Quantitative Determination of Valienamine and Validamine by Thin-Layer Chromatography

Ya-Ping Xue, Yu-Guo Zheng*, Xiao-Long Chen, and **Yin-Chu Shen**
Institute of Bioengineering, Zhejiang University of Technology, Hangzhou 310014, Zhejiang, China

Abstract

A simple and valid thin-layer chromatographic method for the separation and quantitative determination of valienamine and validamine is described. The two compounds are separated using a Silica gel G plate as the stationary phase and a mixture of 1-PrOH–AcOH–H_2O (4:1:1, v/v/v) as the mobile phase. The plate is developed for 1 h at 25°C and dried by a hairdrier, then immersed in 0.1% ninhydrin aqueous solution and heated for 5 min at 121°C. The reacted spots are scanned with a single wavelength at 420 nm in the measurement mode of absorption. The limits of detection of the two compounds are both 0.4 µg. The responses of the densitometry are highly correlated with the amounts of valienamine and validamine in the range of 0.4–2.8 µg. Moreover, the method shows good accuracy and high precision.

Introduction

Valienamine [(1S,2S,3S,4R)-1-amino-5-(hydroxymethyl) cyclohex-5-ene-2,3,4-triol] and validamine [(1S)-(1,2,4/3,5)-1-amino-5hydroxymethyl-2,3,4-cyclohexanetriol] (Figure 1) are two prominent members of the C_7N aminocyclitol family, which is increasingly gaining recognition because of their significant biomedical uses. Because the absolute configurations of the two compounds are similar to that of α-D-glucose, they exhibit strong glucosidase inhibitory and antibiotic activity (1–5). In addition, they are known to be responsible for their biological activities in pseudo-aminosugars and pseudo-oligosaccharides. At present, they are very important chemical intermediates and widely used for the synthesis of other strong glucosidase inhibitors, especially voglibose. In recent years, the method of production of valienamine and validamine has been actively pursued. Valienamine and validamine can be obtained with chemical synthesis (5), *N*-bromosuccinimide cleavage of validoxylamine (6,7), or isolation from the fermentation broth of *streptomyces hygroscopicus* ssp. *Limoneus* (2). The degradation of validamycins to produce valienamine and validamine with microorganism is one of the most promising methods. Several soil bacteria, such as *Pseudomonas denitrificans* (8), *Flavobacterium saccharophilum* (9,10), *Pseudomonas* sp. HZ519 (11), and *Stenotrophomonas maltrophilia* (12), were reported to have the ability to decompose validamycins. Validamycin A, as a major component contained in validamycins, was first hydrolyzed to D-glucose and validoxylamine A, then validoxylamine A was cleaved to produce valienamine and validamine with relevant enzymes. Today in China, the validamycins produced by *Streptomyces hygroscopicus* var. *jinggangensis* (13) are one of the largest yields of antibiotics. This product has been exported to Southeast Asia and other countries. Its fermentation concentration reaches 30,000 µg/mL broth and its cost is very low. Therefore, the method of using validamycins as the substrate to produce valienamine and validamine has great potential.

Gas chromatography (GC) (14) and reversed-phase (RP) high-performance liquid chromatography (HPLC) (15) are reported for quantitative determination of valienamine and validamine. But cumbersome pretreatments, such as solvent extraction and derivatization steps, were inevitably utilized prior to the final analysis, which might reduce the analytical precision. It is also sometimes difficult to perform. This paper describes a simple, valid, and economical thin-layer chromatographic (TLC) method for the separation and quantitative determination of valienamine and validamine. Although the disadvantage of TLC lies in its poor precision, using a sampler device, pre-coated plate, and modern densitometer improves analytical performance markedly.

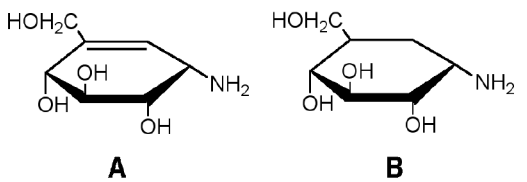

Figure 1. Structures of valienamine (A) and validamine (B).

* Author to whom correspondence should be addressed: email zhengyg@zjut.edu.cn.

Experimental

Microorganism and media

Stenotrophomonas maltrophilia China Center for Type Culture Collection (CCTCC) M 204024, screened in our lab and previously preserved at the CCTCC (Wuhan, China), was used in this study. This microorganism has the ability to produce valienamine and validamine by hydrolysis of validamycin A. The medium consisted of (per liter): 15 g validamycin A, 7.5 g $(NH_4)_2SO_4$, 5 g KCl, 20 g Na_2HPO_4, 0.16 g NaH_2PO_4, and 0.2 g $MgSO_4$. The strain was inoculated into a 250-mL flask containing 50 mL of the medium and incubated for 120 h at 30°C on a rotary shaker (200 rpm).

Apparatus

A TLC system from Camag (Muttenz, Switzerland) was used. It consisted of a semiautomatic TLC sampler (Linomat 5), a twin trough development chamber (10 × 20 cm), a TLC scanner 3, a TLC chromatogram-immersion device III, a TLC sprayer, and a TLC plate heater III (Camag).

Material

A pre-coated Silicagel G plate (10 × 20 cm) was purchased from Qingdao Ocean Chemical Company, (Qingdao, China). D113 resin was purchased from Purolite (China). Validamycin A, which was produced by *Streptomyces hygroscopicus* var. *jinggangensis*, was kindly supplied by Zhejiang Qianjiang Biochem (Haining, China). All the other chemicals were of reagent grade and obtained from commercial sources.

Recommended procedure

TLC calibration

Known amounts of standard valienamine and validamine were dissolved in distilled water to prepare a standard solution in the range of 0.2–1.4 µg/µL. Different concentrations of standard solutions (2.0 µL) were injected in 5 mm bands on the concentration zones of TLC plate at 1.5 cm from the bottom edge of the plate using a semiautomatic TLC sampler (Linomat 5).

The plate was developed for 1 h in a development chamber at 25°C, using 1-PrOH–AcOH–H_2O (4:1:1,v/v/v) as the mobile phase. After air drying, the plate was immersed in 0.1% ninhydrin aqueous solution for one second with a TLC chromatogram-immersion device III. Then the plate was heated for 5 min at 121°C with a TLC plate heater III. The peak area of each spot was determined by using a TLC scanner 3 within 15 min with the following settings: wavelength, 420 nm; slit dimensions, 6.00 × 0.45 mm, micro; scanning speed, 20 mm/s; data solution, 100 µm/step; and measurement mode, absorption. The calibration curve was constructed by plotting the peak area against various concentrations of valienamine and validamine.

Procedure for the assay

As one of the potent methods, valienamine and validamine can be produced by degradation of validamycin A with some microbes. In order to determine the two compounds in the fermentation broth, the following procedure for assay is necessary. The fermentation broth (10 mL) containing valienamine and valienamine was centrifuged (5000 × *g*, 15 min) to discard the cells and the suspension passed through a column of D113 (20 mL, NH_4^+ form) and eluted with 0.5 N aqueous ammonia (100 mL). The eluate was concentrated under vacuum condition at 65°C and diluted to 10 mL with distilled water in a 10-mL volumetric flask, then it was used as a sample for assay. In quantitative TLC, a number of standards were applied to each plate. Each result was calculated on the calibration equation on the basis of values obtained for the standards.

Result and Discussion

Separation of valienamine and validamine

The thin-layer chromatographic separation of valienamine and validamine was carried out using 1-PrOH–AcOH–H_2O (4:1:1, v/v/v) as the mobile phase and a Silica gel G pre-coated plate as the stationary phase. The concentrations of standard valienamine solution and valienamine solution were both 1.0 µg/µL. The standard mixture of valienamine and validamine contained 1.0 µg/µL valienamine and 1.0 µg/µL validamine. Valienamine (2.0 µL), validamine (2.0 µL), and a mixture of the two compounds (2.0 µL) were injected on the concentration zones of TLC plates. They were visualized by reaction with ninhydrin aqueous solution after development. The retention factor values for valienamine and validamine were 0.45 and 0.39, respectively. It can be seen that the two compounds can be separated effectively in this condition.

TLC scanning densitometry

The valienamine and validamine were reacted with 0.1% ninhydrin aqueous solution, and the reacted spots can be detected by visible light scanning. The measurement mode was absorption. In order to increase the sensitivity and selectivity of the detection, the absorption of spectra was measured from 200–700 nm (Figure 2) by a D_2 or W lamp. It

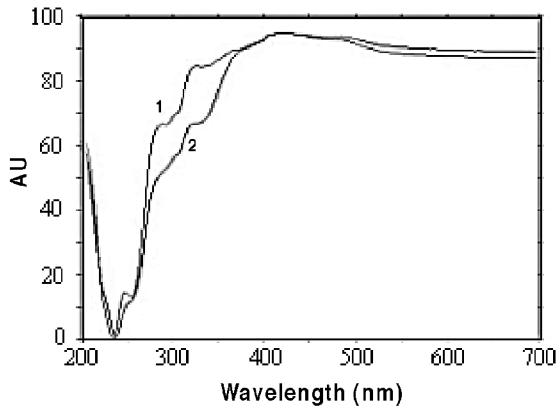

Figure 2. Adsorption spectrum of the reacted spots of valienamine (peak 1) and validamine (peak 2) in the TLC plate (adsorption maximum 420 nm).

was found that the maximum adsorptions of reacted spots of valienamine and validamine were both at approximately 420 nm. At 420 nm, the densitogram of the standard mixture solution containing 1.0 µg/µL valienamine and 1.0 µg/µL validamine is presented in Figure 3A. The detection of valienamine and validamine, which were produced by *S. maltrophilia*, was also preformed. The extraction of valienamine and validamine from the broth was carried out as described earlier, and the densitogram of the sample is shown in Figure 3B.

Limit of detection

The lower detection limits of valienamine and validamine were investigated. The limits of detection (defined as three times of the baseline noise) of the two compounds were the same and calculated to be 0.4 µg/spot. This was the lowest concentration of valienamine or validamine that can be accurately detected and integrated by the instrumentation used. The relative standard deviation (RSD) was 4.05% (n = 6). Below this concentration, the reacted spots of valienamine and validamine were not clearly visible.

Calibration graphs

The calibration graphs for valienamine and validamine were both linear in the range of 0.2–1.4 µg/µL. Linear regression analysis gave correlation coefficients (r^2) 0.993 for valienamine and 0.985 for validamine. The calibration graphs showed good correlation coefficient values, which indicated excellent agreement.

Precision and accuracy

This study was undertaken to document the efficiency of the method. The reproducibility and accuracy of the proposed method were verified by analyzing the aliquots of fermentation broth, which had been added in various amounts to the standard mixture solutions of valienamine and validamine (mixture 1 contained 50 µg valienamine and 50 µg validamine; and mixture 2 contained 100 µg valienamine and 100 µg validamine). Further processing was done as described earlier. The results are shown in Table I, and the average recoveries of valienamine and validamine obtained from six runs were more than 96% and 94%, respectively. It suggested that the method was accurate. The CV (n = 6) of all recoveries for valienamine and validamine were within 5%, illustrating the practical effectiveness and high precision of the method.

Conclusion

The proposed TLC-densitometry method was validated in terms of accuracy, reproducibility, detection, and quantitation limit. It is a simple and sensitive method. The method can be widely used for the quantitative determination of valienamine and validamine, and it can be applied to monitor and control the bioprocesses of valienamine and validamine production.

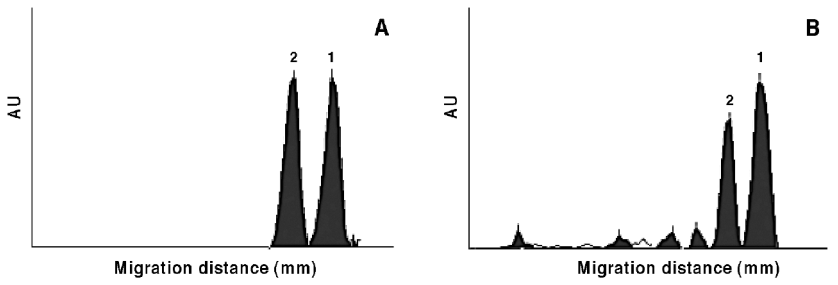

Figure 3. Spectrodensitometric scan at λ = 420 nm of 2 µL standard mixture containing 1.0 µg/µL valienamine and 1.0 µg/µL validamine (A) and of 2 µL sample prepared from the fermentation of both (B). The peak numbers are: valienamine, 1; and validamine, 2.

Table I. Reproducibility and Accuracy of the TLC Densitometry Method

Amounts of valienamine and validamine added (µg)	Amounts of valienamine and validamine found (n = 6) (µg)	Recovery (%)	CV (%)
Mixture 1			
Valienamine: 50	48.2	96.4	2.7
Validamine: 50	48.6	97.2	4.1
Mixture 2			
Valienamine: 100	96.6	96.6	4.4
Validamine: 100	94.1	94.1	3.5

Acknowledgments

This work was supported by the Fund of the Major Basic Research Development Program of China (2003CB716005), the National Natural Science Foundation of China (20176055), and the Natural Science Foundation of Zhejiang Province (Y305242).

References

1. Y. Kameda, N. Asano, M. Yoshikawa, and K. Matsui. Valienamine as an Alpha-glucosidase inhibitor. *J. Antibiot.* **33:** 1575–76 (1980).
2. Y. Kameda, N. Asano, M. Yoshikawa, M. Takeuchi, T. Yamaguchi, K. Matsui, S. Horii, and H. Fukase. Valiolamine, a new alpha-glucosidase inhibiting aminocyclitol produced by *Streptomyces hygroscopicus*. *J. Antibiot.* **37:** 1301–1305 (1984).
3. M. Takeuchi, K. Kamata, M. Yoshida, Y. Kameda, and K. Matsuim. Inhibitory effect of pseudo-aminosugars on oligosaccharide glucosidases I and II and on lysosomal alpha-glucosidase from rat liver. *J. Biochem.* **108:** 42–45 (1990).

4. M. Takeuchi, N. Takai, N. Asano, Y. Kameda, and K. Matsui. Inhibitory effect of validamine, valienamine and valiolamine on activities of carbohydrases in rat small intestinal brush border membranes. *Chem. Pharm. Bull.* **38:** 1970–72 (1990).
5. X.L. Chen, Y.X. Fan, Y.G. Zheng, and Y.C. Shen. Properties and production of valienamine and its related analogues. *Chem. Rev.* **103:** 1955–77 (2003).
6. S. Ogawa, Y. Miyamoto, and A. Nakajima. Cleavage of the imino bonds of validoxylamine A derivatives with *N*-bromosuccinimide. *Chem. Lett.* **18:** 725–28 (1989).
7. S. Ogawa, A. Nakajima, and Y. Miyamoto. Cleavage of validoxylamine A derivatives with N-bromosuccinimide: preparation of blocked synthons useful for the construction of carba-oligosaccharides composed of imino linkages. *J. Chem. Soc. Perkin. Trans.* **I:** 3287–90 (1991).
8. Y. Kameda and S. Horii. Unsaturated cyclitol part of the new antibiotics, the validamycins. *J. Chem. Soc., Chem. Commun.* **12:** 746–47 (1972).
9. Y. Kameda, N. Asano, M. Teranishi, and K. Matsui. New cyclitols, degradation of validamycin A by *Flavobacterium Saccharophilum. J. Antibiot.* **33:** 1573–74 (1980).
10. Y. Kameda, N. Asano, M. Teranishi, M. Yoshikawa, and K. Matsui. New intermediates, degradation of validamycin A by *Flavobacterium Saccharophilum. J. Antibiot.* **34:** 1237–40 (1981).
11. Y.G. Zheng, X.F. Zhang, and Y.C. Shen. Microbial transformation of validamycin A to valienamine by immobilized cells. *Biocatal. Biotransform.* **23:** 71–77 (2005).
12. J.F. Zhang, Y.G. Zheng, Y.P. Xue, and Y.C. Shen. Purification and characterization of the glucoside 3-dehydrogenase produced by a newly isolated *Stenotrophomonas maltrophilia* CCTCC M 204024. *Appl. Microbiol. Biotechnol.* **71:** 638–45 (2006).
13. Agriculture Antibiotic Group in Shanghai Pesticide Institute. Classification and identification of Jinggangmycins producing strains. *Acta. Microbiol.* **15:** 110–13 (in Chinese) (1975).
14. Y. Kameda, and S. Hori. EP0063456 (1984).
15. X.L. Chen, Y.G. Zheng, and Y.C. Shen. Quantitative analysis of valienamine in the microbial degradation of validamycin A after derivatization with *p*-nitrofluorobenzene by reversed-phase high-performance liquid chromatography. *J. Chromatogr.* B. **824:** 341–47 (2005).

Manuscript received November 19, 2005;
revision received May 19, 2006.