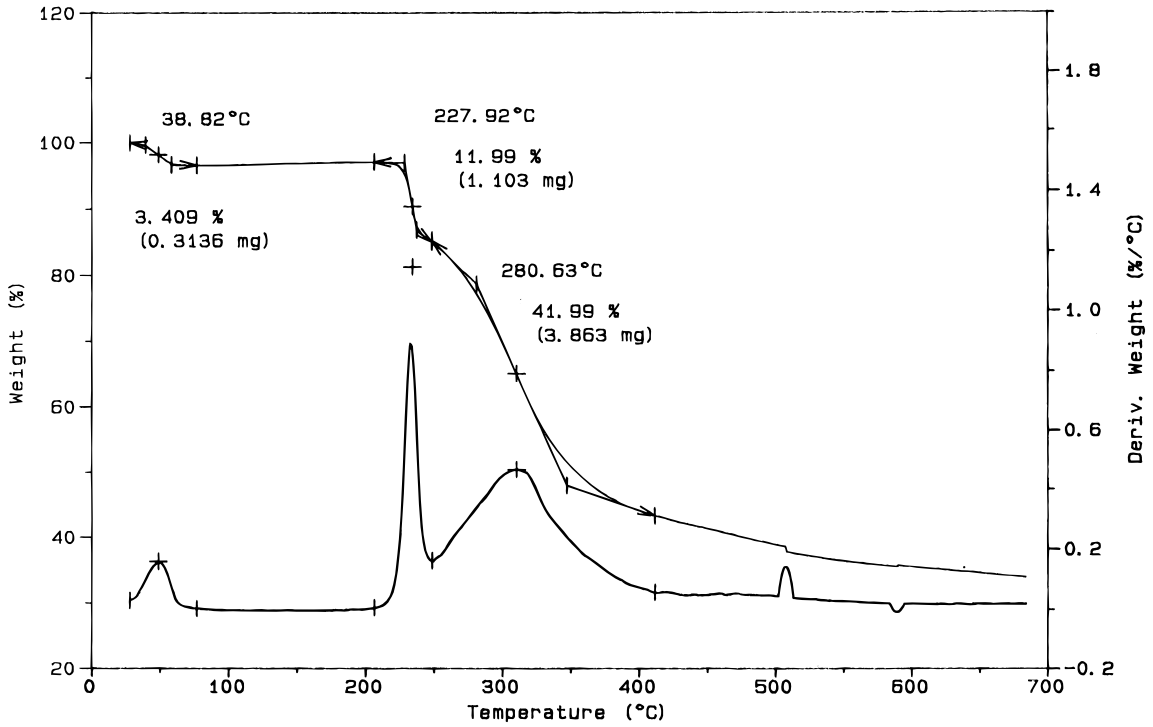

Figure 3. TGA profile of blasticidin S hydrochloride.

(Figure 1), and the chloride ion was adjacent to the amino group of cytosin (Swaminathan et al., 1981).

The TGA diagram showed that there was 11.99% weight loss at 227 °C. This could result from the loss of HCl and H_2O:

$$\frac{MW_{HCl} + MW_{2H} + MW_o \times 100\%}{MW_{\text{blasticidin S monohydrochloride}}} = \frac{36.5 + 18\ g}{458.9\ g} \times 100\% = 11.88\% \quad (3)$$

The HCl might come from the chloride ion and its adjacent H of the amino group of cytosin, and the H_2O might come from the oxygen of carboxylic acid group and its adjacent 2H of the ammonium group (Figure 1, **II**). The DSC diagram showed that this thermodegraded compound might melt at 230.43 °C (Figure 2).

Chromatogram of Blasticidin S Standard. The new HPLC method with photodiode array detection at 265 nm can distinguish real products from imitation products. To demonstrate that the HPLC method was capable of analyzing blasticidin S in commercial products, samples from the market were collected and assayed by the rapid HPLC method and by the conventional bioassay method.

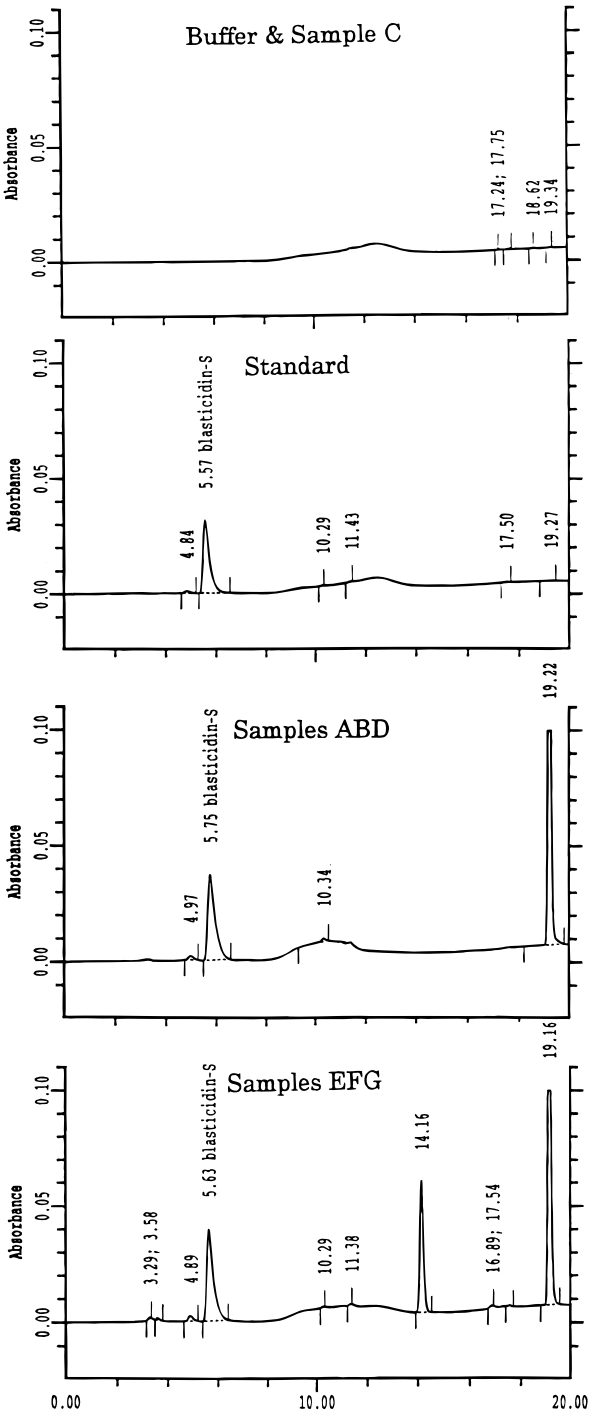

Figure 4. HPLC elution profiles of phosphate buffer, blasticidin S standard, and formulated products. All formulated samples showed blasticidin S peak except sample C.

A typical chromatogram of the blasticidin S standard is shown in Figure 4. The retention times were very consistent, ranging from 5.53 to 5.66 min, with the RSD ranged from 0.02 to 0.38%. The retention time was not influenced by the concentration selected (Table 1).

The blasticidin S was stable in both pH 6 and pH 7 phosphate buffers, indicated that there was no need to use two phosphate buffers for preparing stock solution and working solution and that further investigation could use either pH 6 or pH 7 phosphate buffer.

Chromatogram of Blasticidin S Products. Two HPLC peaks (Figure 4) were found for blasticidin S commercial products of solution (samples A and B) and wettable powder (sample D). The first peak (R_t = 5.75 min) corresponded to the blasticidin S. The second peak (R_t = 19.22 min) was isolated and identified as 4-(*N*-benzylamino)benzenesulfonate (BABS) by the FAB mass spectrum (*m*/*z* 261.80 for $[M\text{-}H]^-$, and *m*/*z* 170.90 for $[261.80\text{-}CH_2\text{-}C_6H_5]^-$). The existence of the BABS peak indicated that the blasticidin S-BABS salt would be dissociated into two parts when contacted with the mobile phase. Also, the bonding between blasticidin S and BABS could be a weak linkage by electrocharge between the sulfonic acid group and the iminium group (MW = 720.9); they were not linked by a covalent bond between S and N (Figure 1). Thus, for the quantitative HPLC analysis in product formulations the MW of the blasticidin S-BABS salt (MW = 720.9) should be calculated back to the MW of blasticidin S (MW = 422.4). For example, an area response in HPLC of 1.0 μg of blasticidin S-BABS salt from a formulated sample would equal to the area response of 0.59 μg of blasticidin S:

Table 1. Precision of Retention Time on HPLC Analysis of Blasticidin S[a]

concn (μg/mL)	retention time (min)	precision (% RSD)
2.2	5.66	0.30
4.5	5.64	0.18
8.9	5.60	0.09
17.8	5.56	0.38
28.6	5.53	0.02
35.8	5.54	0.34
44.7	5.55	0.06

[a] Mean of three measurements.

$$\mathrm{Wt}_{\text{blasticindin S-BABS}} \times \frac{\mathrm{MW}_{\text{blasticidin S}}}{\mathrm{MW}_{\text{blasticidin S-BABS}}} = \mathrm{Wt}_{\text{blasticidin S}} \quad (4)$$

$$1.0\ \mu\text{g} \times (422.4\ \text{g}/720.9\ \text{g}) = 0.59\ \mu\text{g} \quad (5)$$

The same chromatograms were observed for blasticidin S commercial products of emulsifiable concentrates (samples E, F, and G), except one additional peak was found at 14.16 min. This peak possibly reflected a formular composition in the EC formulation, and it had no influence on the separation of active ingredients.

However, there was no peak found for sample C (Figure 4), although the bioassay showed that sample C was active (Table 2). This result indicated that some other antibiotic material instead of blasticidin S was in sample C (i.e., sample C was not an authentic blasticidin S product). This result is very important because the traditional bioassay method is not selective and failed to identify an immitation product. A selective analytical method, such as an HPLC method, is needed.

Sensitivity of the HPLC method. The analysis of the standard solutions from 0.05 to 107.5 μg/mL showed a good linear correlation between the concentration and peak area, with the coefficient of determination (r^2) averaging 0.9993. The MDL was 0.05 μg/mL.

The time to perform the rapid HPLC method for the determination of blasticidin S was compared with that for the traditional bioassay method. It took ~2 days to analyze all seven samples plus create a standard calibration curve by HPLC, whereas it took ~7 days to accomplish the same work by the bioassay method.

Linear Curve from Inhibition Zone. The linear calibration curve could be obtained for the bioassay if the activity was expressed as the inhibition zone (diameter, mm^2). A good correlation was observed from log 5.2 to log 833.6 μg/mL, and the coefficient of determination was 0.9966. The same result was first

Table 2. Comparison between HPLC and Bioassay Determination of Blasticidin S

formulation (% ai claimed)	HPLC (%, RSD)	bioassay %, RSD[e]	bioassay %, RSD[f]	tolerance (%)
A[a] 2% solution (1% blasticidin S)	0.97, 1.52	0.92, 3.33	1.16, 3.52	0.80−1.20
B 2% solution (1% blasticidin S)	1.13, 1.17	1.08, 4.19	1.20, 13.24	0.80−1.20
C 2% solution (1% blasticidin S)	nd[b]		1.71, 9.27	3.72, 31.60	0.80−1.20
D 54% WP[c] (2% blasticidin S)	1.44, 2.83	1.51, 4.57	1.19, 3.36	1.60−2.40
E 2.4% EC (1.2% blasticidin S)	0.79, 0.70	0.95, 1.81	0.93, 3.33	0.96−1.44
F 21.4% EC[d] (0.7% blasticidin S)	0.71, 1.53	0.77, 4.75	0.85, 6.62	0.42−0.84
G 21.4% EC[d] (0.7% blasticidin S)	0.74, 1.01	0.89, 5.00	0.99, 5.60	0.42−0.84

[a] Different manufacturers arranged in alphabetical order. [b] Not detected at a concentration of 0.05 μg/mL). [c] Mixed fungicides (fthalide 50%+4% blasticidin salt). [d] Mixed fungicides (isoprothiolane 20%+1.4% blasticidin salt). [e] Active ingredient is calculated from potency formula. [f] Active ingredient is calculated from the inhibition zone versus log concentration.

Table 3. Effect of Formulation on the Analysis of Blasticidin S by the HPLC Method Evaluated by Standard Addition

formulation (% ai claimed)[a]	extrapolated concn (% ai in formulation)	unspiked concn (% a.i. in formulation)	diff[b] (%)
A 2% solution (1% blasticidin S)	0.84	0.86	−2.4
B 2% solution (1% blasticidin S)	1.14	1.14	0
D 54% WP[c] (2% blasticidin S)	1.42	1.43	0.7
E 2.4% EC (1.2% blasticidin S)	0.76	0.73	3.9
F 21.4% EC[d] (0.7% blasticidin S)	0.63	0.64	−1.6
G 21.4% EC[d] (0.7% blasticidin S)	0.72	0.74	−2.8

[a] Claimed blasticidin S concentration in formulation. [b] Difference between extrapolated concentration and unspiked concentration. [c] Mixed fungicides (fthalide 50% + 4% blasticidin salt). [d] Mixed fungicides (isoprothiolane 20% + 1.4% blasticidin salt).

noted by Yonehara (1984), and a linear relation between the logarithm of concentrations of the blasticidin S and the diameters of inhibition zones was reported in a range 50−1000 μg/mL. But, the RSD values of this method were higher than the potency test (Table 2). Furthermore, the complexity of processes, preparation, and analysis of this method were almost the same as the potency test, and the same disadvantages existed. Thus, determination of a linear curve from the inhibition zone is not an efficient method when compared with the HPLC method.

Influence of Formulations on HPLC Performance. The analysis of blasticidin S in commercial formulation samples was validated by the standard addition method. Commercial samples were fortified with blasticidin S standard. A plot of response versus concentration extrapolated back gave almost the same concentrations as in the original concentration of the samples. For examples, the calculated concentration of blasticidin S in sample A of 2% solution was 0.84% based on the addition method [extrapolated value × 25 mL × dilution factor (10) × 100%/sample weight], and the concentration of blasticidin S in the unspiked sample A was 0.86%. There was only a 2.4% difference (Table 3). For sample B of the same formulation, there was no difference. For the other formulations, the differences were in the range of 3.9 to −2.8%. A typical calibration curve for standard addition is shown in Figure 5.

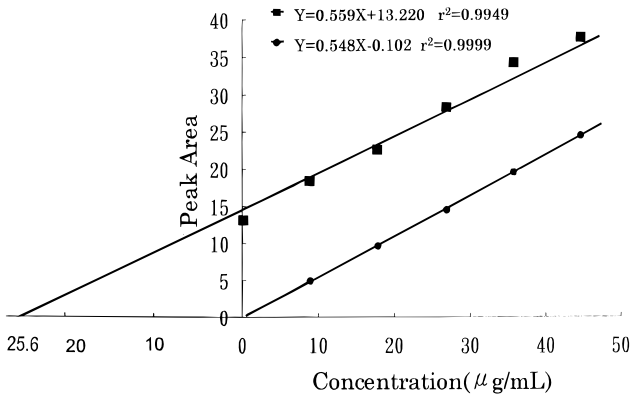

Figure 5. Comparison of the plotting of the standard calibration curve (●) and the standard addition method (■) in blasticidin S product of G (21.4% EC).

Table 4. Effect of Formulation on the HPLC Analysis of Blasticidin S Evaluated by Linear Regression

formulation	slope	intercept	r^2
standard	0.548 (100%)[c]	−0.102	0.9999
A 2% solution	0.607 (110.8%)	12.620	0.9973
B 2% solution	0.547 (99.8%)	15.137	0.9994
D 54% WP[a]	0.532 (97.1%)	9.182	0.9993
E 2.4% EC	0.592 (108.0%)	12.216	0.9998
F 21.4% EC[b]	0.576 (105.1%)	11.715	0.9999
G 21.4% EC[b]	0.559 (102.0%)	13.220	0.9949

[a] Mixed fungicides (fthalide 50% + 4% blasticidin salt). [b]Mixed fungicides (isoprothiolane 20% + 1.4% blasticidin salt). [c] Slope percent of standard curve.

The slope of the standard addition curve of sample B formulation was 0.547, which was almost the same slope value as the standard calibration curve (0.548). The slopes of the standard addition curves of other formulations were in the range 0.532−0.607, which is close to the range of slopes of the standard calibration curve (97.1−110.8%; Table 4).

The coincidence of extrapolated values and the slopes indicated that the formula in the commercial formulation would not interfere with the HPLC method. A simple recovery of spiked sample method could be applied in the future for the quantitation of blasticidin S in formulation.

Conclusion. The HPLC method is a preferred method over the bioassay method not only in sensitivity and selectivity, but also in time consumption and cost reduction. The present official bioassay method should be replaced by the more efficient and accurate HPLC method. Further research with this HPLC method to investigate its application in residue analysis of blasticidin S in the environment is worth considering because blasticidin S is a highly toxic compound with an LD_{50} value of 2.82 mg/kg in mice following intravenous injection. Blasticidin S is also very soluble in water (Yonehara, 1984), a factor that may pose some ecotox problems in the aquatic environment.

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LITERATURE CITED

Anon. Microbiological assay of blasticidin S. Kaken Pharmaceutical Company, Ltd., Tokyo, Japan.

Endo, T.; Furuta, K.; Kaneko, A.; Katsuki, T.; Kobayashi, K.; Azuma, A.; Watanabe, A.; Shimazu, A. Inactivation of blasticidin S by Bacillus cereus. I. Inactivation mechanism. *J. Antibiot.* **1987**, *40*, 1791−1793.

Mierzwa, R.; Cooper, R.; Pramanik, B. Photodiode array detection of peptide-nucleoside antibiotics. *J. Chromatogr.* **1988**, *436*, 259−267.

Otake, N.; Takeuchi, S.; Endo, T.; Yonehara, H. The structure of blasticidin S. *Tetrahedron Lett.* **1965**, *19*, 1411−1419.

Otake, N.; Takeuchi, S.; Endo, T.; Yonehara, H. Chemical studies on blasticidin S part III. The structure of blasticidin S. *Agric. Biol. Chem.* **1966**, *30*, 132−141.

Swaminathan, V.; Smith, J. L.; Sundaralingam, M.; Coutsogeorgopoulos, C.; Kartha, G. Crystal and molecular structure of the antibiotic blasticidin S hydrochloride pentahydrate. *Biochim. Biophys. Acta* **1981**, *655*, 335−341.

Takeuchi, S.; Hirayama; K; Ueda, K.; Sakai, H.; Yonehara, H. Blasticidin S. A new antibiotic. *J. Antibiot.* **1958**, *11*, 1−5.

Yamaguchi, I.; Takagi, K.; Misato, T. The sites for degradation of blasticidin S. *Agric. Biol. Chem.* **1972**, *36*, 1719−1727.

Yamaguchi, I.; Misato, T. Active center and mode of reaction of blasticidin S deaminase. *Agric. Biol. Chem.* **1985**, *49*, 3355−3361.

Yonehara, H. Blasticidin S: properties, biosynthesis, and fermentation. *Drugs Pharm. Sci.* **1984**, *22*, 651−663.

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