Thesis for the Degree of Master of Engineering

Quantification and Validation of Sodium Saccharin in Laver (*Pyropia* sp.) by HPLC

by

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Pukyong National University

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HPLC를 이용한 김(*Pyropia* sp.)의 사카린나트륨 정량분석 및 분석법 검증

Advisor: Prof. Suengmok Cho

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요 약

사카린나트륨은 감미료의 일종으로 다양한 가공식품에 많이 사용되는 대표적인 식품첨가물이다. 설탕보다 450배 정도 단맛을 내어 소량으로도 단맛을 주기 때문에 음료 및 각종 가공식품에 이용된다. 마른김은 아시아에서 주로 생산되며, 대중적으로 많이 섭취하는 조미김의 주원료로 국내에서는 '자연 수산물'로 분류되고 있다. 자연수산물에는 식품첨가물을 넣을 수 없기 때문에 사카린나트륨이 첨가되지 않도록 관리되고 있지만, 마른김에서 사카린나트륨의 검출되는 사례가 보고되고 있다. 또한 기존의 공전상 분석법으로는 정성적인 판단은 가능하나, 사카린나트륨의 회수율이 낮은 것을 확인하였으며 현재 공전상의 분석법의 개선이 필요하다. 지금까지의 사카린나트륨 분석법은 음료 및 가공식품의 감미료 분석에 초점을 두었으며, 김에서 HPLC를 이용한 사카린나트륨 분석에 관한 연구는 거의 없다. 따라서 김의 식품적 특성에 맞는 분석법과 관련한 연구가 필요한 실정이다. 김에는 색소 등 다른 성분들이 존재하기 때문에 전처리 과정을 거치는 것이 필수적인데, 김에서 사카린나트륨을 HPLC로 분석하기 위해서 전처리 방법을 개선하여 분석을 진행하였다. 전처리

과정은 시료를 초음파 처리 및 원심 분리하여 희석한 것을 시험용액으로 하였다. HPLC에 주입하기 전에 용액을 0.45 μm 멤브레인 시린지 필터로 여과하였다. 사카린나트륨 분석을 위한 HPLC 분석 조건은 칼럼 온도 40℃에서 역상계 C_{18} 칼럼 (4.6 mm x 250 mm, 5 μm)을 이용하였고, 이동상은 pH 4.0으로 조정된 30% 메탄올 (10% TPA-OH), 유속 1.0 mL/min, 주입량 10 μm, UV 검출기로 210 nm 파장에서 분석하였다. 사카린나트륨 표준품의 직선성은 회귀곡선 R^2 > 0.99 이상으로 높은 것으로 확인되었다. 검출한계와 정량한계는 각각 1.37 mg/kg, 4.55 mg/kg으로 확인되었다. 정확도는 90-110% 이내의 만족스러운 회수율을 보였으며, 반복성을 평가한 정밀도는 상대표준편차 (RSD, %)가 양호한 값을 나타내었다. 앞서 실험을 통하여 확립된 분석법은 김에서의 사카린나트륨을 정량 및 분석법을 검증하였으며, 이를 바탕으로 시중에 유통되고 있는 마른김을 무작위로 구매하여 분석한 결과 사카린나트륨은 검출되지 않았다. 또한 마른김이 주원료로 사용되는 조미김에도 사카린나트륨이 검출될 가능성이 있기 때문에, 본 실험에서는 조미김에 직접 사카린나트륨을 첨가하여 조미김에서도 검증된 분석법에 잘 적용하는지 확인하였다. 회수율 측면에서 만족스러운 결과를 보였으며, 이는 조미김에서도 사카린나트륨의 분석이 가능함을 시사한다.

본 연구에서 수행한 HPLC 분석법 검증결과는 김 (*Pyropia* sp.)에서 사카린나트륨을 측정하는 데 유용하게 사용될 수 있는 성공적인 분석법임을 확인하였다. 이러한 분석법 검증 및 모니터링 결과를 통하여 먹거리 안전성을 확보하기 위한 기초자료로 활용될 수 있다.

Keywords: Sodium saccharin, Dried laver (*Pyropia* sp.), HPLC, Method validation

Introduction

Sodium saccharin (SS) is a representative food additive widely used as a sugar substitute and sweetener. It is 450 times sweeter than sugar (MFDS, 2017) and provides sweetness in small amounts, so it is widely used in a variety of processed foods and beverages, and its use is increasing (Park et al., 2014; Grembecka et al., 2014; Lee et al., 2001). The daily intake and amounts of artificial sweeteners added to various foods are regulated (Kim et al., 2004; Yun et al., 2022), and the list of sweeteners allowed in foods and their permitted levels varies by country. The food industry must monitor that products containing sweeteners comply with the regulations (Yun et al., 2022).

Dried laver (*Pyropia* sp.), primarily produced in Korea, is a type of seaweed a main ingredient for the seasoned laver that is highly consumed worldwide (Cho et al., 2015). The dried laver has become the top exported seafood product in Korea with the value of laver exports reaching $580 million, and indicating a significant increase in laver consumption (MOF, 2021). In Korea, the addition of sweeteners such as SS and acesulfame potassium to dried laver is prohibited because it is classified as a "natural seafood". However, the detection of SS in dried laver was consistently reported (MFDS, 2021). When SS added to dried laver was analyzed using the Korean Food Code method, a qualitative analysis was possible, but the recovery rates were lower than the actual amount added. These results indicate that there may be errors, presenting that the current method for analyzing laver needs to be improved.

To date, many studies have predominantly focused on the analysis of sweeteners (SS)

in beverages and processed foods. However, there have been no reports regarding the analysis of SS in the dried laver. Therefore, it is necessary to improve an analytical method that can be applied to the characteristics of various foods such as dried laver by analyzing SS using HPLC, which is presently listed in the Korean Food Code method (MFDS, 2023). Furthermore, it is important to consider the characteristics of the sample and perform appropriate pretreatment procedures, including purification and dilution processes. (Yun et al., 2022).

Our study investigated and advanced the HPLC method, including evaluating SS addition and sample preparation procedures tailored to the characteristics of dried laver samples. Furthermore, the validated method was applied to monitor the presence of SS in both the dried laver and seasoned laver products available in the market. The findings of this research can serve as a foundation for ensuring food safety for consumers concerning dried laver consumption.

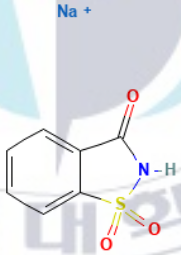
Materials and Methods

1. Chemicals and reagents

The chemicals used in this study were purchased as HPLC grade for analysis, including methanol (MeOH), 10% tetra-propylammonium hydroxide (10% TPA-OH), phosphoric acid, and water. MeOH and water were purchased from Honeywell Burdick & Jackson (Muskegon, MI, USA), 10% TPA-OH was purchased from FUJIFILM (Wako Pure Chemical Corporation, OSA, Japan), and phosphoric acid was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All of the reagents were stored at room temperature.

Reference standard of sodium saccharin (SS, ≥99.0%) was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). The material properties of SS were shown in Table 1 (NCBI, 2023). SS is a colorless to white crystalline powder that is stable in neutrality and unstable in acidic solutions below pH 3.8. It is also 450 times sweeter than sucrose.

Table 1. Physicochemical properties of sodium saccharin

Chemical compound	Formula	Chemical structure	Mass (g/mol)	Sweetness (more than sugar)
Sodium Saccharin	$C_7H_4NNaO_3S$		205.17	450

2. Samples preparation of dried and seasoned laver

The ingredients used in this experiment were obtained from an online and local market in Busan and tested on dried laver (*Pyropia* sp.). The dried laver sample was used to verify that SS was not detected. SS is primarily used as food additives, SS was purchased as a food additive grade from ES Food Ingredients. The analytical method was based on the KFDA (MFDS, 2023), and the conditions were set according to relevant references. After reviewing the SS assay method using the references, the experiment was conducted as follows.

Samples of 2-5 g were accurately weighed to the nearest 1 mg and placed in a 50 mL conical flask. Subsequently, 20 mL water was added and mixed well with the sample. To extract the sample components using an ultrasonic extractor for 20 min, the temperature of extractor was set at room temperature. Centrifuged at 4,000 rpm for 15 minutes, take the supernatant was diluted 10 times, and filtered with a 0.45 μm Nylon syringe membrane filter before injecting into the HPLC.

For the seasoned laver sample application test, this experiment used dried laver (*Pyropia* Sp., Mokpo), corn oil (100% corn, Baeksul), and flavored salt (90.3% refined salt, Daesang). One sheet of dried laver (2.5 g) was heated at 200℃ for 10 seconds after spreading oils and sprinkled flavored salt (ratio of 55% laver, 40% oil, and 5% flavored salt). The seasoned laver directly sprinkled with sodium saccharin was used for sample applicability test.

3. Preparation of the standard solution

The standard solution was prepared by using the reference standard sodium saccharin and dissolved in distilled water. A stock solution with a concentration of 1 mg/mL (1,000 ppm) was prepared and used for the calibration curve. The stock solution was diluted with water to obtain the seven concentrations of 1, 5, 10, 25, 50, 100, and 200 mg/L as a standard solution for the calibration curve. Then, before HPLC injection, the SS diluted solutions were then filtered through a 0.45 μm Nylon syringe membrane filter. An internal standard was used by adding sodium saccharin standard.

4. Chromatographic method

To optimize chromatographic HPLC conditions, we assessed parameters such as a column, column oven temperature, mobile phase, flow rate, and injection volume. The instrument operating conditions for the SS quantification of the Korean Food Code (KFDA) were used as a reference (MFDS, 2023). In this study, HPLC-UV was used to analyze and validate SS in dried laver. The SS investigated in the sample identified by comparing retention times and their chromatograms.

The HPLC used for analysis was performed on a Hitachi CM5000 series HPLC system (Hitachi Ltd., Tokyo, Japan) consisting of a CM-5110 binary pump, CM-5280 autosampler, and CM-5410 UV-detector. A Discovery C18 (4.6 mm x 250 mm, 5 μm) analytical reverse column was used for chromatographic separation, the column

temperature was at 40℃ and monitored at 210 nm. The flow rate was 1.0 mL/min, and the injection volume was 10 μL for all experiments. The mobile phase was prepared by putting 10% TPA-OH adjusted at pH 4.0 to a mixture of water and MeOH 7:3 (v/v) treated with vacuum-degassed. The HPLC conditions for the analysis of SS in dried laver are shown in Table 2.

5. Quantification test

Quantification is important in quantitative methods using HPLC and must be calculated under a number of conditions. When the peak obtained in the chromatogram matches the retention time of the standard peak, the area of the peak is integrated with the calibration curve to determine the concentration (mg/kg) of the test solution. For the quantification of SS, it was considered the purity of the reference standard solution, the volume of the test solution, and the dilution factor. The contents of SS were calculated by following the procedure above.

Table 2. HPLC chromatographic operating conditions for sodium saccharin

Parameters	HPLC condition
Column	Discovery C_{18} (4.6 mm x 250 mm, 5 μm)
Flow rate	1.0 mL/min
Temperature	40 ℃
Mobile phase	MeOH : Water = 3 : 7 10% TPA-OH, pH 4.0
Wavelength	210 nm
Injection volume	10 μL
Total run time	15 min

6. Statistical analysis

Statistical processing was performed using the IBM SPSS program (SPSS Inc. Chicago, IL, USA) and Minitab 21.0 program. Results were statistically validated by one-way ANOVA (Analysis of variance), followed by Tukey's multiple comparison tests at 95% confidence intervals, and discovered significant differences between samples. All data were expressed as the mean ± standard deviation (SD) obtained from triplicate experiments.

Results and Discussions

1. Pretreatment method for sodium saccharin measurement

The proper sample pretreatment depends on the matrix of food samples. Therefore, it is crucial to select an appropriate pretreatment method to enhance the analytical sensitivity by refining the sample (Lee et al., 1994). Additionally, the pretreatment method should be quick and simple and involves centrifugation, filtration, and dilution. It should be able to recover the analyte quantitatively (Lee et al., 1994). So, the sample solution was diluted to account for the characteristics that laver contains pigments and other impurities.

In consideration of the characteristics of laver, including the pigments and other impurities, the sample solution was diluted to address these factors. The pretreatment method was assessed by evaluating the recovery rate at each step of the procedure, including extraction, centrifugation, and dilution to extract the sample solution. Compared with the recovery rate of the blank test, the results confirmed that diluting the sample solution after centrifugation can increase the recoveries of SS. The results are shown in Table 3 with recovery rates. The SS recovery of dried laver was relatively low at 80.98 ± 6.17% in the KFDA method, however, the pretreatment with dilution showed good results at 90.31 ± 4.69%. Furthermore, considering that laver is a high protein food source (Hwang et al., 2013), the effectiveness of adding Carrez reagent is known for its ability to dissolve protein components. However, the addition of Carrez

Table 3. Comparison of pretreatment method for sodium saccharin measurement

KFDA method	Mean ± S.D[1)] (%)
Blank	95.55 ± 0.79
Extraction	17.00 ± 2.28
Centrifugation	80.98 ± 6.17
Pretreatment method test	**Mean ± S.D[1)] (%)**
Extraction & dilution	31.13 ± 0.53
Centrifugation & dilution	90.31 ± 4.69
Carrez solution	60.72 ± 11.77
Carrez & dilution	74.32 ± 10.44

[1)] Mean ± S.D (n=3)

reagent resulted in relatively low recovery rates ranging from 60.72 ± 11.77% to 74.32 ± 10.44%. These findings indicate that the recovery of SS can be improved by appropriately diluting the sample solutions.

2. Optimization of analytical conditions for sodium saccharin measurement

This study conducted experiments to evaluate the instrument analysis conditions for HPLC analysis. The HPLC conditions for the quantitative determination method of the KFDA (MFDS, 2023) were used as a reference. The recovery evaluation was performed by adding SS to dried laver samples.

The optimal mobile phase conditions, which affect the retention time (RT) and recovery rate, were compared by adjusting the concentration of MeOH and the amount of 10% TPA-OH. Five random conditions tests were set by adjusting the MeOH concentration to 20-50% and the 10% TPA-OH amount to 10-50 mL, based on the existing mobile phase solution of MeOH 30% with the addition of 10% TPA-OH (20.3 mL) and adjusted to pH 4.0. The prepared mobile phase was used after sonication, degassing, and filtration processes, and the RT was performed using SS standard solution with a concentration of 100 mg/L. The results were assessed by examining the RT and recovery rates of SS. It was observed that increasing MeOH content in the mobile phase tended to lead to faster RT, as seen in samples No. 2 and 5. In contrast, No. 1 and 4 showed either delayed RT or considerably lower recovery rates. Thus, No.

2 and 3 exhibited good results in terms of RT and recovery; however, at 50% MeOH content, the column pressure increased and showed instability. After reviewing a number of factors, we determined that the existing mobile phase conditions were the best for RT, recovery rates. The comparison results of the HPLC mobile phase are presented in Table 4.

3. Validation of HPLC for sodium saccharin measurement

Validation of the analytical HPLC method was performed to assess specificity, linearity, the limit of detection (LOD) and quantitation (LOQ), accuracy, and precision according to KFDA (MFDS, 2016), AOAC guidelines (AOAC, 2012), and ICH guidelines (ICH, 2005). The accuracy and precision were evaluated on intra-day and inter-days. All validation procedures were performed in triplicate tests. The results were checked by referring to the KFDA Food Additive Test Method Guideline (MFDS, 2016), and the verification parameters and criteria are shown in Table 5.

Table 4. Optimization for analytical conditions of HPLC for sodium saccharin measurement

No.	Mobile phase	RT	Recovery rate (%)
KFDA method	10% TPA-OH 20.3 ml Meth 30% (pH 4.0)	5.477	101.10
1	10% TPA-OH 10 ml Meth 20% (pH 4.0)	11.197	103.78
2	10% TPA-OH 10 ml Meth 50% (pH 4.0)	3.653	96.97
3	10% TPA-OH 30 ml Meth 35% (pH 4.0)	5.387	102.27
4	10% TPA-OH 50 ml Meth 20% (pH 4.0)	4.42	6.72
5	10% TPA-OH 50 ml Meth 50% (pH 4.0)	3.72	96.94

Table 5. Validation parameters and criteria of Food Additives test methods

Parameters	Criteria
Accuracy	70% ≤ recovery ≤ 125%
LOD (Limit of detection)	Based on 3.3xσ/S of method blanks
LOQ (Limit of quantification)	Based on 10xσ/S of method blanks
Linearity	Linearity(R^2) ≥ 0.99
RSD_r	$RSD_r \leq 20\%$
RSD_R	$RSD_R \leq 25\%$

Source: MFDS (Ministry of Food and Drug Safety). (2016). Guidelines on standard procedures for preparing analysis methods.

3.1. Specificity

In chromatographic analysis, to develop a separation, it is necessary to demonstrate the specificity of the analytical method, which is the ability to accurately measure the analyte in the sample components. Specificity is an essential characteristic of a reliable chromatographic method and the ability to selectively and accurately measure the analyte in the matrix. To demonstrate specificity, the standard solution and sample solution were analyzed by HPLC with the same retention time (RT) and peaks, and if the RT is the same, they are considered to be the same substance.

Figure 1 was shown the chromatograms of standard SS and SS in dried laver. It expressed the specificity of the HPLC-UV method. Two peaks obtained from the chromatogram were detected at RT 5.6-7 min, so the two substances were identified as the same material. This means that this analytical method for SS in dried laver, has high separation efficiency and selectivity, without interference from other matrices.

3.2. Linearity

Linearity refers to the ability to obtain measurement values within a certain range for the analyte in the sample at seven concentrations. The range is the interval between the high and low limits of analyte concentration, which has been demonstrated to be measured with precision, accuracy, and linearity using the method as written. It was

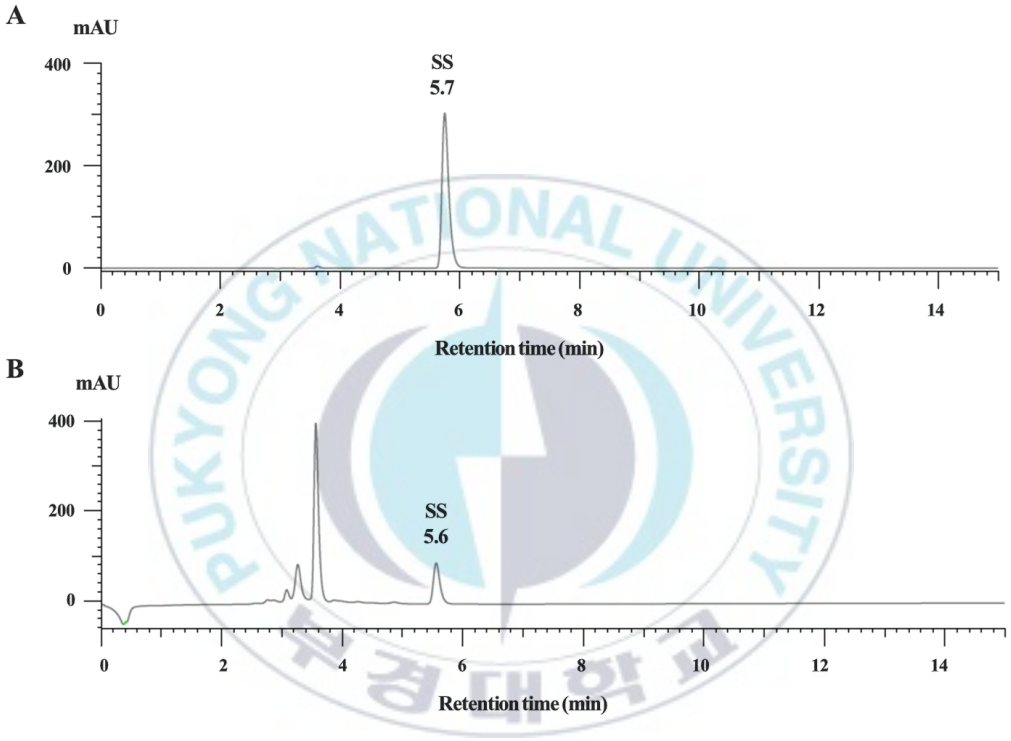


Figure 1. Chromatogram of (A) sodium saccharin reference standard and (B) sodium saccharin in dried laver.

expressed in the same units as the test result obtained by the method. In addition, at least five concentration levels and a specific minimum specified range should be provided, according to the ICH guidelines. For quantitative assays, the minimum specified range is typically 80-120% of target concentration.

For linearity, SS standard solution was tested using seven concentrations in the range from 1 to 200 mg/L. The calibration curve was obtained by adding reference standard concentration against peak area and over the defined range for analyte SS. Each standard solution was taken and diluted to 7 concentrations and injected into the HPLC according to the analytical conditions and the calibration curve was constructed from the height and area of the peaks obtained. The correlation coefficient of linearity was determined.

The linear equation of SS was $y = 55033x - 248084$ and the calibration curve indicated high linearity ($R^2 > 0.999$). The calibration curve of the SS standard is shown below in Figure 2. A calibration curve for the SS food additive was constructed using the same method as the SS standard for linearity evaluation. As the results, the linear equation of SS food additive was $y = 49794x - 183284$ and the calibration curve indicated high linearity ($R^2 > 0.999$). The calibration curve of the SS food additive is shown below in Figure 2.

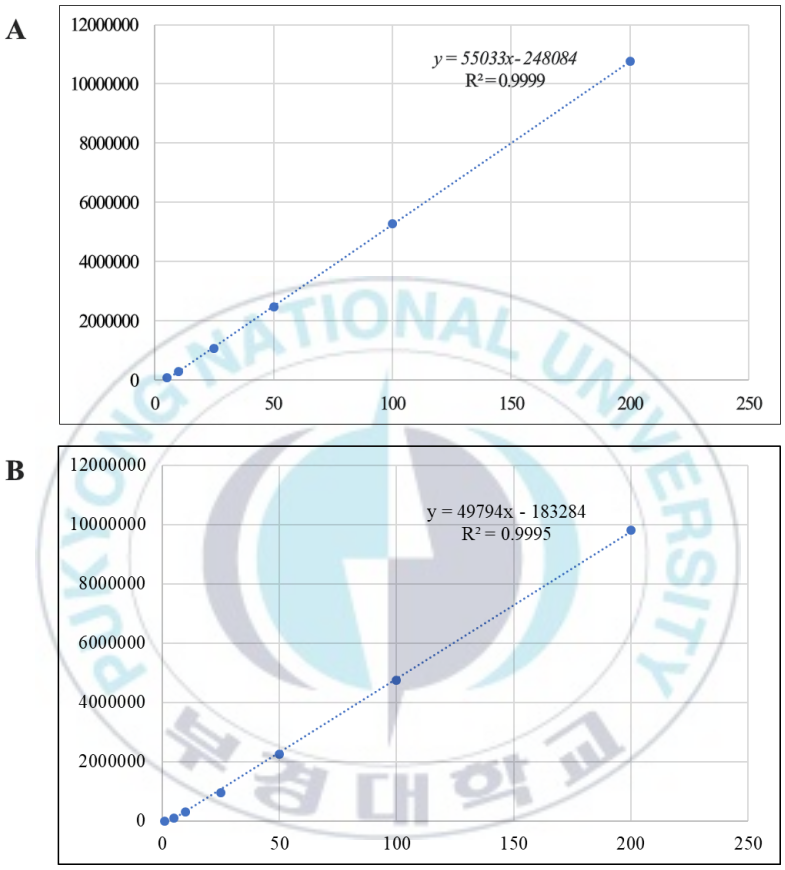


Figure 2. Graph of calibration curve of (A) sodium saccharin reference solution and (B) sodium saccharin foods additives.

3.3. Limit of detection and quantification

The minimum amount or concentration of an analyte that can be estimated with acceptable confidence is commonly referred to as the limit of detection (LOD) (AOAC, 2012). It is typically determined experimentally by analyzing samples with known low concentrations of the analyte and calculating the signal-to-noise ratio. The LOD is an important parameter in an analytical method as it defines the sensitivity and provides a practical limit for detecting and quantifying analytes. The LOD is the lowest concentration of an analyte in a sample that can be measured precisely and accurately using an analytical method under specified operating conditions. It represents the lower limit at which the analyte can be reliably detected and quantified with confidence (Shabir, 2003).

The LOD and LOQ of SS were calculated by repeatedly analyzing the reference standard solution of SS at 7 concentrations three times. And, determining the LOD and LOQ for the y-intercept standard deviation (σ, sigma) and slope (s) of the regression equation obtained from each calibration curve were used to calculate the following formula.

$$\text{The limit of detection} = 3.3*\sigma/s$$

$$\text{The limit of quantification} = 10*\sigma/s.$$

The LOD and LOQ were 1.37 mg/kg and 4.55 mg/kg, respectively. It means to enable reliable detection and quantification for SS in dried laver at low concentrations. The LOD and LOQ results are represented in Table 6.

3.4. Method detection limit

The method detection limit (MDL) was defined as the minimum measured concentration of an analyte that can be determined with 99% confidence distinguishable from the blank test of the validated method and also a type of LOD and does not necessarily require the analyte in the sample to be quantifiable at a minimum concentration to be detected. The MDL for SS was calculated by the standard deviation of seven replicate measurements of the SS at 10 mg/kg by 3.14 based on a regression equation obtained from the calibration curve. The method is based on EPA guidelines. (EPA, 2016).

$$\text{Method detection limit (MDL)} = 3.14*\sigma/s$$

Table 7 below shows the results of the MDL. The MDL for SS was determined to be 1.24 mg/kg.

Table 6. Results of linearity, LOD and LOQ by HPLC

Sample	Regression Equation	Linearity (R^2)	LOD[1)] (mg/kg)	LOQ[2)] (mg/kg)
Sodium Saccharin	*y = 55128x-260263*	0.9998	1.37	4.55
	y = 56694x-247840	0.9999		
	y = 53277x-236149	0.9999		
	y = 55033x-248084	0.9999		

[1)] LOD, Limit of detection

[2)] LOQ, Limit of quantification

Table 7. Results of MDL by HPLC-UV for dried laver

Sodium saccharin		
Spike conc.	10 mg/kg	
Replicate	Analysis results (mg/kg)	Recovery (%)
1	10.13	101.34
2	9.04	90.42
3	9.63	96.30
4	10.37	103.73
5	9.89	98.95
6	9.63	96.27
7	9.87	98.70
Average	9.80	
Std[1)]	0.39	
Df[2)]	6.00	
t(n-1)	3.143	
MDL[3)] (mg/kg)	1.24	

[1)] Std : Standard deviation

[2)] Df : Degree of freedom

[3)] MDL : Method detection limit

3.5. Accuracy and Precision

Accuracy can be evaluated by analyzing a sample of a known amount and comparing the measured value with the true value (Shabir et al., 2003). Accuracy was calculated by adding the standard to the dried laver at three level concentrations and calculating the recovery rates (%). The spiked samples are prepared at three levels spanning the range of 50-150% of the target concentration. In this study, recovery studies were performed to evaluate the accuracy by spiking the sample standard solution of three concentrations (25, 50, and 100 mg/L) at the sample solution. The accuracy criteria for the analytical method is that the average recovery at each level should be within 80-120% of the target concentration. Analytical results were obtained from three replicate experiments (i.e. nine measurements) for each sample in three concentrations according to ICH guidelines (Shabir et al., 2003). Table 8 showed the accuracy test results of SS in dried laver.

After adding SS standard solution to the dried laver sample, using a validated analytical method including sample preparation and determination by HPLC. As the results of measuring intra-day and inter-day accuracy tests, the SS recovery ranged from 96.32 ± 1.27%-112.53 ± 4.72%, respectively. This represents a high recovery rate, close to the 90-110% recovery range for SS. At all concentration levels, recoveries ranged from 70 to 125% and satisfied the validation factors of KFDA's guidelines for food additives analytical methods (MFDS, 2016) ensuring accuracy. It means that SS was successfully detected and quantified in dried laver.

Table 8. Results of accuracy and precision for sodium saccharin measurement in dried laver

Sample	Added standard (mg/L)	No.	Recovery rate (%)	Inter-day		Day	Recovery rate (%)	Intra-day	
				Accuracy (%)	Precision (RSD[1)], %)			Accuracy (%)	Precision (RSD, %)
Dried laver	25	1	110.36	112.53 ± 4.72[ab]	0.86	1	111.32	111.99 ± 1.68[ab]	1.23
		2	113.72			2	108.53		
		3	111.89			3	117.73		
	50	1	101.65	101.45 ± 2.48[ab]	1.00	1	100.66	102.73 ± 2.30[ab]	1.83
		2	101.17			2	104.22		
		3	105.37			3	99.46		
	100	1	103.78	96.32 ± 1.27[ab]	1.08	1	95.11	102.23 ± 1.49[ab]	1.19
		2	100.82			2	96.2		
		3	102.08			3	97.64		

[1)] RSD, relative standard deviation

[a] Mean ± S.D (n=3)

[b] Inter-day and intra-day accuracy were not significantly different by one-way ANOVA in Tukey's multiple comparison tests (p<0.05)

Repeatability, also known as intra-assay precision, refers to the consistency of the results obtained from an analytical method under identical conditions within a short time interval. The precision was indicated as the relative standard deviation (RSD, %) obtained by dividing the RSD of repeated experiments by the mean value, and both accuracy and precision were performed in the triplicate test. The RSD was expressed as the percent for a statistically significant value of samples. Intermediate precision can be evaluated for different days of the week, analysts, equipment, and other factors in the laboratory (Shabir et al., 2003). The precision criterion for an analytical method is typically ≤ 2% intra-assay precision.

Analytical results were obtained from nine replicate experiments for each sample. Precision conducted the inter-day and intra-day assays. The RSD for precision was inter-day 0.86-1.08% and intra-day 1.19-1.23%, respectively. It means that SS was successfully detected and quantified in dried laver. The recovery rates were within the range of 70-125% and the relative standard deviation (RSD) was below 20% at all concentration levels, satisfying the validation criteria for method validation parameters in the guidelines (MFDS, 2016). The results of the accuracy and precision tests were summarized in Table 8.

4. Monitoring of sodium saccharin in commercial dried laver products

The detection of sodium saccharin in commercially available dried laver was still common, so there was a need for monitoring. Seven different types of commercially available dried seaweed were randomly purchased and analyzed for the presence of SS. The analysis was performed in the same way as the three replicate tests conducted previously. As natural seafood products like laver are not added with SS, all commercially available dried lavers showed ND (non-detected), indicating no detection of SS. It was shown in Table 9.

It has been reported to be detected in commercially available dried laver, especially in *Pyropia dentata*, but it is not a risk for human intake. Furthermore, in our study, no SS was detected in the dried laver samples that were purchased and analyzed. However, it is expected that monitoring and management would be necessary to ensure that products with SS added to dried lavers do not continue to be detected. Based on the results of monitoring contribute to enhancing the food safety of consumers who consume lavers.

Table 9. Monitoring of the validated HPLC-UV method for sodium saccharin measurement in commercial dried laver

Sample No.	Concentration (mg/kg)
	SS[1)]
1	ND[2)]
2	ND
3	ND
4	ND
5	ND
6	ND
7	ND

[1)] SS, Sodium saccharin

[2)] ND, Not detected

5. Measurement of sodium saccharin in seasoned laver

Due to the potential for SS to be detected in seasoned laver consisting primarily of dried laver, we investigated the applicability and recovery of established analytical methods. For this experiment, a sample of seasoned laver prepared by adding SS directly was used. As seasoned laver is a high-fat food that contains a lot of oil, the experiment was conducted by adding a process to remove the fat component to the existing pretreatment process (Kim et al., 2020). In the case of foods containing fat, the sample must be pretreated after removing the fat. Therefore, to remove the fat component, diethyl ether was used to separate the fatty components from the water after extraction and centrifugation procedures. And then, take the supernatant, transfer it to a separation funnel, add 50 mL of diethyl ether, take the water layer, and repeat the above operation twice (MFDS, 2023).

Applying the HPLC-UV method to the seasoned laver, the recovery test of the evaluation showed that the rate of SS was 96.01 ± 2.97%-98.92 ± 2.63%. These results indicated that the established method was acceptable for the quantification of SS in seasoned laver, though. The results were represented in Table 10.

Table 10. Sample applications of the validated HPLC-UV method for sodium saccharin in seasoned laver

Sample	No.	Recovery (%)	Mean ± S.D (%)	RSD[1)] (%)
Seasoned Laver	1	99.13	98.13 ± 1.27[ab]	1.06
		96.70		
		98.57		
	2	96.43	97.34 ± 0.98[ab]	0.82
		97.22		
		98.38		
	3	96.18	97.60 ± 1.37[ab]	1.17
		97.65		
		98.97		
	4	98.08	98.19 ± 1.03[ab]	0.86
		97.21		
		99.26		
	5	96.28	96.01 ± 2.97[ab]	2.53
		92.91		
		98.84		
	6	96.94	97.36 ± 0.70[ab]	0.59
		96.98		
		98.17		
	7	97.41	98.92 ± 2.63[ab]	1.40
		98.58		
		100.76		

[1)] RSD, relative standard deviation
[a] Mean ± S.D (n=3)
[b] Sample application tests were not significantly different by one-way ANOVA in Tukey's multiple comparison tests ($p<0.05$)

Conclusion

This study was conducted to develop HPLC-UV method for the detection of SS in dried laver. An established method to pretreat the sample according to the characteristics of dried laver in a simpler way. The optimal analytical conditions for HPLC were determined, resulting in good linearity for SS analysis, with LOQ and LOQ being 1.37 mg/kg and 4.55 mg/kg, respectively and MDL was 1.24 mg/kg. The recovery rates of SS were evaluated by the spiking method with the improved pretreatment method and showed satisfactory recoveries of 96.32 ± 1.27%-112.53 ± 4.72%. The precision was found to be high with RSD of inter-day 0.86-1.08% and intra-day 1.19-1.23%, respectively. These results demonstrate that the HPLC analytical method is sufficiently repeatable and precise for the quantification of SS.

In this paper, the method validation results proved satisfactory linearity, accuracy, and precision. These results indicate that the developed method offers more accuracy compared to existing methods in KFDA and provides improved sample pretreatment methods and recovery rates. The developed method was successfully conducted and could be useful for the determination of SS in dried laver. Considering that dried laver is a primary ingredient in seasoned laver that is mainly consumed in Korea, if SS is detected in dried laver by using HPLC, there is a high probability that SS is detected in seasoned laver. Therefore, the recovery rate was high 96.01%-98.92% when the established analytical method was applied to seasoned laver. In addition, SS was not detected in commercially available dried laver products, indicating that they are safely

managed. The method used in this study was validated and reviewed for SS detection in dried laver and seasoned laver.

Accordingly, the validated HPLC method can be used as a basis for further research related to the analysis of SS in laver and contribute to food safety, and the detection of SS in laver should be managed and monitored continuously.

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