Journal of Pharmaceutical and Biomedical Analysis 49 (2009) 957–963

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Quantitative analysis of valiolamine through pre-column derivatization with phenylisocyanate using high-performance liquid chromatography with UV detection: Selection of reagent, identification of derivative and optimization of derivatization conditions

He Li[a], Jae Ran Lee[a], Do Quynh Anh Nguyen[a], Sung Bae Kim[a], Yang-Gon Seo[a], Yong Keun Chang[b], Soon-Kwang Hong[c], Myung Hee Moon[b], In-Young Chung[d], Chang-Joon Kim[a,*]

[a] Department of Chemical & Biological Engineering and ERI, GyeongSang National University, 900 Gajwadong, Jinju, Gyeongnam 660-701, Republic of Korea
[b] Department of Chemical & Biomolecular Engineering, Korea Advanced Institute of Science and Technology, Daejeon, Republic of Korea
[c] Division of Bioscience and Bioinformatics, Myung Ji University, Yongin, Republic of Korea
[d] Department of Electronic and Communications Engineering, KwangWoon University, Seoul, Republic of Korea

ARTICLE INFO

Article history:
Received 4 December 2008
Received in revised form 13 February 2009
Accepted 14 February 2009
Available online 27 February 2009

Keywords:
Valiolamine
Phenylisocyanate
Derivatization
HPLC
UV detection

ABSTRACT

This report describes the improved quantitative determination of valiolamine in a medium for microbial culture using high-performance liquid chromatography with UV detection. Valiolamine aqueous solution was dried, dissolved in dimethyl sulfoxide and derivatization performances of phenylisocyanate (PHI), 1-fluoro-2,4-dinitrobenznene and 1-naphthylisothiocyanate were compared in the presence of triethylamine. The PHI was chosen as the most suitable derivatization reagent and the valiolamine–PHI derivative was identified by thin-layer chromatography and electrospray ionization mass spectrometry. The derivative eluted at 10.5 min on a reverse-phase column using a mobile phase composed of 10% acetonitrile in water containing 0.5 mM sodium octyl sulfate (pH 3.0), at a column flow rate of 1.0 mL/min with UV detection at 240 nm. The optimum conditions for derivatization were a reaction temperature of 30 °C, reaction time of 30 min, and PHI concentration higher than 33.6 mM. Calibration curves were linear in the range of 0.99–19.95 μg/mL for the standard solutions and 24.9–99.7 μg/mL for the spiked sample. The proposed method was validated and proven to be selective, accurate and precise and suitable for the quantitative analysis of valiolamine in medium for microbial cultures.

1. Introduction

Valiolamine (Fig. 1(a)), a natural aminocyclitol (aminocarbasugar), is a strong α-glucosidase and maltase inhibitor. Voglibose, a commercially available anti-diabetic medicine, can be synthesized by chemical modification of valiolamine [1–3]. Valiolamine was first isolated from the fermentation broth of *Streptomyces hygroscopicus* [3]. It can be prepared by the biodegradation of validamycin into a mixture of validamine and valienamine (Fig. 1(b)) [4], which is then chemically converted into valiolamine [5]. Several chemical synthetic processes of valiolamine have been also reported [6–10].

Nowadays, as an alternative approach, intensive studies have been conducted to identify and characterize gene clusters responsible for validamycin biosynthesis, followed by the elucidation of their biosynthetic pathways. This allows for the generation of large amounts of valiolamine and its novel derivatives by the fermentation of metabolically engineered strains [10–13]. Therefore, the quantitative analysis of commercially value-added valiolamine is crucial for monitoring and thus quality control of its production process by chemical synthesis or fermentation, as well as voglibose production process. Thin-layer chromatography (TLC) is currently used for the analysis of valiolamine. But its sensitivity was shown to be comparatively poor [14] and this cannot provide an accurately quantitative measure [15].

High-performance liquid chromatography (HPLC) method is one of the most popular methods of quantifying natural compounds. However, valiolamine has no significant UV absorption or fluorescence and thus derivatization by chromophoric reagents is inevitable for the sensitive detection of valiolamine by HPLC with UV or fluorescence detectors. Many reports have been published on derivatization of aminoglycosides or other drugs containing primary or secondary amine groups in human plasma. Although o-phthaldialdehyde (OPA), fluorescent-labeling agent, had been used for the past decades, the instability of OPA-drug derivatives has

* Corresponding author. Tel.: +82 55 751 5391; fax: +82 55 753 1805.
E-mail address: cj.kim@gsnu.ac.kr (C.-J. Kim).

0731-7085/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2009.02.020

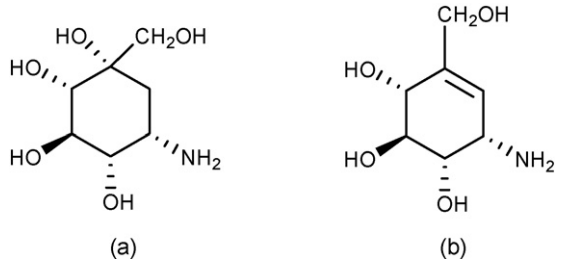

Fig. 1. Chemical structures of valiolamine (a) and valienamine (b).

been a disadvantage [16–19]. Currently 9-fluorenylmethyl chloroformate has been evaluated for its performance [20,21].

The commonly used derivatizing reagents for UV detection are 1-fluoro-2,4-dinitrobenzene (FDNB) [15,22–27], phenylisocyanate (PHI) [28,29], and its derivative 1-naphthylisothiocyanate (NITC) [30,31] or phenylisothiocyanate (PITC) [32–34]. However, a comparison between the performances of these derivatizing reagents has not been made. Although pre-column derivatizations using PHI, NITC, or PITC were reported to offer relatively high sensitivity and selectivity, most assays using these reagents were poorly reproducible because water was not removed during the sample preparation in spite of degradation of these reagents upon contact with water [23,32,33]. And also the effect of concentrations of phenylisocyante on the formation of derivative was not demonstrated.

This study aimed to develop an improved derivatization method for reliable and sensitive quantitative HPLC/UV determination of valiolamine in microbial culture medium. The derivatization performances by PHI, FDNB, or NITC were compared in the newly formulated reaction mixture without water and the derivative was identified by thin-layer chromatography and electrospray ionization mass spectrometry. Derivatization conditions were optimized to improve the sensitivity. The proposed method was validated with respect to linearity, intra- and inter-day accuracy and precision, as well as analyte stability. It was proven to be selective, accurate and precise. This is the first paper demonstrating the quantitative analysis of valiolamine using HPLC.

2. Materials and methods

2.1. Chemicals and reagents

Valiolamine (purity: 99.7%) and valienamine were purchased from Shenzhen fangye industries Co. Ltd. (Shenzhen, China). Acetonitrile, phenylisocyanate, triethylamine (TEA), dimethyl sulfoxide (DMSO), 1-fluoro-2,4-dinitrobenzene, 1-naphthylisothiocyanate, methanol, and other chemicals were all analytical grade and purchased from Sigma chemical Co. (St. Louis, MO, USA).

2.2. Sample preparation and chemical derivatization

Microbial culture medium consisting of glucose (50 mg/mL), soybean flour (36 mg/mL), peptone (5 mg/mL) and calcium carbonate (4 mg/mL) [35] was sterilized and insoluble materials were removed by centrifugation at 9950 × *g* for 10 min. The supernatant was used for further experiments. The standard stock solution of valiolamine was prepared by dissolving accurately weighed valiolamine in distilled water to give a final concentration of 1 mg/mL. This solution was then successively diluted with water or culture medium to obtain standard solutions or spiked samples. Each standard solution or spiked sample (500 μL) was added to 500 μL acetonitrile, one of the widely used protein precipitants [36,37], and then centrifuged (9950 × *g* for 5 min) to remove most of the proteins present [30]. The supernatant (800 μL) was transferred into a 20-mL vial and dried at 50 °C under vacuum, after which 750 μL of DMSO was added. After the dried sample was dissolved well, 100 μL PHI dissolved in acetonitrile and 150 μL TEA (15 mg/mL in acetonitrile) were added and mixed. The vial was then placed in an incubator at 30 °C or water bath if higher temperatures were required.

2.3. HPLC analysis

The analysis of valiolamine derivatives was carried out using HPLC system consisting of 515 HPLC pump and a 486 UV detector (Waters, USA). The isocratic mobile phase was composed of acetonitrile and water containing 0.5 mM sodium octyl sulfate (10:90). The pH of the mobile phase was adjusted to 3.0 using phosphoric acid. A 20 μL filtered sample obtained using a 0.2 μm disposable syringe filter (DISMIC-13_{HP}, ADVANTEC, Tokyo, Japan) was injected into the J'sphere ODS-H80 (250 mm × 4.6 mm, YMC Co., Ltd., Kyoto, Japan) column, eluted with the mobile phase at 1.0 mL/min at room temperature and detected at wavelength of 240 nm [28] because at this wavelength phenyl urea analogues, derivatized product, absorbed strongly [38–40].

2.4. Thin-layer chromatography and electrospray ionization mass spectrometry

The reaction mixture was concentrated using a vacuum evaporator and then spotted onto silica gel thin-layer sheets $60F_{254}$ (Merck, Darmstadt, Germany). Chromatograms were developed using an ascending solvent of 1-butanol–methanol–$CHCl_3$–25% ammonia solution (4:5:2:5, v/v/v/v) [41]. The spots were visualized using a cerium- and molybdate-containing reagent: 25 g phosphomolybdic acid, 10 g cer(IV)-sulfate, 60 mL H_2SO_4 in 1000 mL of distilled water [42].

Mass spectrometric data were obtained using a 3200 QTRAP LC/MS/MS system equipped with a Turbo V™ source and Turbo ion spray probe (500 °C) (Applied Biosystems, Forster, CA, USA). BioAnalyst™ (version 1.4.2) and Analyst software (version 1.4.2) were used for instrument control and data acquisition, respectively. The reaction mixture (1000 μL) was evaporated using a vacuum evaporator at 100 °C to remove DMSO, and then diluted with 300 μL of acetonitrile. This solution was directly injected into the ion source using a syringe pump at the flow rate of 10 μL/min. The spectra were collected in positive ionization mode. Nitrogen was used as both the nebulizing and drying gas with a flow rate of 2 mL/min at 60 psi. The source voltage was set at 5.5 kV and the sample was heated at 500 °C.

2.5. Method validation

The aqueous standard solutions were prepared to provide concentrations of 0.99, 4.95, 9.97 and 19.95 μg/mL. For spiked sample analysis, the medium for microbial culture was chosen as the control blank. The standard stock solution was added to the control blank to provide concentrations of 24.9, 49.9, 74.8 and 99.7 μg/mL. These calibration solutions were derivatized and calibration curves were obtained by a least-square regression evaluation of the peak area (for neat standard solution) or blank-subtracted peak area (for spiked samples) versus the corresponding concentration. Intra- and inter-day assays were performed using aqueous standard solutions at 2.99, 6.98 and 14.96 μg/mL or spiked samples at 29.9, 59.8, and 89.8 μg/mL. The sample concentrations were calculated from this linear regression equation. The accuracy and precision were then evaluated as the % relative error (% R.E.) and % relative standard deviation (% R.S.D.) respectively, using the following equations [34]:

Table 1
Derivatization of valiolamine using different reagents.

Reagent	Phenylisocyante	1-Fluoro-2,4 -dinitrobenzene	1-Naphthyl -isocyanate
Reaction temperature (°C)	30	80	70
UV detection (nm)	240	365	230
Retention time (min)	10.5 ± 0.02	46.7 ± 0.9	61.3 ± 0.7
Peak height ($\times 10^4$)	13.6 ± 0.2	8.6 ± 0.2	11.7 ± 0.7
Peak area ($\times 10^4$)	380.5 ± 3.3	590.8 ± 12.1	108.7 ± 7.6

The concentration of valiolamine standard solution was 99.7 μg/mL. 120 mM of derivatization reagent and 1.25 mg/mL of triethylamine were used for derivatization.

$$\%\,\text{R.E.} = \frac{\text{measured value (mean)} - \text{spiked value}}{\text{spiked value}} \times 100$$

$$\%\,\text{R.S.D.} = \frac{\text{standard deviation}}{\text{mean value}} \times 100$$

For intra-day assays, three or four replicates of each sample were analyzed. The inter-day assay evaluation was performed on five continuous days.

The stability of valiolamine derivatives obtained from spiked samples at two concentrations (59.8 and 89.8 μg/mL) was investigated under a variety of storage conditions: 24 h storage under room temperature, three cycles of freeze (−20 °C)–thaw (room temperature), and 30 days storage of the derivatized products of valiolamine in the −20 °C freezer. The stability was evaluated by comparing the relative mean peak areas of stored samples with freshly prepared samples under identical assay conditions.

3. Results and discussion

3.1. Evaluation of derivatizing reagents for the determination of valiolamine in standard solution

Valiolamine has a primary amine in its structure. The PHI, FDNB, and NITC have a strong reactivity to primary or secondary amines. Therefore, these reagents were evaluated for their ability to derivatize the valiolamine standard solution at 99.7 μg/mL. Initially, 500 μL of an aqueous standard solution was added to 250 μL of PHI solution (5 mg/mL in acetonitrile) and 250 μL of TEA solution (5 mg/mL in acetonitrile) following the procedure described by Kim et al. [28]. However, we have been unable to reproduce this assay because the mixture became turbid in the presence of higher amounts of PHI, thereby resulting in a low peak intensity of the derivatized product. PHI was reported to react rapidly with water at room temperature, giving rise to diphenyl urea, which can combine with PHI to form triphenylbiuret [40]. This may interfere with the analysis as well as the consumption of PHI required for valiolamine derivatization.

Upon combining 800 μL of aqueous standard solution and 300 μL of NITC solution (80 mM) using the procedure suggested by Chen et al. [30], the reaction mixture became turbid and the new peak obtained was too small to detect using HPLC analysis. It was also reported that NITC derivatization was water-sensitive and thus samples had to be dried prior to analysis [32,33]. FDNB is sparingly soluble in water. Therefore, water had to be removed by evaporation in a vacuum oven and the dried solid had to be solubilized in an appropriate solvent.

Methanol, acetonitrile, and DMSO were tested for their suitability as reaction solvents for the derivatization procedure. Although methanol and acetonitrile could dissolve the dried solids obtained from aqueous standard solution, they could not completely dissolve the dried solid obtained from the medium that was spiked with valiolamine. Furthermore, methanol was reported to give reaction by-products [40]. DMSO was selected because it completely and efficiently dissolved dried solids and was generally used as a solvent for chemical reactions.

It was reported that the derivatization reactions of primary or secondary amines with PHI, NITC, or FDNB were favorable in an alkaline medium [25,32,43]. Therefore, TEA was added to make the reaction environment alkaline. Reaction temperatures and UV detection wavelengths were set based on previous reports [25,28,30]. Reactions were performed using different derivatizaton reagents while their molar concentrations and reaction times were the same. As shown in Table 1, new peaks of derivatized products by PHI, FDNB, or NITC appeared at 10.5, 46.7, and 61.3 min, respectively. The peak areas increased in the order of FDNB, PHI and NITC whereas the peak heights increased in the order of PHI, NITC, and FDNB. Considering peak area, peak sharpness, analysis time and mild reaction temperature, PHI was chosen as the most optimum derivatization reagent. The selection of a basic medium is also important for the derivatization reaction. Use of basic aqueous buffer solutions was avoided because PHI was found to be sensitive to water. For example, serious degradation of derivative of isothiocyante was reported in aqueous Na_2CO_3 buffer [44]. When organic solvents, pyridine and TEA were tested, no significant differences in derivatization efficiencies by PHI were found (data not shown). TEA was chosen because it was commonly used as a basic medium for derivatization reaction by PHI or other reagents [28,29,44–47].

Fig. 2(a) shows the TLC analyses. The single spot for the valiolamine standard was observed at R_f value of 0.16. When valiolamine was allowed to react with PHI, a new spot (R_f = 0.53) was observed instead of valiolamine, whereas this new spot was missing in the reaction mixture that lacked valiolamine.

Fig. 2(b)–(d) depicts the mass spectrum resulting from direct infusion of the derivatization reaction mixture. Two ions were observed at m/z 313.2 and 335.3. A prominent fragmentation ion at m/z 194.4 ([valiolamine + H]$^+$) was observed with loss of 118.8 (PHI) from ion at m/z 313.2. Fragmentation of the ion at m/z 335.3 resulted in abundant ion at m/z 216.4 ([valiolamine + Na]$^+$) with loss of 118.9. Therefore, ions at 313.2 and 335.3 corresponded to the protonated and sodium forms of valiolamine–PHI derivative, respectively.

This evidence clearly indicated that valiolamine were successfully derivatized by chemical reaction with PHI. It was reported that a variety of primary and secondary amino acids, peptides and amino alcohols were phenyl isocyanated through the nucleophilic substitution in alkaline medium [43]. Combining our result and this demonstration, we suggest the putative reaction scheme for the formation of valiolamine–PHI derivative in Fig. 3.

3.2. Optimization of derivatization conditions

The optimal reaction temperature and time were determined by investigating the effects of these factors on derivatization of valiolamine in spiked sample (199.4 μg/mL) at a fixed concentration of PHI (50.4 mM) to improve sensitivity. Comparison was made in terms of relative peak area of the derivatized product when the reaction temperature was varied between 30, 50, and 70 °C while reaction time was varied at 15, 30, and 60 min. As shown in Table 2, the derivatization reaction was almost completed in 30 min irrespective of temperature. The relative peak area reached a maximum value at 30 °C and then decreased with further increase in the tem-

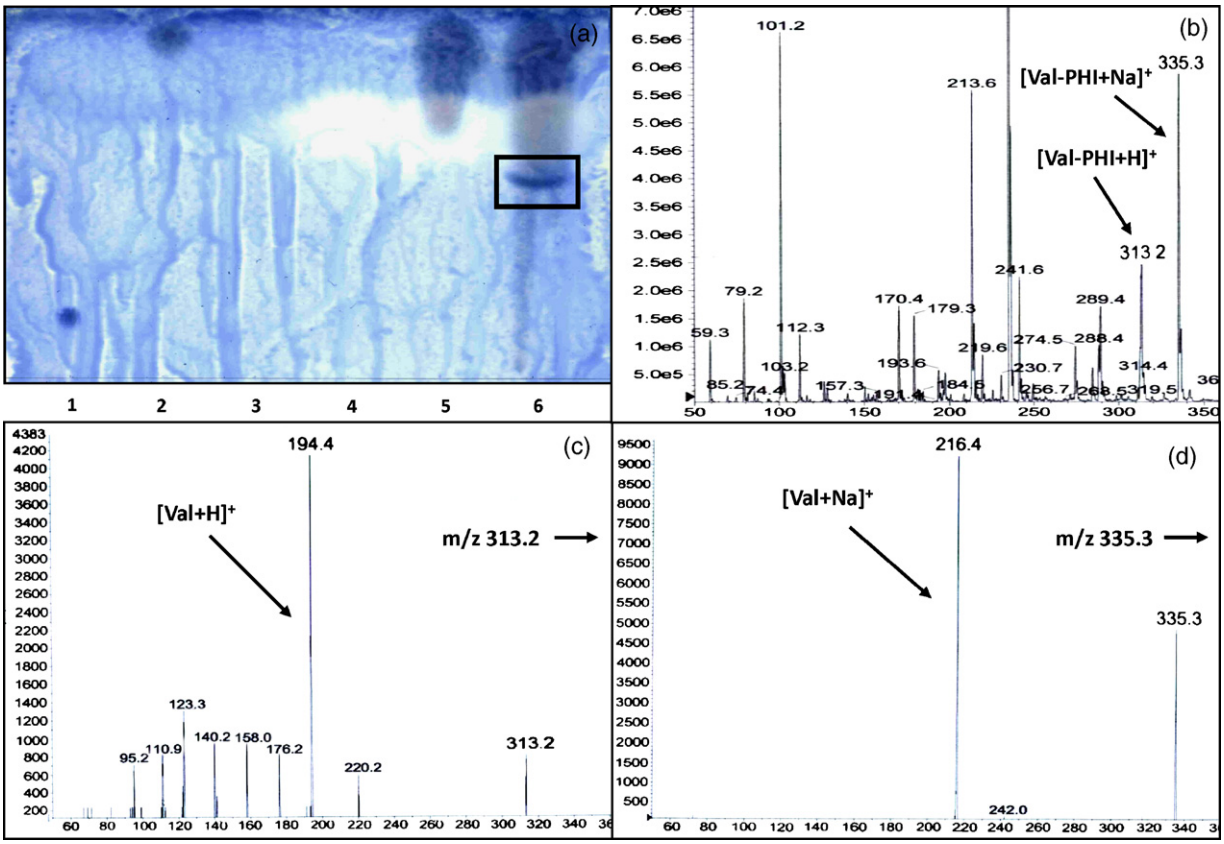

Fig. 2. Identification of valiolamine derivatized with phenylioscyante. (a) Thin-layer chromatography of valiolamine standard solution (1) PHI (2) TEA (3) DMSO (4) and derivatized reaction solution without (5) or with (6) valiolamine. Positive ESI mass spectra of the phenylisocyanate-derivatized valiolamine. (b) MS scan of infused derivatized reaction solution. (c) MS^2 scan of product ion at m/z 313.2. (d) MS^2 scan of product ion at m/z 335.3. Val and Val-PHI represent valiolamine and phenylisocyante-derivatized valiolamine, respectively.

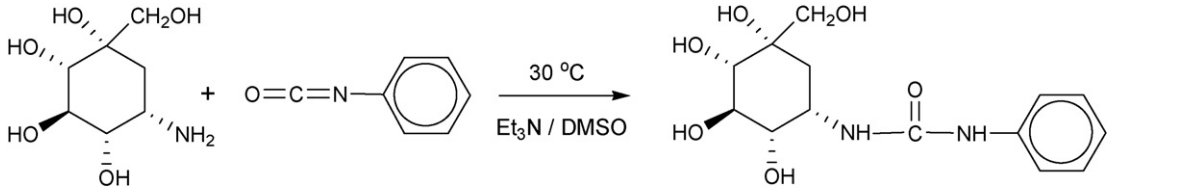

Fig. 3. Putative reaction scheme for the derivatization reaction of valiolamine with phenylisocyanate.

Table 2
Optimization of reaction temperature and time for derivatization of valiolamine with phenylisocyanate.

Run[a]	Temperature (°C)	Time (min)	Relative peak area (%)[b]
1	30	15	74.9 ± 7.0
2		30	100.0 ± 11.3
3		60	97.4 ± 10.7
4	50	15	47.7 ± 13.9
5		30	61.3 ± 20.2
6	70	15	56.7 ± 11.2
7		30	55.8 ± 15.2

[a] Triplicate reactions were performed in the presence of 50.4 mM phenylisocyanate and 2.25 mg/mL triethylamine for derivatization of medium spiked with valiolamine (199.4 μg/mL).

[b] Relative peak area corresponds to the peak area divided by peak area of run 2.

perature probably due to a side reaction [38] or degradation of the derivatized product. This result implied that the derivatization of valiolamine was a fast reaction with PHI at mild temperature [28]. This is one of the advantages of using PHI over other reagents. For example, derivatization reactions using FDNB, a commonly used reagent, requires high temperature and long reaction times, which may cause difficulties in quantitative analysis because solvent evaporation, side reaction or degradation of derivatized product may occur [23,27]. Based on this result, the optimal reaction temperature and time were set to 30 °C and 30 min.

In the following experiments, a different amount of PHI over a range of 2.1–67.2 mM was allowed to react with the spiked sample at 30 °C for 30 min to determine the optimum amount of PHI. Fig. 4 shows the amount of PHI required for derivatization. The relative peak areas of the derivatized products increased with an increase in PHI concentrations, up to 33.6 mM and then no significant increase was observed. This result indicated that more than 33.6 mM of PHI was required for the derivatization of 1 mM of vali-

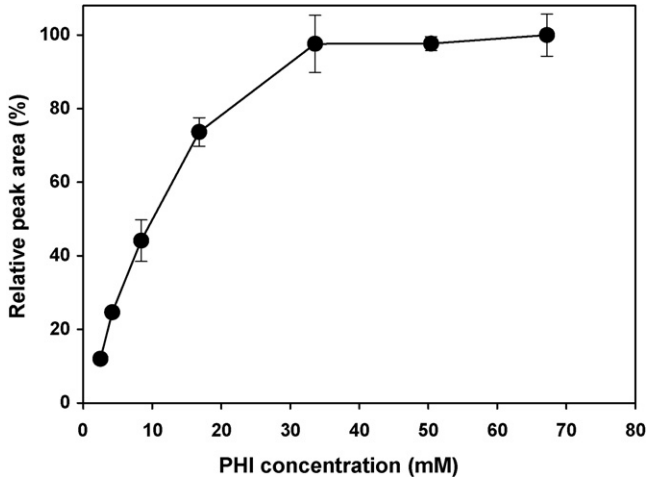

Fig. 4. The effect of concentration of phenylisocyanate on the formation of valiolamine derivative. Medium spiked with valiolamine (199.4 μg/mL) was derivatized at 30 °C for 30 min in the presence of various concentrations of phenylisocyante and 2.25 mg/mL of triethylamine.

olamine. The medium for cultivation of microorganisms contained a number of compounds with amino groups including proteins and amino acids, which can react with PHI [43,48]. Most proteins in the medium were already removed by precipitation with acetonitrile. But amino acids possibly existed in reaction mixtures, which might have contributed to further consumption of PHI. Therefore, an additional amount of PHI may be required to compensate for possible consumption of the derivatizing reagent by these amino compounds. Furthermore, the excess amount of PHI may increase the rate of reaction for the formation of derivatives with respect to the reaction kinetics [30].

The effect of the concentration of derivatizing reagent has previously been reported during derivatization reactions using FDNB, NITC or PITC [15,23,25,27,30,32]. Even though excess amounts of PHI were used, however, unreacted valiolamine was observed in the reaction mixture by mass spectrometric analysis. When the spiked concentration was reduced to 99.7 μg/mL in the mixture, complete conversion to valiolamine derivative was observed by mass spectrometric analysis (Supplementary 1). Therefore, all reactions were performed in the presence of 50.4 mM PHI and less than 99.7 μg/mL of spiked valiolamine at 30 °C for 30 min in the following experiments.

3.3. Selectivity

Valienamine, the starting material for the synthesis of valiolamine, has a primary amine and its chemical structure is very similar to valiolamine. Derivatization reactions were performed in a medium spiked with both valiolamine (99.7 μg/mL) and valienamine (97 μg/mL) to examine the possibility of interference by the presence of valienamine. Fig. 5 shows the representative chromatograms of standards or spiked samples. The derivatized products of valiolamine and valienamine eluted at 10.5 and 17.5 min, respectively. No shifts in retention times of the peaks of these compounds were observed when a mixture of these pure compounds was spiked into the medium. This indicated that derivatization products of these compounds resolved well and thus the presence of valienamine did not interfere with HPLC analysis of valiolamine.

However, a small peak in the blank medium was observed at the retention time of valiolamine, indicating possible interference of endogenous substances in the medium with the analysis of

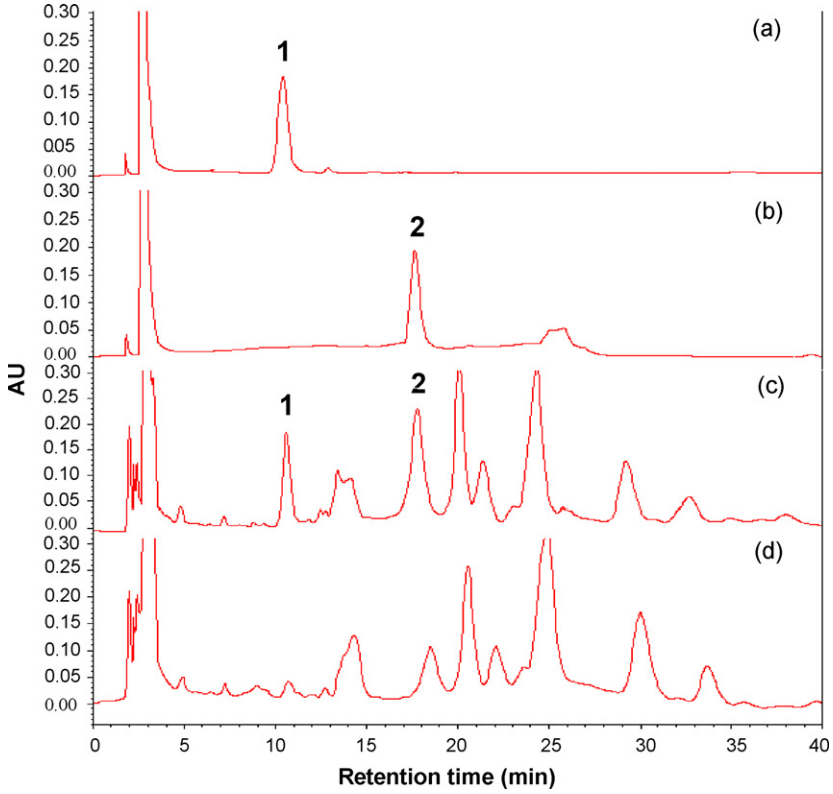

Fig. 5. Representative chromatograms of the phenylisocyanate derivative of valiolamine and valienamine: (a) standard valiolamine (99.7 μg/mL); (b) standard valienamine (97 μg/mL); (c) medium spiked with valiolamine (99.7 μg/mL) and valienamine (97 μg/mL); (d) blank medium. Peaks: 1, valiolamine derivative; 2, valienamine derivative.

Table 3
ANOVA results for lack of fit.

	Source of variation	Sum of square	Degree of freedom	Mean of square	*F*-ratio
Standard solutions	Residual	2.71×10^9	9	3.01×10^8	0.05
	Lack of fit	3.47×10^7	2	1.74×10^7	
	Pure error	2.67×10^9	7	3.82×10^8	
Spiked samples	Residual	1.49×10^{11}	9	1.65×10^{10}	0.8
	Lack of fit	2.77×10^{10}	2	1.39×10^{10}	
	Pure error	1.21×10^{11}	7	1.73×10^{10}	

The critical value of *F*-ratio is 4.74 at $\alpha = 0.05$. Test ranges were 0.99–19.95 μg/mL for standard solutions and 24.9–99.7 μg/mL for spiked samples.

Table 4
Precision and accuracy of the analysis of valiolamine standards or spiked samples.

Concentration (μg/mL)		Intra-day repeatability[a]			Inter-day repeatability[b]		
Standards	Spiked samples	Assay value[c] (μg/mL)	Precision (% R.S.D)	Accuracy (% R.E.)	Assay value[c] (μg/mL)	Precision (% R.S.D)	Accuracy (% R.E.)
2.99	–	2.94 ± 0.38	13.0	−1.7	2.71 ± 0.07	2.7	−9.5
6.98	–	6.54 ± 0.42	6.4	−6.3	6.27 ± 0.31	4.9	−10.3
14.96	–	14.14 ± 1.66	11.7	−5.5	13.87 ± 0.69	4.9	−7.3
–	29.9	25.3 ± 5.9	23.2	−15.4	31.9 ± 10.4	32.6	6.5
–	59.8	67.3 ± 0.5	0.8	12.5	63.4 ± 7.8	12.3	5.9
–	89.8	87.1 ± 4.0	4.6	−3.0	84.1 ± 6.8	8.1	−6.3

[a] Triplicate (for standard solutions) or quadruplicate (for spiked samples) for intra-day assays were performed in the presence of 50.4 mM of phenylisocyantae at 30 °C for 30 min.
[b] Five-separate inter-day assays were performed in the presence of 50.4 mM of phenylisocyantae at 30 °C for 30 min.
[c] Values are expressed as average ± standard deviation.

valiolamine. For the quantitative analysis of valiolamine, therefore, corrections were made for interference by endogenous substances; the blank peak area corresponding to retention time of valiolamine was subtracted from peak area of the spiked medium.

3.4. Linearity

Calibration curves were constructed using four series of standards or spiked samples. The linear regression evaluation of the peak area (Y) versus concentration (X) relationship for intra-day assay as an example gave $Y = 44561.3(\pm 1450.8)X - 10645.6(\pm 2130.1)$ for standard solutions and $Y = 24757.1(\pm 734.1)X + 245618.6(\pm 68422.3)$ for spiked samples. The correlation coefficients of the two calibration curves were 0.999. However, a regression coefficient close to unity is not necessarily indicative of a linear relationship and so calibration curves should also be evaluated using an *F*-test for lack of fit [49–52]. The *F*-ratio for lack of fit was calculated by dividing lack-of-fit mean square by pure error mean square. As shown in Table 3, the calculated *F*-ratio values for standard solutions and spiked samples were 0.05 and 0.8, respectively. These values were lower than the tabulated critical value (4.74 at $\alpha = 0.05$), which obviously implied that the regression models were linear.

The linearities of calibration curves for inter-day assay were also confirmed (data not shown). The lowest concentration used in the construction of the calibration curve for standard sample was 0.99 μg/mL whereas that for the spiked sample was as high as 24.9 μg/mL due to the interference of the blank peak at lower concentrations.

3.5. Assay precision and accuracy, and analyte stability

Table 4 shows the precision and accuracy of the intra- and inter-day assays for the analysis of standard solutions or spiked samples. For standard solutions, the intra- and inter-day % R.S.D. ranged from 6.4 to 13.0 and from 2.7 to 4.9, respectively. Moreover, % R.E. ranged from −1.7 to −6.3 and from −7.3 to −10.3, respectively. These data indicate high accuracy and repeatability of this method for the assay of standard solutions in the concentration ranges of 2.99–14.96 μg/mL.

For spiked samples, the intra- and inter-day % R.S.D. ranged from 0.8 to 4.6 and from 8.1 to 12.3 at concentrations above 59.8 μg/mL whereas corresponding values were 23.2 and 32.6 at 29.9 μg/mL. The intra- and inter-day % R.E. ranged from −3.0 to 12.5 and from −6.3 to 5.9 at concentrations above 59.8 mg/L whereas corresponding values were −15.4 and 6.5 at 29.9 μg/mL. These data suggest that the accuracy and precision of this method are satisfactorily acceptable for the determination of valiolamine in the medium at concentrations higher than 59.8 μg/mL, considering that the normal acceptance criteria for % R.S.D. and % R.E. is less than 15% according to United States Food and Drug Administration (FDA) Guideline [53]. The accuracy and precision of the technique would be improved for analyzing lower concentrations of valiolamine in spiked samples if the sample preparation method were developed for removing interfering endogenous substances present in the medium.

Table 5 summarizes the stability of valiolamine–PHI under a variety of storage conditions. The valiolamine–PHI in the reaction mixture was shown to be stable for at least 24 h at room temperature and was also stable after three cycles of freeze–thaw. The derivative was possibly stored for at least 1 month in the −20 °C freezer without showing significant losses in the quantified values.

Table 5
Stability of valiolamine–PHI derivative in spiked samples.

	Concentration spiked (μg/mL)	Relative peak area (%)[a]	
		Initial	Final
Three freeze–thaw cycles	59.8	100.0 ± 2.5	118.9 ± 11.7
	89.8	100.0 ± 7.5	115.2 ± 2.2
Stability for 24 h at room temperature	59.8	100.0 ± 7.5	111.4 ± 11.5
	89.8	100.0 ± 3.8	109.3 ± 9.2
Stability for 30 days at ≤ −20 °C	59.8	100.0 ± 5.1	87.7 ± 15.4
	89.8	100.0 ± 11.0	99.9 ± 5.4

[a] Relative peak area corresponds to the each peak area divided by each peak area of initial run.

These results indicated that no stability-related problems would be expected during routine analysis of valiolamine.

4. Conclusions

This is the first demonstration on the comparison between the derivatization performances of FDNB, PHI, and NITC. Eliminating water and protein, selection of an appropriate dissolving solvent, and optimization of PHI concentrations for HPLC analysis of aminoglycosides in aqueous solution by derivatization with PHI were not performed in previous publications although these factors strongly influenced the sensitivity of a number of derivatization reactions. This method described in the present report involved deprotenation and replacement of water with DMSO in the reaction mixture, optimization of derivatization temperature, time, and PHI concentration. This novel method was found to be very sensitive, and reliable for the HPLC determination of valiolamine in medium for microbial culture based on pre-column derivatization with PHI.

This is the first publication describing the quantitative HPLC analysis of valiolamine and can be applied for analysis of the valiolamine production process as well as analysis of another aminoglycoside compounds in the pharmaceutical industry.

Acknowledgements

This work was supported by grant from 21C frontier R&D programs (Microbial Genomics & Application Center), and partially supported by the second stage of the Brain Korea 21 project in 2008. We thank Prof. Sim-Seong Lee (Department of Chemistry, GyeongSang National University, Jinju, Korea) for the valuable advice provided, with regards to the mass spectrometry data analysis.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpba.2009.02.020.

References

[1] Y.Z. Shu, J. Nat. Prod. 61 (1998) 1053–1071.
[2] L.E. Blidi, M. Ahbala, J. Bolte, M. Lemaire, Tetrahedron Asymmetry 17 (2006) 2684–2688.
[3] Y.K. Kameda, N. Asano, M. Yoshikawa, M. Takeuchi, T. Yamaguchi, K. Matsui, J. Antibiot. 37 (1984) 1301–1307.
[4] N. Asano, M. Takeuchi, K. Ninomiya, Y. Kameda, K. Matsui, J. Antibiot. 37 (1984) 859–867.
[5] S. Horii, H. Fukase, Y. Kameda, Carbohydr. Res. 140 (1985) 185–200.
[6] S. Ogawa, Y. Ohishi, M. Asada, A. Tomoda, A. Takahashi, Y. Ooki, M. Mori, M. Itoh, T. Korenaga, Org. Biomol. Chem. 2 (2004) 884–889.
[7] S. Ogawa, M. Kanto, J. Nat. Prod. 70 (2007) 493–497.
[8] O. Sellier, P. Van de Weghe, D. Nouen, C. Strehler, J. Eustache, Tetrahedron Lett. 40 (1999) 853–856.
[9] X. Chen, Y. Fan, Y. Zheng, Y. Shen, Chem. Rev. 103 (2003) 1955–1977.
[10] T. Mahmud, Nat. Prod. Rep. 20 (2003) 137–166.
[11] Z. Deng, L. Bai, Nat. Prod. Rep. 23 (2006) 811–827.
[12] L. Bai, L. Li, H. Xu, K. Minagawa, Y. Yu, Y. Zhang, X. Zhou, H.G. Floss, Z. Deng, Chem. Biol. 13 (2006) 387–397.
[13] Y. Yu, L. Bai, K. Minagawa, X. Jian, L. Li, J. Li, S. Chen, E. Cao, T. Mahmud, H.G. Floss, X. Zhou, Z. Deng, Appl. Environ. Microbiol. 71 (2005) 5066–5076.
[14] D.A. Stead, J. Chromatogr. B 747 (2005) 69–93.
[15] X. Chen, Y. Zheng, Y. Shen, J. Chromatogr. B 824 (2005) 341–347.
[16] T.A.C. Vermeij, P.M. Edelbroek, J. Chromatogr. B 810 (2004) 297–303.
[17] D.F. Chollet, L. Goumaz, C. Juliano, G. Anderogg, J Chromatogr. B 746 (2000) 311–314.
[18] Q. Jiang, S. Li, J. Chromatogr. B 727 (1999) 119–123.
[19] P.J. Kijak, J. Jackson, B. Shaikh, J. Chromatogr. B 691 (1997) 377–382.
[20] T.M. Huang, C.H. Deng, N.Z. Chen, Z. Liu, G.L. Duan, J. Sep. Sci. 29 (2006) 2296–2302.
[21] G. Bahrami, S. Mirzaeei, B. Mohammadi, A. Kiani, J. Chromatogr. B 822 (2005) 322–325.
[22] H. Jalalizadeh, E. Souri, M.B. Tehrani, A. Jahangiri, J. Chromatogr. B 854 (2007) 43–47.
[23] E. Souri, H. Jalalizadeh, A. Shafiee, Chem. Pharm. Bull. 55 (2007) 1427–1430.
[24] S. Nicoli, P. Santi, J. Pharm. Biomed. Anal. 41 (2006) 994–997.
[25] M. Zhou, G. Wei, Y. Liu, Y. Sun, S. Xiao, L. Lu, C. Liu, D. Zhong, J. Chromatogr. B 798 (2003) 43–48.
[26] N. Isoherranen, S. Soback, Clin. Chem. 46 (2000) 837–842.
[27] J. Lu, M. Cwik, T. Kanyok, J. Chromatogr. B 695 (1997) 329–335.
[28] B.H. Kim, S.C. Lee, H.J. Lee, J.H. Ok, Biomed. Chromatogr. 17 (2003) 396–403.
[29] B.H. Kim, Y.K. Kim, J.H. Ok, J. Chromatogr. B 752 (2001) 173–177.
[30] S.H. Chen, Y.C. Liang, Y.W. Chou, J. Sep. Sci. 29 (2006) 607–612.
[31] C.H. Feng, S.J. Lin, H.L. Wu, S.H. Chen, J. Chromatogr. B 780 (2002) 349–354.
[32] I.A. Tekko, M.C. Bonner, A.C. Williams, J. Pharm. Biomed. Anal. 41 (2006) 385–392.
[33] A. Aghazadeh-Habashi, S. Sattari, F. Pasutto, F. Jamali, J. Pharm. Pharm. Sci. 5 (2002) 176–180.
[34] Z.L. Liang, J. Leslie, A. Adebowale, M. Ashraf, N.D. Eddington, J. Pharm. Biomed. Anal. 20 (1999) 807–814.
[35] Y.K. Kameda, S. Horii, US Patent 4,923,975 (1990).
[36] C. Polson, P. Sarkar, B. Incledon, V. Raguvaran, R. Grant, J. Chromatogr. B 785 (2003) 263–275.
[37] S. Souverain, S. Rudaz, J.L. Veuthey, J. Pharm. Biomed. Anal. 35 (2004) 913–920.
[38] W. Chu, K.H. Chan, C.T. Jafvert, Y.S. Chan, Chemosphere 69 (2007) 177–183.
[39] B. Sahasrabuddhey, A. Jain, K.K. Verma, Analyst 124 (1999) 1017–1021.
[40] S. Rakotomanga, A. Baillet, F. Pellerin, D. Baylocq-Ferrier, Chromatographia 32 (1991) 125–129.
[41] Y. Kameda, N. Asano, M. Yoshikawa, M. Takeuchi, T. Yamaguchi, K. Matsui, J. Antibiot. 37 (1984) 1301–1307.
[42] A. Drepper, R. Peitzmann, H. Pape, FEBS Lett. 388 (1996) 177–179.
[43] S. Chen, Chromatographia 59 (2004) 697–703.
[44] O.P. Kleidernigg, W. Lindner, Chromatographia 44 (1997) 465–472.
[45] S. Zhao, H. Wang, R. Zhang, L. Tang, Y.M. Liu, Electrophoresis 27 (2006) 3428–3433.
[46] M.R. Criado, S.P. Torre, I.R. Pereiro, R.C. Torrijos, J. Chromatogr. A 1024 (2004) 155–163.
[47] J.C. Dunphy, D.G. Pessler, S.W. Morrall, K.A. Evans, D.A. Robaugh, G. Fujimoto, A. Negahban, Environ. Sci. Technol. 35 (2001) 1223–1230.
[48] A.M. El-Brashy, S.M. Al-Ghannam, Analyst 122 (1999) 147–150.
[49] J.M. Sonnergaard, Int. J. Pharm. 321 (2006) 12–17.
[50] G.M. Hadad, A. El-Gindy, W.M.M. Mahmoud, Chromatographia 66 (2007) 311–317.
[51] C. Kousoulos, G. Tsatsou, Y. Dotsikas, C. Apostolou, Y.L. Loukas, Biomed. Chromatogr. 22 (2008) 494–501.
[52] G.E.P. Box, W.G. Hunter, J.S. Hunter, Statistics for Experimenters: An Introduction to Design, Data analysis and Model Building, John Wiley & Sons, New York, 1978.
[53] FDA, Guidance for industry bioanalytical method validation, US Department of Health Services Food and Drug Administration, 2001.