creative commons
COMMONS DEED

저작자표시-비영리-변경금지 2.0 대한민국

이용자는 아래의 조건을 따르는 경우에 한하여 자유롭게

- 이 저작물을 복제, 배포, 전송, 전시, 공연 및 방송할 수 있습니다.

다음과 같은 조건을 따라야 합니다:

저작자표시. 귀하는 원저작자를 표시하여야 합니다.

비영리. 귀하는 이 저작물을 영리 목적으로 이용할 수 없습니다.

변경금지. 귀하는 이 저작물을 개작, 변형 또는 가공할 수 없습니다.

- 귀하는, 이 저작물의 재이용이나 배포의 경우, 이 저작물에 적용된 이용허락조건을 명확하게 나타내어야 합니다.
- 저작권자로부터 별도의 허가를 받으면 이러한 조건들은 적용되지 않습니다.

저작권법에 따른 이용자의 권리는 위의 내용에 의하여 영향을 받지 않습니다.

이것은 이용허락규약(Legal Code)을 이해하기 쉽게 요약한 것입니다.

Disclaimer

dCollection

약학석사 학위논문

Validation of HPLC-UV analysis and preformulation studies of Psammaplin A and Halichondramide, derived from marine organisms

해양천연물에서 유래한 Psammaplin A와 Halichondramide의 HPLC-UV 분석법 확립과 Preformulation 연구

2013 년 8 월

서울대학교 대학원

약학과 약제과학 전공

박 지 현

Validation of HPLC-UV analysis and preformulation studies of Psammaplin A and Halichondramide, derived from marine organisms

해양천연물에서 유래한
Psammaplin A와 Halichondramide의
HPLC-UV 분석법 확립과 Preformulation 연구

지도교수 김 대 덕

이 논문을 약학석사 학위논문으로 제출함
2013 년 5 월

서울대학교 대학원
약학과 약제과학 전공
박 지 현

박지현의 석사 학위논문을 인준함
2013 년 6 월

위 원 장 심 창 구 (인)
부위원장 정 석 재 (인)
위 원 김 대 덕 (인)

Abstract

Validation of HPLC-UV analysis and preformulation studies of Psammaplin A and Halichondramide, derived from marine organisms

Jihyun, Park
Dept. of Pharmaceutics, College of Pharmacy
The Graduate School
Seoul National University

Marine natural products are attractive resources for pharmaceutical industry due to their unique mode of action and nearly unlimited quantity. Preformulation process is prerequisite in the early stages of new molecular entity (NME) development to avoid costly failure in later stages. This study was performed to develop a suitable analytical method and investigate the various physical-chemical properties of Psammaplin A and Halichondramide, which were isolated from marine organism and provided by the lab of Professor Jongheon Shin in Seoul National Unversity. The HPLC-UV analytical methods for quantification of the compounds were developed and validated by linearity, accuracy and precision. The physicochemical characterization of the materials included lipophilicity ($LogP_{oct/wat}$) and aqueous

solubility at equilibrium state. PAMPA tests were also conducted to assess the permeability ($LogP_e$).

For Psammaplin A, the HPLC-UV method using a C18 column and mixture of acetonitrile and DDW (55:45, v/v) as a mobile phase was developed and validated for the quantification. A peak of Psammaplin A was obtained after 5.53 min on 207 nm of detection wavelength. The lower limit of quantification was 0.5 μg/mL. Solubility in DDW was 4.57 ± 0.06 μg/mL (n=3) and $LogP_{oct/wat}$ was 1.29 ± 0.07 (n=5). The $LogP_e$ value as a parameter for the permeability was -6.50 ± 0.23 cm/sec (n=9). For Halichondramide, chromatographic separation was performed with a mobile phase composed of acetonitrile and sodium phosphate buffer (40 mM, pH 6.0) (50:50, v/v) using a CN column. The peak of Halichondramide was detected at 5.37 min on 232 nm. The lower limit of quantification was 100 ng/mL. Aqueous solubility was 3.99 ± 0.02 μg/mL (n=3) and $LogP_{oct/wat}$ was 1.16 ± 0.04 (n=5). The $LogP_e$ value as a parameter for permeability was -6.94 ± 0.33 cm/sec (n=9). Finally, comfortable and reliable analytical method was developed and validated. These results of HPLC-UV analysis and characterization of physical-chemical properties could be useful in the NME development process of Psammaplin A and Halichondramide.

Keywords: HPLC-UV analysis validation, Preformulation, Psammaplin A, Halichondramide, Marine natural product

Student number: 2011-23729

Contents

List of Tables

List of Figures

1. Introduction

The natural products are attractive sources for drug leads consistently [1]. Although chemically synthetic drugs have drawn a lot of attention of scientists in pharmaceutical industry because of their stable supply of compound libraries[2-4], natural pharmacy showed a much better correlation to final approval for clinical stages due to greater structural diversity than standard combinatorial chemistry [2,5-7].

Therefore, considerable ratio of new drugs is originated or designed from natural product data base [8] and even half of the best-selling non-protein drugs are associated with natural products [9]. Especially terrestrial organisms have served as the major source of therapeutics for a long time for easier fermentation and collecting techniques [10] whereas the ocean consists of 70% of the world's surface and has tremendous biological diversity [11]. The pharmacologically active compounds derived from marine natural products are expected consistently to play a key role due to their various advantages. Since the marine environment provides the tough living conditions, the organisms have developed innovative defense pathways and biologically active chemical diversity related to symbiosis [12]. This leads to superior chemical novelty [13], biological diversity and unique and potent mechanism of action [14-17]. In recent, a large number of marine drug candidates have been discovered due to advancement of new technologies [18-20]. The development in spectroscopy, analytical technology [21,22] and high-throughput screening [18] helps to reduce the amount required for identification and

activity tests of compounds. Aquaculture [23-25] as well as collection technique with robotic arms [26,27] can aid sustainable supply which was the biggest problem to investigate marine environment. Also, semi-synthesis and genome mining can overcome mass production problem [28-31].

There already exist several kinds of medicines launched in market originating from marine environment. The initial reports were Bergmann's studies about two unusual nucleosides isolated from marine sponges collected in Florida, USA. By chemical modification, ara-A® (vidarabine) and ara-C® (cytarabine) were derived from these compounds [2,32-34]. For the treatment of chronic pain, Ziconotide was approved by FDA in 2004 under the trade name Prialt® [14,15]. The first marine drug launched for anticancer therapeutics, trabectedin (Yondelis®) was approved by the EU in 2007 [35]. Other marine natural products are currently in the pipeline, being tested in clinical trials all over the world [36,37].

For drug candidate molecules to reach their active site effectively, they should be able to be dissolved sufficiently in body fluid and penetrate some barriers from epithelial cell surrounded gastric-intestinal tract to cell membrane around active site [38]. Therefore, several pharmacologically effective compounds which have poor pharmacokinetic-related characteristics, such as solubility and permeability, have been wiped out in developing process. Since even well designed formulation can hardly overcome inherent short points of candidate molecules, in case developers notice those problems late after many studies has progressed, all the capital and time become useless and they

should start from searching proper targets again [38,39]. Thus, it is essential to conduct researches about physical-chemical characteristics of candidates prior to formulation study for screening compounds that have unsuitable properties even with potent remedial effect in order to enhance efficiency of development process [39]. Therefore, preformulation studies including establishment of analytical method and investigation of the physicochemical properties would be important. Moreover, development and validation of the analytical method for drug candidates are considered as a crucial and fundamental step in new drug discovery process to verify identity, purity and quality control of pharmacologically effective molecules [40,41]. The rapid, accurate and sensitive analytical method should be established firmly to be applied in related consecutive studies. Since high performance liquid chromatography (HPLC) with UV detector system has been generally used to develop a quantitative analysis of drug compounds, this preformulation study also adopted it due to handiness.

To assess the possibility for marine natural products to be developed to the drug candidates, Psammaplin A (Figure 1a) and Halichondramide (Figure 1b) were selected as the objects of the study since they could be offered with a sufficient amount of supply from the laboratory of professor Jongheon Shin in Seoul National University. The former was obtained first from *Psammaplinaplysilla* sponge in 1987 with the molecular formula $C_{22}H_{24}S_2Br_2N_4O_6$ (662.96 g/mol) and it shows structure–activity relationship like its analogues related to bromotyrosine structure [42]. This compound features anticancer activity through the inhibition of enzymes essential to cell growth, for example,

histone deacetylase (HDAC) [43] and DNA methyl transferase (DNMT) [44] and the induction of apoptosis by activating peroxisome proliferator-activated receptor (PPAR) [45]. Moreover, the hindrance of DNA gyrase, chitinase and mycothiol-sulfur conjugate amidase incurs antimicrobial potency [46]. The latter which was discovered from *Chondrosia corticata* Thiel in 1986, with the molecular formula $C_{44}H_{60}N_4O_{12}$ (836.97 g/mol), has drawn interest of scientists in pharmaceutical industry [47]. Cell growth inhibiting properties based on depolymerization of actin, cytoskeletal protein essential for cellular mortality, result in anticancer and antifungal activity [48,49]. In addition, Eribulin mesylate synthesized from Halichondrin B which is an analogue of Halichondramide was approved by US FDA for the treatment of metastatic breast cancer with the trade name Halaven™ [50]. Halichondramide also becomes a promising drug candidaate due to structural similarities.

The objective of this study was to develop and validate an accurate, precise and effective HPLC-UV analytical method of Psammaplin A and Halichondramide. The quantification method was then applied for obtaining physicochemical characteristics of these compounds, including solubility, lipophilicity and permeability.

2. Materials and Methods

2.1. Materials

Psammaplin A was gathered from Poecillastra sp. and Halichondramide was collected from Chondrosia corticata Thiel. Both were provided from the laboratory of Professor Jongheon Shin in Seoul National University. L-α-Phosphatidyl choline from egg yolk (Type XVI-E), octanol and dodecane were obtained from Sigma. Dimethylsulfoxide (Cryoserv®) was purchased from Bionichepharma. Methanol and acetonitrile were obtained from Fischer. Phosphate buffered saline was purchased from Lonza. Double distilled water was produced by Milli-Q water purification system (Millipore Corp., Bedford, MA, USA) and used for HPLC procedure.

2.2. Operation conditions for HPLC-UV analysis

Quantitative determination of compounds was achieved with the Waters high performance liquid chromatography series equipped with a binary pump 1525, auto-sampler 717plus and UV detector 2487 (Waters, Milford, MA, USA). For Psammaplin A, an isocratic mobile phase was eluted on a Shiseido® CAPCELL PAK C18 MG S-5 column (5 μm, 250 mm × 4.6 mm i.d., Shiseido, Tokyo, Japan). A mobile phase composed of acetonitrile and double distilled water (55:45, v/v) was delivered isocratically with a flow rate of 1.0 mL/min and injection volume was 70 μL. The effluent absorbance wavelength was 207 nm. In case of Halichondramide,

the chromatographic separation was performed on a Phenomenex® Luna 5μ CN 100A (5μm, 250 mm × 4.6 mm i.d., Phenomenex, Torrance, CA, USA) column. A mixture of acetonitrile and sodium phosphate buffer (pH 6.0, 40 mM) (50:50, v/v) was applied as a mobile phase at a flow rate of 1.0 mL/min. The UV detector wavelength was 232 nm and injection volume was 50 μL. The chromatography response was integrated using Empower® software (Waters, Milford, MA, USA) software. Every mobile phase solution was used after being degassed and filtered (0.2 μm; Whatman). The maximum absorbance wavelength of UV light was attained by using UV full scan function of UV/VIS spectrophotometer U-3210 machine.

2.3. Preparation of sample solutions

A primary stock solution of Psammaplin A was prepared by dissolving accurately weighed 42.3 mg of Psammaplin A in 42.3 mL of MeOH and diluted with a mobile phase to give a series of standard solutions. Concentration of each sample for calibration curve and linearity study was included in a range from 0.5 to 5 μg/mL. Each concentration of quality control samples was determined at four level in 0.5, 1.5, 2.5, 4.5 μg/mL. Every solution was also yielded by serial dilution with a mobile phase. After dissolving accurately weighed 56.8 mg of Halichondramide in 56.8 mL of methanol to yield a primary stock solution (1 mg/mL), working solutions were prepared by serial dilution in the mobile phase. Concentration of each point for calibration standard curve was decided to cover a range from 100 to 1000 ng/mL. Quality control samples were arranged at four different

concentration (100, 300, 500, 900 ng/mL) to verify liability of over a range of standard curve.

2.4. Analytical method validation

2.4.1. Specificity

The specificity is used to evaluate the ability to measure the components selectively. It was determined by comparing chromatograms of blank sample which was compound-free with containing analytes.

2.4.2. Linearity and limits of quantification (LOQ)

Six points of calibration standard samples were separately prepared and analyzed in triplicate for three consecutive days to check linearity. Responses were back calculated to matching concentrations from a linear regression equation. Calibration curves were plotted from the relationship between UV absorbance peak-area and the analyte concentrations ranging from 0.5 μg/mL to 5 μg/mL for Psammaplin A and 100 ng/mL to 1000 ng/mL for Halichondramide, respectively. Correlation coefficients (R^2) should be over 0.99. The limit of quantification (LOQ) of each compound was determined by signal-to-noise ratio of 10:1 and both precision and accuracy were less than or equal to 20%.

2.4.3. Accuracy and precision

Accuracy and precision of this method were assessed from the obtained results of quality control samples at four concentration

levels covering the range. For intra-assay precision and accuracy, all quality control samples were analyzed in three replicates on a single day. Three times repeated analysis of the homogeneous sample was conducted at every quality control sample in three different days for inter-assay precision and accuracy. The precision indicates the relative standard deviation (coefficient of variation) and accuracy is expressed as the deviation between the found concentration and the true value. To evaluate acceptance of analytical method by US FDA and ICH guidelines [51,52], the precision of quality control samples was required to be less 15% except for LLOQ sample, while accuracy values of all examined cases apart from LLOQ sample should be within ±15%. Lower limit of quantification was determined based on two parameters; signal response to noise response ratio over than 10 and precision represented as %RSD below 20% and accuracy percent within ±20%.

2.5. Preformulation studies

2.5.1. Solubility studies

To achieve equilibrium solubility, the excess amount of each compounds dissolved in the pure distilled water was shaken at room temperature for 24 hours and centrifuged at 13,200 rpm for 10 minutes. The supernatant of the sample was gathered and diluted with the same composition of the mobile phase. The concentration of the compounds was determined by HPLC-UV spectroscopy analysis.

2.5.2. Lipophilicity studies

Apparent 1-octanol/water partition coefficient ($LogP_{oct/wat}$) of each compounds was determined at room temperature by the most reliable shake-flask method. In advance, pre-saturated DDW and pre-saturated 1-octanol were separated after shaking same volume of these solvents overnight to reach the equilibrated state. The stock solution of the material (1 mg/mL) was added to the pre-mixed solvents together and distributed through vortexing for 2 hours and centrifuging at 13,200 rpm for 10 minutes at room temperature. The concentration of the solute in each layer was measured by HPLC-UV spectroscopy.

$$Log\,P_{oct/wat} = Log\left(\frac{[solute]_{octanol}}{[solute]_{water}}\right)$$

2.5.3. Permeability studies

Trans-cellular permeation could be obtained rapidly through PAMPA tests. For application of the artificial membrane on the donor side of the 96-well filter plate, 10 μL of 0.5 % egg lecithin in dodecane was added to the each well. After 150 μL of drug solution in 5% DMSO/PBS was added to donor side and 300 μL of aqueous buffer (5% DMSO/PBS) to acceptor side, the donor plate was placed onto the acceptor plate carefully and incubated at 30°C for 16 hours to contact the membrane with the buffer in acceptor wells. UV/Vis absorption from 250 to 500 nm was measured for 100 μL of the compound solution in the donor side and 250 μL of the acceptor side. Equation used to calculate the

permeability rate (P_e) and the terms for the equation are shown below.

$$P_e = -C \times \ln\left\{1 - \left(\frac{[compound]_{acceptor}}{[compound]_{equilibrium}}\right)\right\}$$

, where $C = \frac{(V_D \times V_A)}{(V_D + V_A) \times Area \times time}$

V_D is the volume of donor compartment, V_A is the volume of acceptor compartment, area is the surface area of the membrane, time is the incubation time for the assay, $[compound]_{acceptor}$ is the final concentration of solute in the acceptor compartment and the $[compound]_{equilibrium}$ is the concentration of solute at theoretical equilibrium. Synergy HT multi-mode microplate reader of Biotek® was used to measure absorbance of samples of PAMPA tests at UV absorbance mode.

3. Results and Discussion

3.1. Analytical method validation

3.1.1. Specificity

The chromatographic spectrums shown in Figure 2 and Figure 3 proved that there was no interference for detection of Psammaplin A and Halichondramide, respectively. Although methanol has a strong UV absorbance around early 200 nm wavelength, a peak of Psammaplin A at 5.53 min was separated effectively with methanol peak in short running time. In case of Halichondramide, distinctive peak was detected at 5.37 min.

3.1.2. Linearity and limits of quantification (LOQ)

The linearity in the range from 0.5 μg/mL to 5 μg/mL was obtained by HPLC-UV analytical method for Psammaplin A. The calibration equation was drown at 0.5, 1, 2, 3, 4, 5 μg/mL of concentration (Figure 4) and equation was y = 253957x + 45987 (R^2=0.998), where y represents the peak area of UV absorbance and x indicates the concentration of the compound. In case of Halichondramide, the HPLC-UV method was linear over the range from 100 ng/mL to 1000 ng/mL. The equation of calibration curve (Figure 5) achieved at 100, 200, 400, 600, 800, 1000 ng/mL was as follows: y = 1180.9 x - 15921 and the correlation coefficient was 0.999.

The limit of quantification of Psammaplin A and Halichondramide were 0.5 μg/mL and 100 ng/mL, respectively, which is sufficient for satisfying two criteria of determination.

The signal to noise ratio was over 10 and its precision and accuracy of inter day analysis were under 20%RSD, which are acceptable for FDA guidance and ICH guidelines.

3.1.3. Accuracy and precision

Inter-assay and intra-assay precision and accuracy of all quality control samples at four different concentrations were analyzed to verify the method. The mean observed value for each sample was back-calculated on regression equation. The intra-assay precision of three replicates for three concentrations indicates the repeatability and the inter-assay precision means the intermediate precision of analyzed data from three consecutive days. Results are shown in Table 1 and Table 2. The intra-assay accuracy of Psammaplin A showed 99.0~101% with 0.52~2.97% of precision for three replicates of each concentration of samples (Table 1). Moreover, accuracy of the inter-assay was within 99.9~102% with 0.93~1.95% of precision. For Halichondramide, the intra-assay accuracy was included in the range of 102~104% with 1.11~4.39% of precision for three replicates (Table 2). In addition, accuracy of the inter-assay was within 104~105% with 0.59~4.61% of precision when conducted for three consecutive days. Since all values of quality control samples are less than 15%RSD and those of limit of quantification are below 20%RSD, HPLC-UV methods developed in this study are validated successfully and can be considered effective, accurate and precise.

3.2. Preformulation studies

3.2.1. Solubility studies

Aqueous solubility measured at equilibrium state was 4.57 ± 0.06 μg/mL (n=3) for Psammaplin A and 3.99 ± 0.02 μg/mL (n=3) for Halichondramide, respectively (Table 3). These results indicated that both compounds are poorly soluble and need to establish strategies for enhancement of solubility.

3.2.2. Lipophilicity studies

Lipophilicity was assessed as a $LogP_{oct/wat}$ value from the result of apparent partition coefficient, which was calculated from distribution ratio between 1-octanol and water. The log value of partition coefficient of Psammaplin A was 1.29 ± 0.07 (n=5) and that of Halichondramide was 1.16 ± 0.04 (n=5) (Table 3). Since $LogP_{oct/wat}$ over 1 indicated that both compounds were distributed much in octanol compared to water by about 10 times, these compounds could be regarded as lipophilic.

3.2.3. Permeability studies

In PAMPA test, the $LogP_e$ value determined as a parameter for permeability for Psammaplin A was -6.50 ± 0.23 cm/sec (n=9) and the result of Halichondramide was -6.94 ± 0.33 cm/sec (n=9) (Table 3). Lipid layer formed of phosphatidyl choline (Figure 6a) roles as a barrier to disturb passage of compound molecule like a cell layer in the body. After donor plate with the drug solution was layed on top of acceptor plate, sufficient incubation let some

molecules pass the lipid layer (Figure 6b). High permeability means that absorption fraction is over 90% by absolute bioavailability studies in permeation models and mass balance studies. These measured values were assessed as indicator of low permeability when compared to propranolol which is used as a standard parameter.

4. Conclusion

As collection and artificial culture techniques have improved, marine natural products become more attractive resources for medicine due to their unique mode of action and nearly unlimited quantity. Among marine natural products, Psammaplin A and Halichondramide draw attention of scientists in pharmaceutical industry because of their potent anticancer and antibacterial activities. Preformulation study is essential in the early stages of new molecular entity (NME) development to avoid costly failure in later stages. In this study, a sensitive, precise and accurate HPLC-UV analytical methods for these compounds were developed and validated. In addition, physical-chemical properties including the solubility, lipophilicity and permeability were investigated. Both compounds are relatively lipophilic, poorly soluble in DDW and have low permeability. Thus, it is expected to establish various pharmaceutical strategies such as structure modification and additives, for improving the physiochemical properties of these compounds and modifying their pharmacokinetics in the future drug development process.

Table 1. Intra-assay and inter-assay of precision and accuracy of Psammaplin A

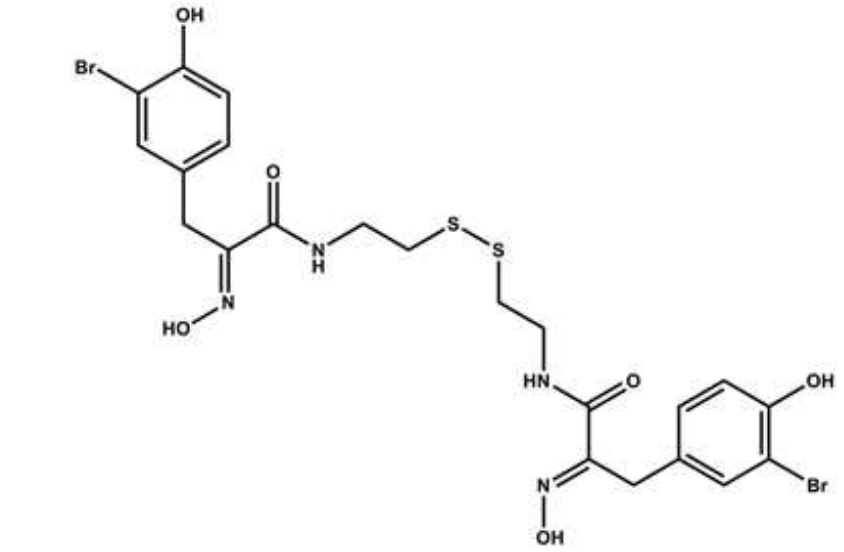
Nominal concentration (μg/mL)	Found concentration (μg/mL)	Precision (%,CV)	Accuracy (%)
Intra-assay (n=3)			
0.5	0.43 ± 0.01	2.84	86.7
1.5	1.50 ± 0.01	0.90	100
2.5	2.47 ± 0.01	0.52	99.0
4.5	4.54 ± 0.08	2.97	101
Inter-assay (n=3)			
0.5	0.45 ± 0.01	3.32	89.8
1.5	1.52 ± 0.03	1.95	102
2.5	2.50 ± 0.02	0.93	99.9
4.5	4.54 ± 0.08	1.86	101

Table 2. Intra-assay and inter-assay of precision and accuracy of Halichondramide

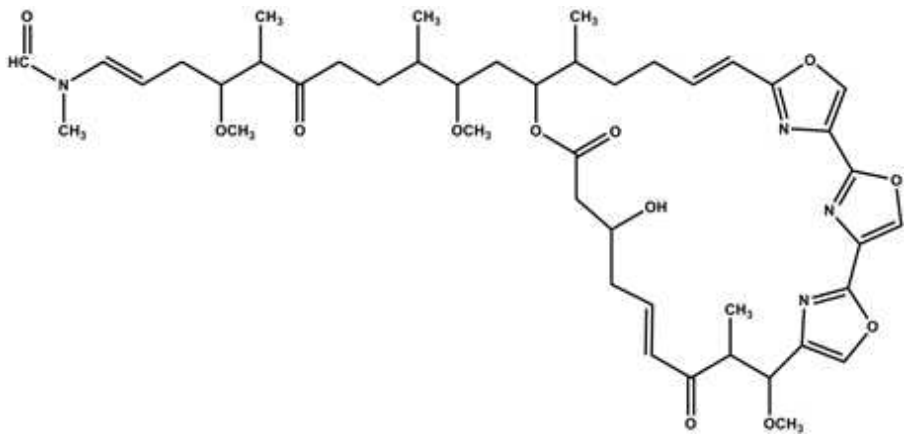
Nominal concentration (ng/mL)	Found concentration (ng/mL)	Precision (%,CV)	Accuracy (%)
Intra-assay (n=3)			
100	105 ± 3.91	3.71	105
300	305 ± 9.21	3.01	102
500	519 ± 22.8	4.39	104
900	933 ± 10.4	1.11	104
Inter-assay (n=3)			
100	111 ± 3.27	2.94	111
300	313 ± 2.85	0.91	104
500	523 ± 24.1	4.61	105
900	944 ±5.61	0.59	105

Table 3. Physicochemical properties of Psammaplin A and Halichondramide

	Psammaplin A	Halichondramide
Solubility (μg/mL, n=3)	4.57 ± 0.06	3.99 ± 0.02
Lipophilicity ($LogP_{oct/wat}$, n=5)	1.29 ± 0.07	1.16 ± 0.04
Permeability ($LogP_e$, cm/sec, n=9)	-6.50 ± 0.23	-6.94 ± 0.33
UV λ_{max}(nm)	207	232



(a)



(b)

Figure 1. Chemical structures of Psammaplin A (a) and Halichondramide (b)

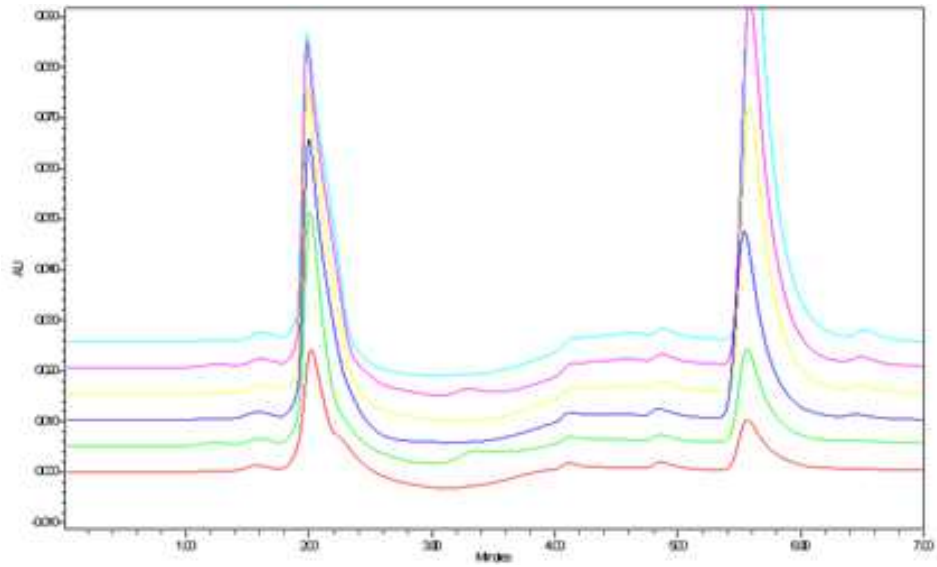


Figure 2. Chromatographic spectrum of Psammaplin A. Since methanol which was used for solubilization of compound in stock solution has strong absorbance at around 200 nm, a peak of left side is as large as right one. A Peak at 5.53 min represents Psammaplin A at UV absorbance wavelength of 207 nm.

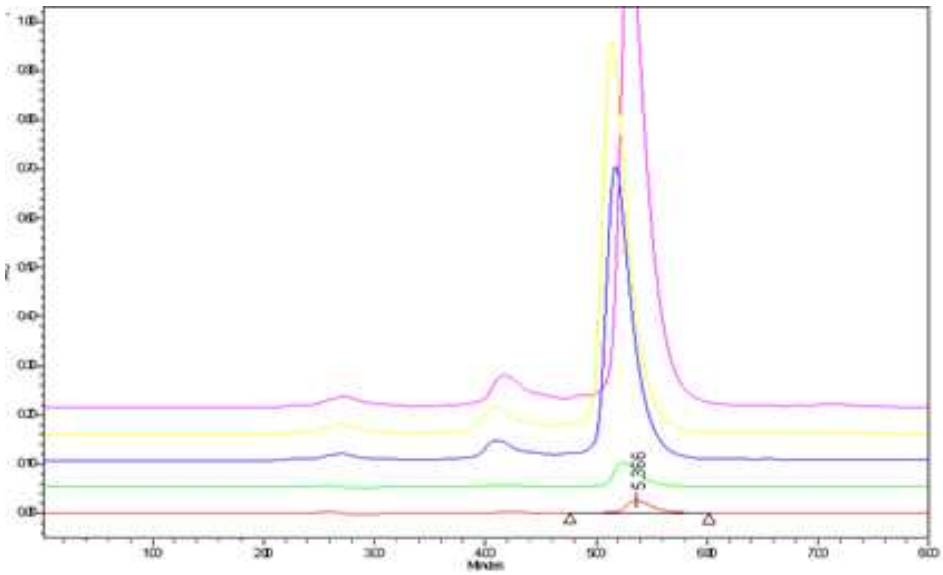


Figure 3. Chromatographic spectrum of Halichondramide. A peak at 5.37 min represents Halichondramide at UV absorbance wavelength of 232 nm.

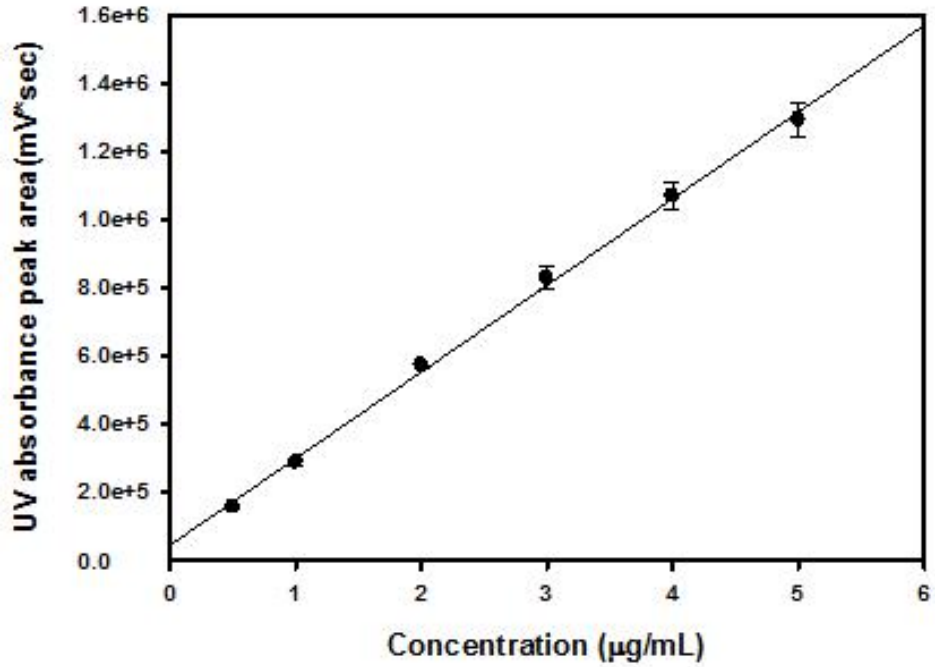


Figure 4. Calibration curve of Psammaplin A. Regression equation is y = 253957x + 45987 and correlation coefficient (R^2) was 0.998.

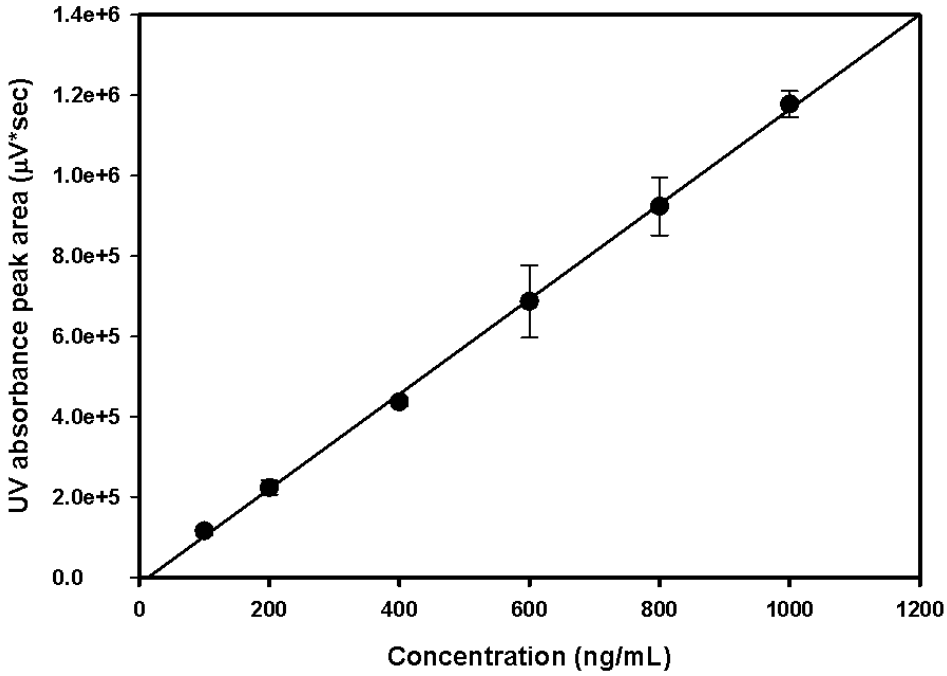
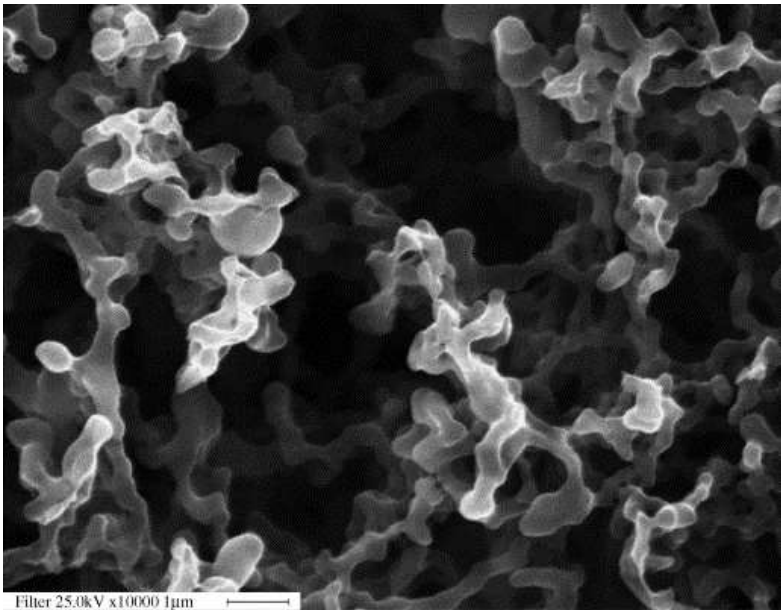
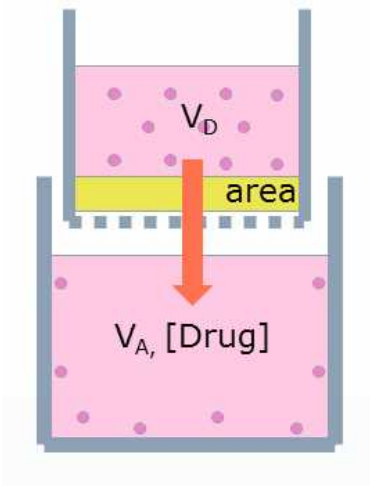


Figure 5. Calibration curve of Halichondramide. Regression equation is y = 1180.9 x – 15921 and correlation coefficient (R^2) was 0.999.



(a)



(b)

Figure 6. Electron microscope picture of lipid later formed with phosphatidyl choline (a) and mimetic diagram of PAMPA test (b)

References

1. A. Harvey, Drug Discovery Today, 5 (2000) 294-300.

2. R. Montaser, H. Luesch, Future Medicinal Chemistry, 3 (2011) 1475-1489.

3. R.A. Houghten, Current Biology, 4 (1994) 564-567.

4. J.-Y. Ortholand, A. Ganesan, Current Opinion in Chemical Biology, 8 (2004) 271-280.

5. T.F. Molinski, D.S. Dalisay, S.L. Lievens, J.P. Saludes, Nature Reviews Drug Discovery, 8 (2008) 69-85.

6. M. Feher, J.M. Schmidt, Journal of Chemical Information and Computer Sciences, 43 (2003) 218-227.

7. T. Henkel, R.M. Brunne, H. Müller, F. Reichel, Angewandte Chemie International Edition, 38 (1999) 643-647.

8. D.J. Newman, G.M. Cragg, K.M. Snader, Journal of Nnatural Pproducts, 66 (2003) 1022-1037.

9. D.J. Newman, G.M. Cragg, Journal of Natural Products, 70 (2007) 461-477.

10. B.K. Carté, Bioscience, 46 (1996) 271-286.

11. T.L. Simmons, E. Andrianasolo, K. McPhail, P. Flatt, W.H. Gerwick, Molecular Cancer Therapeutics, 4 (2005) 333-342.

12. C.C. Thornburg, T.M. Zabriskie, K.L. McPhail, Journal of Natural Products, 73 (2010) 489-499.

13. D.-X. Kong, Y.-Y. Jiang, H.-Y. Zhang, Drug Discovery Today, 15 (2010) 884-886.

14. J.G. McGivern, Drug Discovery Today, 11 (2006) 245-253.

15. B.M. Olivera, H. Terlau, Physiological Reviews, 84 (2004) 41-68.

16. M. D'Incalci, C.M. Galmarini, Molecular Cancer Therapeutics, 9 (2010) 2157-2163.

17. J.A. Smith, L. Wilson, O. Azarenko, X. Zhu, B.M. Lewis, B.A. Littlefield, M.A. Jordan, Biochemistry, 49 (2010) 1331-1337.

18. F.E. Koehn, G.T. Carter, Nature Reviews Drug Discovery, 4 (2005) 206-220.

19. B. Haefner, Drug Discovery Today, 8 (2003) 536-544.

20. A. Rayl, Scientist, 13 (1999), 1 – 3.

21. M. Fellenberg, A. Çoksezen, B. Meyer, Angewandte Chemie International Edition, 49 (2010) 2630-2633.

22. T.F. Molinski, Current Opinion in Biotechnology, 21 (2010) 819-826.

23. R.A. Long, F. Azam, Applied and Environmental Microbiology, 67 (2001) 4975-4983.

24. C. Cuevas, M. Pérez, M.J. Martín, J.L. Chicharro, C. Fernández-Rivas, M. Flores, A. Francesch, P. Gallego, M. Zarzuelo, F. de la Calle, Organic Letters, 2 (2000) 2545-2548.

25. G.M. Cragg, S.A. Schepartz, M. Suffness, M.R. Grever, Journal of Natural Products, 56 (1993) 1657-1668.

26. W. Fenical, P.R. Jensen, Nature Chemical Biology, 2 (2006) 666-673.

27. W. Fenical, P.R. Jensen, M.A. Palladino, K.S. Lam, G.K. Lloyd, B.C. Potts, Bioorganic & Medicinal Chemistry, 17 (2009) 2175-2180.

28. A.L. Lane, B.S. Moore, Natural Product Reports, 28 (2011) 411-428.

29. G.L. Challis, Journal of Medicinal Chemistry, 51 (2008) 2618-2628.

30. D.W. Udwary, L. Zeigler, R.N. Asolkar, V. Singan, A. Lapidus, W. Fenical, P.R. Jensen, B.S. Moore, Proceedings of the National Academy of Sciences, 104 (2007) 10376-10381.

31. J. Piel, Current Medicinal Chemistry, 13 (2006) 39-50.

32. W. Bergmann, D.C. Burke, The Journal of Organic Chemistry, 21 (1956) 226-228.

33. W. Bergmann, R.J. Feeney, The Journal of Organic Chemistry, 16 (1951) 981-987.

34. W. Bergmann, M.F. Stempien Jr, The Journal of Organic Chemistry, 22 (1957) 1575-1577.

35. A. Mayer, K.B. Glaser, C. Cuevas, R.S. Jacobs, W. Kem, R.D. Little, J.M. McIntosh, D.J. Newman, B.C. Potts, D.E. Shuster, Trends in Pharmacological Sciences, 31 (2010) 255-265.

36. J.W. Blunt, B.R. Copp, M.H. Munro, P.T. Northcote, M.R. Prinsep, Natural Product Reports, 23 (2006) 26-78.

37. J.W. Blunt, B.R. Copp, M.H. Munro, P.T. Northcote, M.R. Prinsep, Natural Product Reports, 27 (2010) 165-237.

38. Barich, D. H., Munson, E. J., & Zell, M. T. Drug Delivery: Principles and Applications (2005), 1-14, 57-71.

39. R. Gopinath, International Journal of Pharmaceutical & Biological Archive, 2 (2011).

40. S.S. Bharate, R.A. Vishwakarma, Expert Opinion on Drug

Delivery, (2013) 1-19.

41. M.J. Hageman, Combinatorial Chemistry & High Throughput Screening, 13 (2010) 90-100.

42. Y. Park, Y. Liu, J. Hong, C.-O. Lee, H. Cho, D.-K. Kim, K.S. Im, J.H. Jung, Journal of Natural Products, 66 (2003) 1495-1498.

43. D.H. Kim, J. Shin, H.J. Kwon, Experimental & Molecular Medicine, 39 (2007) 47-55.

44. M.Y. Ahn, J.H. Jung, Y.J. Na, H.S. Kim, Gynecologic Oncology, 108 (2008) 27-33.

45. F.D. Mora, D.K. Jones, P.V. Desai, A. Patny, M.A. Avery, D.R. Feller, T. Smillie, Y.-D. Zhou, D.G. Nagle, Journal of Natural Products, 69 (2006) 547-552.

46. J. Tabudravu, V. Eijsink, G. Gooday, M. Jaspars, D. Komander, M. Legg, B. Synstad, D. Van Aalten, Bioorganic & Medicinal Chemistry, 10 (2002) 1123-1128.

47. M.R. Kernan, D.J. Faulkner, Tetrahedron Letters, 28 (1987) 2809-2812.

48. J. Shin, H.-S. Lee, J.-Y. Kim, H.J. Shin, J.-W. Ahn, V.J. Paul, Journal of Natural Products, 67 (2004) 1889-1892.

49. S.-C. Chung, S.-H. Lee, K.H. Jang, W. Park, J.-e. Jeon, H. Oh,

J. Shin, K.-B. Oh, Bioorganic & Medicinal Chemistry Letters, 21 (2011) 3198-3201.

50. E. Muñoz-Couselo, J. Pérez-García, J. Cortés, OncoTargets and Therapy, 4 (2011) 185-192.

51. U. FDA, Rockville, MD: CDER, (2001).

52. I.H.T. Guideline, in: International Conference on Harmonisation, Geneva, Switzerland, www. ich. org, 2005.

국문초록

해양천연물 유래물질은 독특한 작용기전과 개발 가능성이 풍부한 공급원 등의 이점 때문에 제약 산업에서도 유망한 자원으로 주목받고 있다. 효과적인 신약 개발을 위해서는 개발 초기부터 preformulation 연구를 진행하여 약물의 기본적 물성을 충분히 이해해야 한다. 이는 약효만 고려할 것이 아니라 취약한 물성이나 약물동태학적 특성 등을 파악하여 효과적으로 극복할 수 있는 전략을 세우고 접근하는 것이 후기에 초래되는 시간적, 비용적 낭비를 미연에 방지할 수 있기 때문이다. 따라서 해양천연물의 일종인 Psammapliln A와 Halichondramide를 대상으로 편리하고 효율적인 분석법을 개발하고 두 가지 물질의 물리화학적 특성을 이해하는 preformulation 연구를 진행하였다. 각 물질의 정량을 위해 HPLC-UV 분석법을 개발하고 직선성, 정확성, 정밀성을 평가하여 밸리데이션하였다. 여러 가지 물리화학적 특성 중에는 물에 대한 용해도와 더불어 겉보기 분배계수 시험을 통해 지용성을 조사하고 PAMPA test로 막 투과도 역시 평가하였다.

Psammaplin A의 HPLC 상에서의 분리는 C18 컬럼에서 아세토나이트릴과 증류수의 혼합액 (55:45, v/v)을 이동상으로 이용하여 이루어졌다. 207 nm의 최대흡광파장에서 5.53분에 피크가 관찰되었고 최저정량한계는 0.5 μg/mL로 얻어졌다. 물에 대한 용해도는 4.57 ± 0.06 μg/mL (n=3) 이고 지용성의 척도가 되는 옥탄올-물 분배계수 ($LogP_{oct/wat}$)는 1.29 ± 0.07 (n=5) 인공지질막 투과도($LogP_e$)는 -6.50 ± 0.23 cm/sec (n=9) 였다. Halichondramide의 경우는 아세토나이트릴과 인산 나트륨 완충용액 (40 mM, pH6.0) 의 혼합액을 이동상 (50:50, v/v)을 시안 컬럼에 흘려 분리하였다. 232 nm의 최대흡광파장에서 5.37분에 피크가 관찰되었으며 최저정량한계는 100 ng/mL로 나타났다. 물에 대한 용해도는 3.99 ± 0.02 μg/mL (n=3), 지용성

의 척도가 되는 옥탄올-물 분배계수 ($LogP_{oct/wat}$)는 1.16 ± 0.04 (n=5), 인공지질막 투과도($LogP_e$)는 -6.94 ± 0.33 cm/sec (n=9) 라는 결과를 얻었다.

두 가지 물질에 대한 기존의 연구는 약효와 작용기전에 중점을 두었으나 이 연구를 통해 신속하고 정확하여 신뢰할 수 있는 분석법의 밸리데이션이 완료되었고 신약 개발 가능성을 타진하기 위해 필수적인 물리화학적 특성도 새로이 밝혀졌다는 것에 의의가 있다. 또한 다른 신약 후보 물질의 preformulation 연구에도 본 연구의 개념과 방법을 적용할 수 있을 것이다.

주요어: HPLC-UV 분석법 밸리데이션, Preformulation, Psammaplin A, Halichondramide, 해양천연물

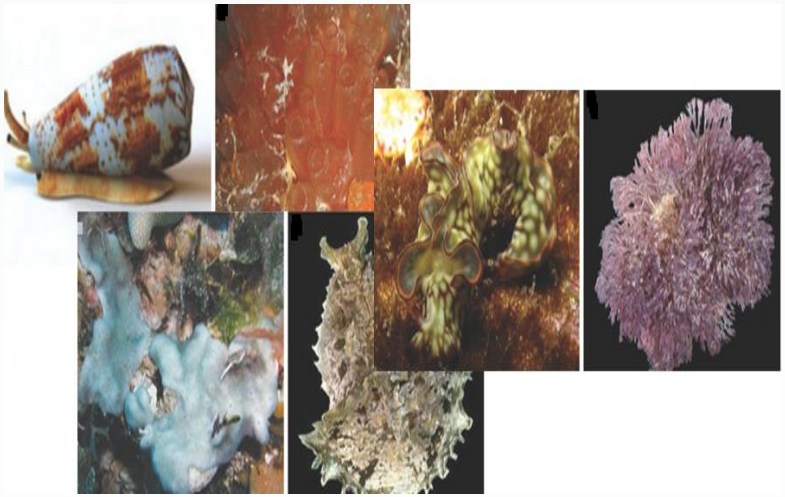
학 번: 2011-23729

Validation of HPLC-UV Analysis & Preformulation Studies of Psammaplin A and Halichondramide

Ji Hyun Park
Department of Pharmaceutics
College of Pharmacy
Seoul National University

Introduction

Marine natural products



✓ **Advantages**

- Limitation of terrestrial resource
- Good possibility to discover new compounds by technical development
- Strong biological activity through unique mechanism

✓ **Halaven**®(Eisai, FDA approved)

- 3rd line treatment for metastatic breast cancer
- Derived from **Halichondrin B**
- Mechanism of action:

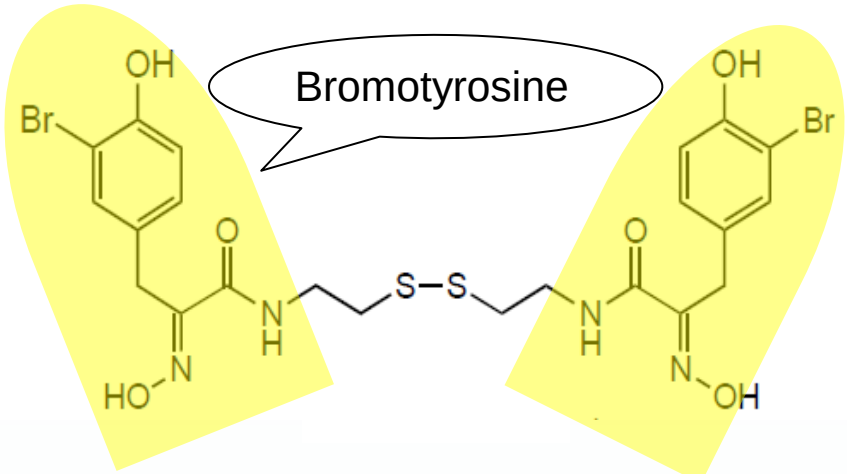
Inhibition of the growth phase of microtubules → tubulin-based antimitotic mechanism → G2/M cell-cycle block, disruption of mitotic spindles → apoptosis

Botanical and plant-derived drugs:global markets, *BCC research*

Introduction

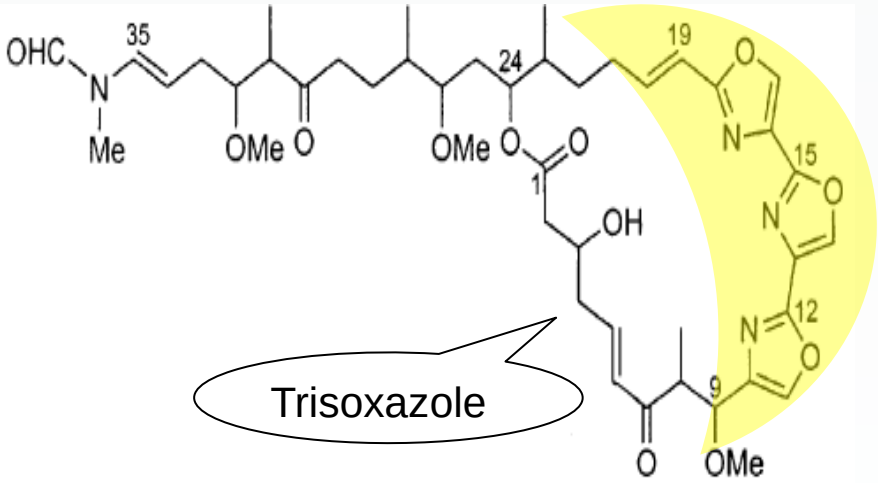
Psammaplin A & Halichondramide

✓ Psammaplin A

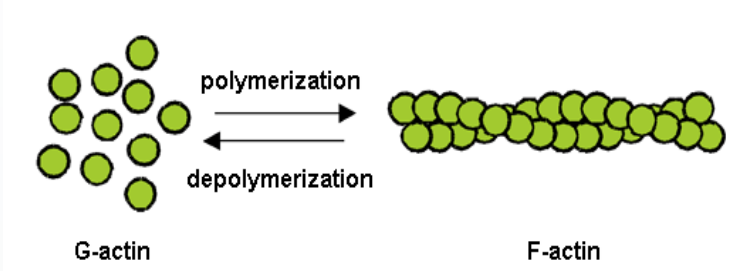


- **Molecular Formula ; $C_{22}H_{24}S_2{}^{79}Br_2N_4O_6$ (MW ; 662.96)**
- **White powder**
- **Anticancer/antimicrobial activity**
  - **Induction of apoptosis by activation of PPARr**
  - **Inhibition of enzymes involved in cell growth**
    - **HDAC and DNMT**
    - **DNA gyrase, chitinase, MCA**

✓ Halichondramide



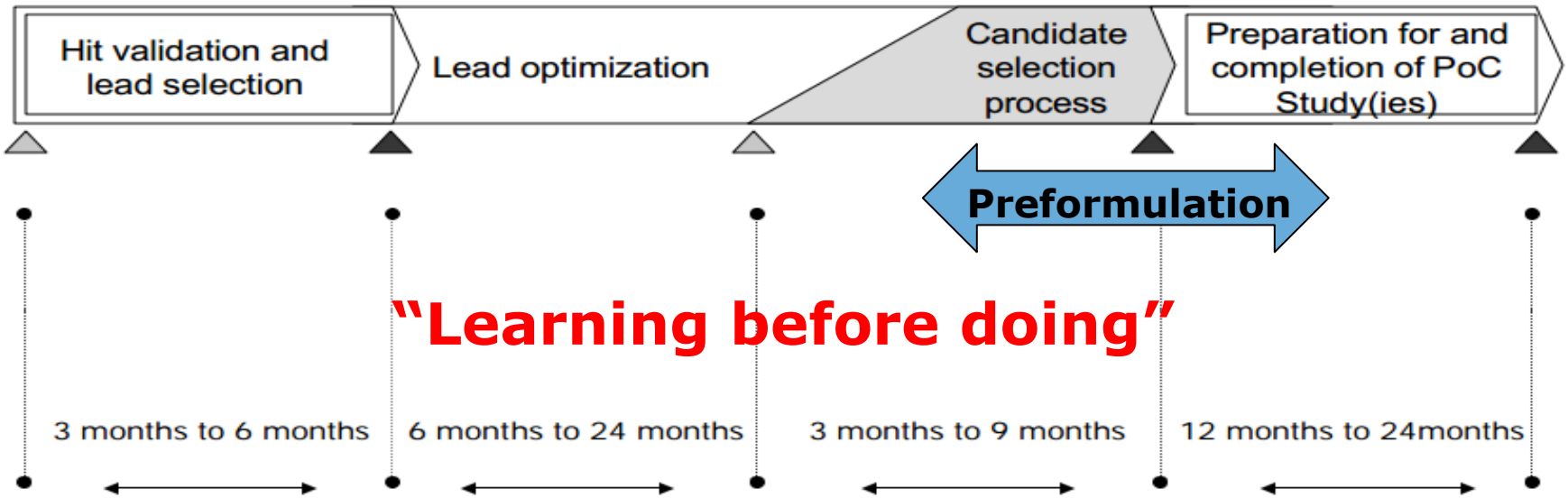
- **Molecular Formula ; $C_{44}H_{60}N_4O_{12}$ (MW ; 836.97)**
- **Colorless amorphous solid**
- **Anticancer/antifungal activity**
  - **Depolymerization of actin**



New Macrolides from the Sponge *Chondrosia corticata, J. Nat. Prod. 2004,* 67, 1889-1892
Activated chemical defense in marine sponges-a case study on *Aplysinella* sp., *J Chem Ecol*,2008, 34:1242–1252

Introduction

What is "Preformulation"?



- **Fundamental tests :**

Spectroscopy(UV simple assay), solubility(intrinsic aqueous solubility, pH effect), lipophilicity($k_{o/w}$), melting point(DSC polymorphism), assay development, stability(thermal, hydrolysis, pH, oxidation, photolysis)

- **Derived tests :**

Microscopy(particle size, morphology), bulk density, flow properties, compression properties, excipient compatibility

http://www.pharmacy.utah.edu/pharmaceutics/pdf/Preformulation.pdf

A review of preformulation studies of drugs, IJPRD, 2012, 4, 64-74

Objectives

Preformulation of PsA & Hali

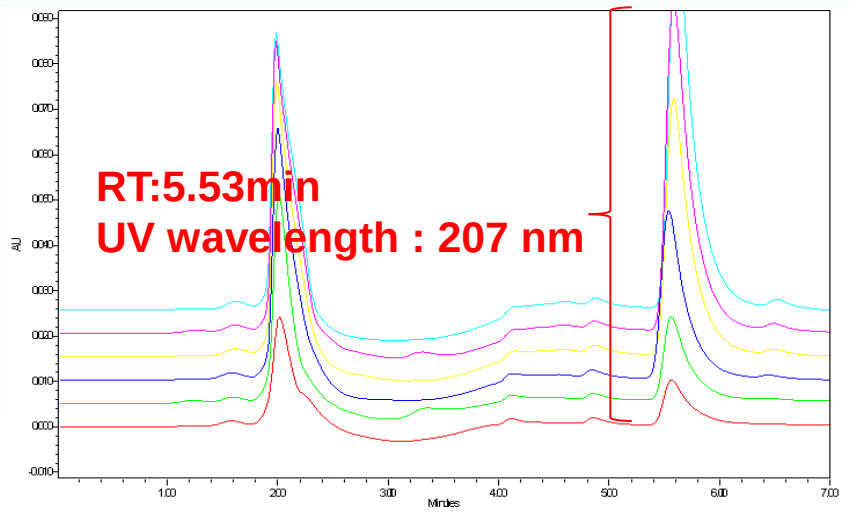
- ✓ **HPLC/UV analysis method validation**
  - Linearity
  - Intra/Inter-day variability(precision, accuracy)

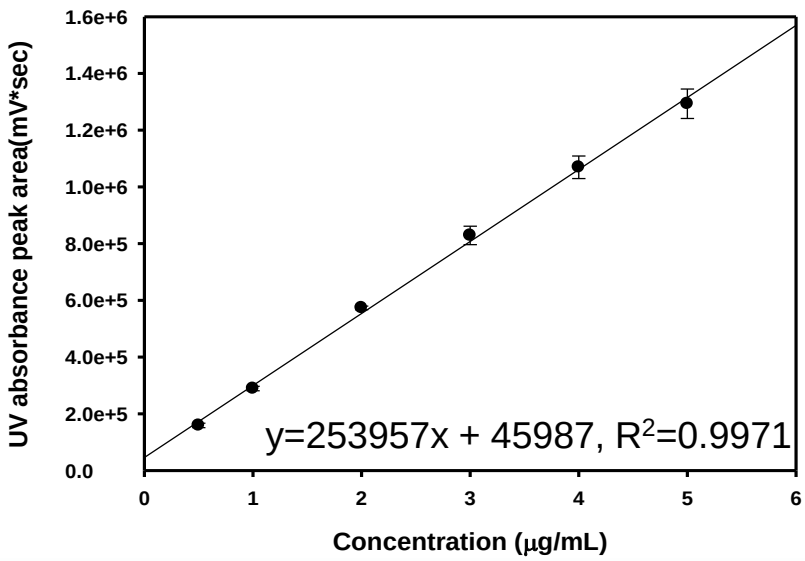
- ✓ **Characterization of physical-chemical properties**
  - Lipophilicity(Apparent partition coefficient)
  - Solubility
  - Permeability

Methods & Results

Analysis of Psammaplin A

Using 55% ACN solution as a mobile phase on a C18 column with a flow rate of 1 mL/min





Theoretical concentration(ug/mL)	QL 0.5	LQC 1.5	MQC 2.5	HQC 4.5
(A) Intra-day variability				
Found conc.(mean ± S.D.)	0.434±0.0123	1.50±0.0134	2.47±0.0129	4.54±0.0828
Precision(CV, %)	2.84	0.897	0.521	2.97
Accuracy(%)	86.7	100	99.0	101
(B) Inter-day variability				
Found conc.(mean ± S.D.)	0.449±0.0149	1.52±0.0298	2.50±0.0233	4.54±0.0842
Precision(CV, %)	3.32	1.95	0.93	1.86
Accuracy(%)	89.8	102	99.9	101

***QL(Quantitation Limit): 0.5 ug/mL**

- 시각적 평가
- S/N ratio : 10
- QL=10 x σ / S

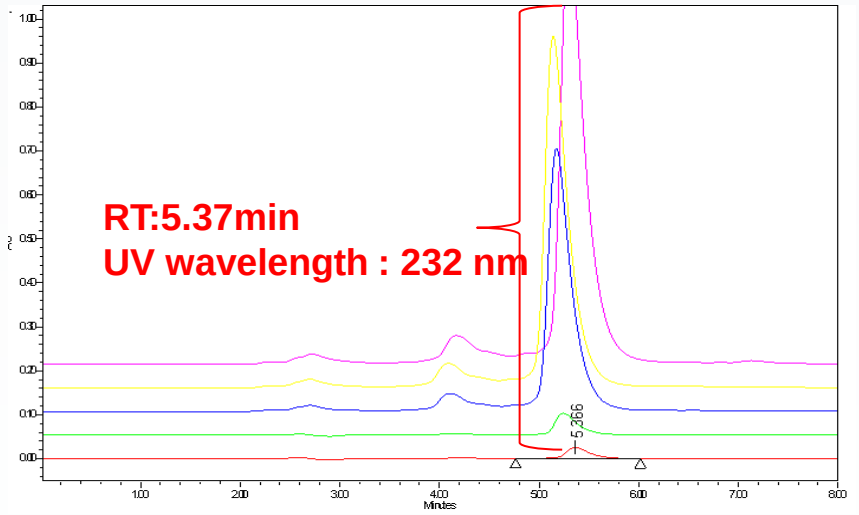
(σ:검량선 y절편 STDEV
S:검량선의 기울기)

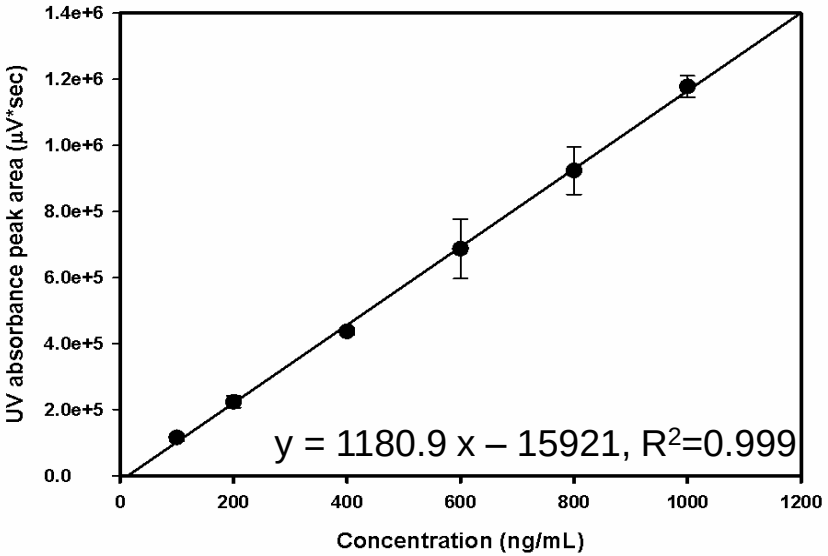
Validated

Methods & Results

Analysis of Halichondramide

Using 50% ACN : sodium phosphate buffer (40mM, pH 6.0) solution as a mobile phase on a CN column with a flow rate of 1 mL/min





Theoretical concentration(ng/mL)	QL 100	LQC 300	MQC 500	HQC 900
(A) Intra-day variability				
Found conc.(mean ± S.D.)	105±3.91	305±9.21	519±22.8	933±10.4
Precision(CV, %)	3.71	3.01	4.39	1.11
Accuracy(%)	105	102	104	104
(B) Inter-day variability				
Found conc.(mean ± S.D.)	111±3.27	313±2.85	523±24.1	944±5.61
Precision(CV, %)	2.94	0.913	4.61	0.594
Accuracy(%)	111	104	105	105

***QL(Quantitation Limit): 100 ng/mL**

- 시각적 평가
- S/N ratio : 10
- QL=10 x σ / S

(σ:검량선 y절편 STDEV
S:검량선의 기울기)

Validated

Methods & Results

Physical-chemical properties

❖ Solubility

- ✓ Evaporate the solvent of stock solution 500μL and add DDW 100μL
- ✓ Centrifuge for 10min at RT, 13200rpm after Vortexing for 24hrs at RT
- ✓ Analyze the collected saturated solution samples by HPLC/UV analysis method

❖ Lipophilicity(Apparent Partition Coefficient)

- ✓ Drug solution 50μL + pre-saturated water 450μL + pre-saturated octanol 500μL
- ✓ Centrifuge for 10min at RT, 13200rpm after vortexing for 2hrs at RT
- ✓ Separate each layer and make matched matrix sample
  (sample 50μL + other phase 50μL + mobile phase 900μL)
- ✓ Analyze by HPLC/UV analysis method

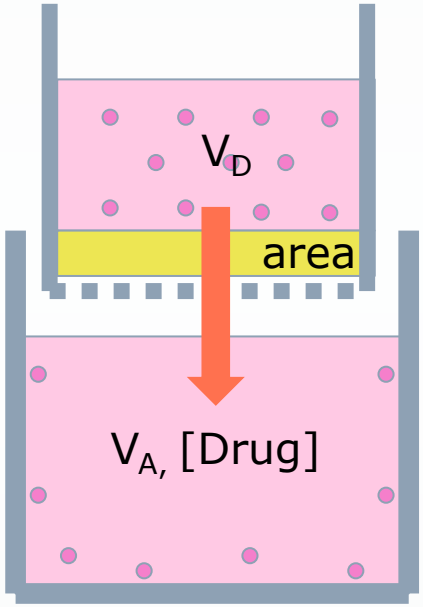
❖ Permeability

- ✓ Prepare 0.5 mM drug solution in 5% DMSO/PBS and 0.5%(w/v) lecithin solution in dodecane by sonication
- ✓ Pipette lecithin solution carefully into each donor plate well to set thin lipid membrane and Immediately add drug solution to each well of donor plate
- ✓ Put 5% DMSO/PBS in each well of acceptor plate
- ✓ Place the donor plate into the acceptor plate
- ✓ Measure UV/Vis absorption for samples after incubation at room temperature for 16 hrs

Methods & Results

Physical-chemical properties

RESULTS	Solubility (ug/mL, n=3)	Lipophilicity ($logP_{oct/wat}$, n=5)	Permeability ($logP_e$, cm/sec, n=9)
Psammaplin A	4.57 ± 0.0592	1.29 ± 0.0668	-6.50 ± 0.178
Halichondramide	3.99 ± 0.0164	1.16 ± 0.0370	-6.94 ± 0.341



※ $\log P_e = \log\{(-C) * \ln(1 - r)\}$

$$C = \frac{V_D * V_A}{(V_D + V_A) * \text{area} * \text{time}}$$

$$r = \frac{[\text{Drug}]_{acceptor}}{[\text{Drug}]_{equilibrium}}$$ → When there is layer
→ When there is no layer

Permeability	log Pe
High	≥-5.15
Medium	-5.15~-6.4
Low	≤-6.4

⇒ **Both compounds are poorly soluble in DDW, lipophilic and have low permeability.**

Conclusion

- ✓ As collection and artificial culture techniques have improved, marine natural products become more attractive resources for medicine due to their unique mode of action and nearly unlimited quantity.
- ✓ Among marine natural products, **Psammaplin A and Halichondramide** draw attention of scientists in pharmaceutical industry because of their potent anticancer and antibacterial activities.
- ✓ To effectively develop new drugs, preformulation process should be conducted in the early stages to understand specific characteristics of target compound for further drug development.
- ✓ Through this study, a sensitive, precise and accurate HPLC-UV analytical methods for these compounds were developed and validated. In addition, some physical-chemical properties were investigated. Both are relatively lipophilic but poorly soluble in DDW and have low permeability.
- ✓ Therefore, it is imperative to establish various strategies, such as structure modification, additives for improving solubility and effective drug delivery system, to overcome expected poor pharmacokinetic problem. The concept and scheme of this study can be applied to other compounds usefully for drug development.

Preformulation of Psammaplin A

Ji-Hyun Park, Suk-Jae Chung, Chang-Koo Shim and Dae-Duk Kim

Department of Pharmaceutics, College of Pharmacy, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul 151-742, South Korea

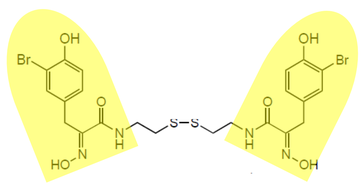
Abstract

Objectives Psammaplin A, a marine natural product, shows anticancer and antimicrobial activity. The purpose of this study was the validation of an analytical method and the characterization of Psammaplin A for as part of preformulation. **Methods** The HPLC-UV method using a C18 column and acetonitrile : DDW (55:45, v/v) as a mobile phase was developed and validated for the quantification of Psammaplin A. The apparent partition coefficient was calculated from the distribution ratio between n-octanol and DDW. In addition, solubility in DDW at equilibrium state was measured and permeability was evaluated through a PAMPA test. **Results** A peak of Psammaplin A was obtained after 5.53 min at a flow rate of 1 mL/min on 207 nm of detection wavelength. The method was linear over the range of 0.5~4.5 μg/mL and for each quality control sample, inter- and intra-assay precision and accuracy were below 15% RSD. The lower limit of quantitation was 0.5 μg/mL. Solubility in DDW was 4.57 ± 0.06 μg/mL (n=3) and log Poct/wat was 1.29 ± 0.07 (n=5). The log Pe value as a parameter for the permeability was -6.50 ± 0.18 cm/sec (n=7). Conclusions The accurate and precise HPLC-UV method for the analysis of Psammaplin A was developed and validated. Physicochemical characteristics including solubility, lipophilicity and permeability were investigated through this study.

Introduction

Psammaplin A

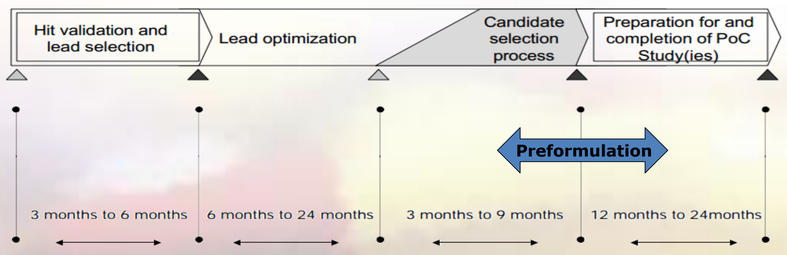
- Terrestrial plants and microbes have been a traditional source of drug candidates. However, recently attention has somewhat shifted to marine derived materials, which are much less investigated than their terrestrial counterparts.
- There are good possibilities to discover new compounds from world oceans due to technical improvement of the screening methods. Furthermore, given the sheer number of biological active marine compounds, the likelihood of finding compounds with anticancer activity is quite high.
- Psammaplin A was collected from *Psammaplinaplysilla* sponge in 1987. The molecular formula is $C_{22}H_{24}S_2Br_2N_4O_6$(662.96 g/mol). Its anticancer activity is caused by induction of apoptosis via activating PPAR(peroxisome proliferating activated receptor) and inhibition of enzymes related to cell growth, for example, HDAC(histone deacetylase) and DNMT(DNA methyl transferase). Moreover, it has antibacterial activity because of the inactivation of DNA gyrase, chitinase and mycothiol-S-conjugate amidase.



<Psammaplin A>

Preformulation

- When pharmacokinetic problems of target molecules are found late in the drug discovery process, those can easily become severe obstacles, because at this stage the costs of improving poor features may be too high. Therefore, it is important to screen problematic candidates in earlier stages of development through rapid and reliable research prediction.



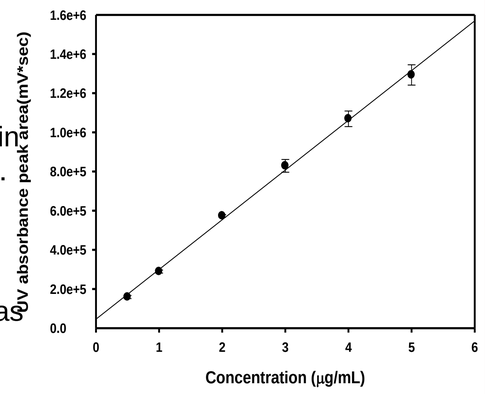
Result

Development of an analytical method

- HPLC-UV system using a C18 column
- 55% acetonitrile aqueous solution as a mobile phase
- Peak was detected at 5.53 min with 1 mL/min of flow rate at 207 nm detection wavelength.

Validation of HPLC-UV analysis

- This method was linear over the range of 0.5~4.5 μg/mL. Equation of calibration curve was y = 254957x + 45987,(n=3, R^2=0.998).
- Precision and accuracy of all quality control samples (n=3) were below 15% RSD.



<Calibration curve>

Theoretical concentration (μg/mL)	Intra-day (n=5) Calculated concentration (μg/mL) (mean ± S.D.)	Precision (%, RSD)	Accuracy (%)	Inter-day (n=3) Concentration found (μg/mL) (mean ± S.D.)	Precision (%, RSD)	Accuracy (%)
0.5	0.41 ± 0.03	6.68	82.90	0.47 ± 0.08	16.75	94.06
1.5	1.50 ± 0.03	2.14	100.02	1.54 ± 0.06	3.90	102.85
2.5	2.49 ± 0.08	3.37	99.65	2.56 ± 0.06	2.34	102.20
4.5	4.54 ± 0.14	2.97	100.90	4.50 ± 0.20	4.52	100.08

<inter-, intra assay variability>

Investigation of physical-chemical properties

- Aqueous solubility at equilibrium state was measured. Lipophilicity was assessed as a $logP_{oct/wat}$ value, calculated from distribution ratio between octanol and water. Log P_e acquired from PAMPA tests was used as a parameter of permeability. Specific results are presented in table below.

Solubility	Lipophilicity($logP_{oct/wat}$)	Permeability($logP_e$)
4.57 ± 0.06 μg/mL	1.29 ± 0.07	-6.50 ± 0.18 cm/sec
(n=3)	(n=5)	(n=7)

<Basic physical-chemical properties>

Method

Development and validation of analytical method

- Using HPLC-UV system
- Checking linearity, accuracy and precision of quality control samples

Investigation of physical-chemical properties

- Solubility
- Lipophilicity : Apparent partition coefficient test (log $P_{oct/wat}$)
- Permeability : PAMPA test (log Pe)

Conclusion

As collection and artificial culture techniques have improved, marine natural products become more attractive resources for the pharmaceutical industry due to their nearly unlimited quantity and unique mechanisms of action. Preformulations in the early stages of the drug development process helps to understand specific characteristics of target compound for further drug development. In this preformulation study about Psammaplin A, which shows anticancer and antibacterial activity, a sensitive, precise and accurate HPLC-UV analytical method for this compound was developed and validated. Furthermore, some physical-chemical properties like solubility, lipophilicity and permeability were also obtained. Psammaplin A was found to belong to BCS class IV.

Preformulation of Halichondramide

Ji-Hyun Park, Suk-Jae Chung, Chang-Koo Shim and Dae-Duk Kim

Department of Pharmaceutics, College of Pharmacy, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul 151-742, South Korea

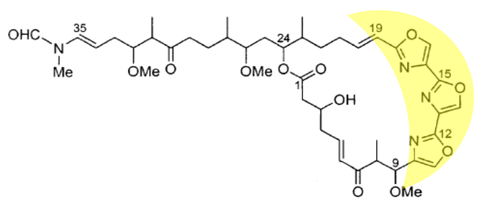
Abstract

Objectives Halichondramide is one of marine natural products, which has anticancer and antifungal effects. This preformulation study was performed to develop a suitable analytical method and investigate the physical-chemical properties of the material. **Methods** The HPLC-UV analytical method for quantification of Halichondramide was developed and validated by linearity, accuracy and precision. The physical-chemical characterization of the material included lipophilicity (log $P_{oct/wat}$) and aqueous solubility equilibrium state. PAMPA tests were also conducted to assess the permeability (Pe). **Results** Chromatographic separation was performed with a mobile phase composed of acetonitrile and sodium phosphate buffer (40 mM, pH 6.0) (50:50, v/v) using a CN column. The HPLC-UV method was linear over the range of 100 ~ 900 ng/mL and fast with a retention time of 5.37 min on 232 nm. Inter- and intra-assay precision and accuracy of all quality control samples were less than 15% RSD and the lower limit of quantitation was 100 ng/mL. Aqueous solubility was 3.99 ± 0.02 μg/mL (n=3) and log $P_{oct/wat}$ was 1.16 ± 0.04 (n=5). The log Pe value as a parameter for permeability was -6.94 ± 0.34 cm/sec (n=7). **Conclusions** Preformulation of Halichondramide, involving the development and validation of HPLC-UV analytical method and investigation of some physicochemical properties was successfully conducted.

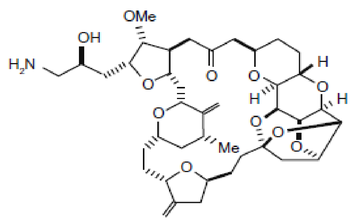
Introduction

Halichondramide

- Currently many countries are interested in marine natural products due to the limitation of terrestrial resources and good possibility to discover new compounds thanks to advances in screening methods. Since these materials usually have strong biological activity based on unique structures and mechanisms. There exists the possibility of finding marine natural products with desirable properties like anticancer activity.
- Halichondramide is a marine natural product, discovered from *Chondrosia corticata* Thiel in 1986. With the molecular formula $C_{44}H_{60}N_4O_{12}$(836.97g/mol), cell growth inhibiting properties based on depolymerization of actin, a cytoskeletal protein essential for cellular mortality, resulting in anticancer and antifungal activity.
- Eribulin mesylate(Halaven™), a derivative of Halichondrin B which is a synthetic analogue of Halichondramide is FDA approved for the treatment of metastatic breast cancer. Therefore Halichondramide becomes a promising drug due to structural similarities.



<Halichondramide>



<Eribulin mesylate>

Preformulation

- Many drug candidate molecules fail due to poor pharmacokinetic-related properties, such as solubility and permeability. If pharmacokinetic problems are found late in the discovery process, those failures may inflict high costs despite otherwise potent properties.
- Therefore, it is important to screen possibly problematic candidates in early stages of the development process through rapid and reliable research prediction.

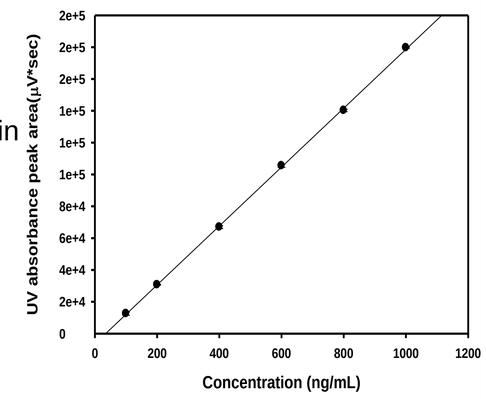
Result

Development of an analytical method

- HPLC-UV system using a CN column
- Acetonitrile with sodium phosphate buffer (pH6.0, 40mM, 50%) as a mobile phase
- Peak was detected at 5.37 min with 1 mL/min flow rate at 232 nm detection wavelength.

Validation of HPLC-UV analysis

- Linearity was acquired at the range of 100~900 ng/mL. Equation of calibration curve was y = 185.09x + 6373.7 (n=3, R^2=0.999).
- Precision and accuracy of all quality control samples (n=3) were below 15% RSD.



<Calibration curve>

Theoretical concentration (ng/mL)	Intra-day (n=5) Calculated concentration (ng/mL) (mean ± S.D.)	Precision (%, RSD)	Accuracy (%)	Inter-day (n=3) Concentration found (ng/mL) (mean ± S.D.)	Precision (%, RSD)	Accuracy (%)
100	115.44 ± 4.78	4.14	115.44	113.60 ± 7.17	6.33	113.60
300	323.00 ± 14.57	4.51	107.67	312.09 ± 24.94	7.99	103.03
500	540.68 ± 24.16	4.47	108.14	516.46 ± 37.39	7.24	103.29
900	949.11 ± 12.29	1.30	105.46	926.18 ± 57.95	6.26	102.91

<inter-, intra assay variability>

Investigation of physical-chemical properties

- Aqueous solubility at equilibrium state was measured. Lipophilicity was assessed as a $logP_{oct/wat}$ value, calculated from distribution ratio between octanol and water. Log P_e acquired from PAMPA tests was used as a parameter of permeability. Specific results are presented in table below.

Solubility	Lipophilicity($logP_{oct/wat}$)	Permeability($logP_e$)
3.99 ± 0.02 μg/mL	1.16 ± 0.04	-6.94 ± 0.34 cm/sec
(n=3)	(n=5)	(n=7)

<Basic physical-chemical properties>

Method

Development and validation of analytical method

- Using HPLC-UV system
- Checking linearity, accuracy and precision of quality control samples

Investigation of physical-chemical properties

- Solubility
- Lipophilicity : Apparent partition coefficient test (log $P_{oct/wat}$)
- Permeability : PAMPA test (log Pe)

Conclusion

Marine natural products are good resources for pharmaceutical industry due to their unique mode of action and nearly unlimited quantity. To effectively develop new drugs, preformulation process should be conducted in the early stages to avoid costly failure in later stages. In this study, Halichondramide, which shows anticancer and antifungal activity, was selected as a marine natural product and a rapid as well as accurate analytical method for this compound was developed and validated. In addition, some physical-chemical properties including solubility, lipophilicity and permeability were also investigated as a part of a preformulation study. Halichondramide was found to belong to BCS class IV.