

CHUNG SHAN MEDICAL UNIVERSITY



and



Taipei City Department of Health



Société Générale de Surveillance
(General Surveillance Society)

Laboratory Intern.

Techniques Hematology analyzer, biochemical analyzer, PCR, ELISA,
Laboratory Information System (LIS)

Jun-Sep, 2020

Internship Report
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Supervised by:

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Abstract

This study aims to analyze the use of preservatives in food and their compliance with safety standards. Preservatives are food additives that delay or prevent food spoilage and inhibit or kill microorganisms. They are classified into natural preservatives (such as garlic, chili, tea, etc.) and artificial preservatives.

(such as benzoic acid, sorbic acid, and salts), which must meet the relevant standards of food safety regulations. This study detected preservatives in spring roll wrappers and oyster sauce samples using high-performance liquid chromatography (HPLC) following direct dilution and shaking extraction methods.

According to the "Standards for the Scope, Limit, and Specifications of Food Additives," the results indicated that the preservative content in the samples did not exceed the maximum allowable limit.

Furthermore, the study analyzed the Taipei City Government inspection statistics for food preservatives from 2016 to 2020. Out of 1,392 samples tested, only 13 were non-compliant, with a non-compliance rate of less than 1

The findings of this study provide data support for food safety regulation and offer a reference for the legal use of preservatives and detection methods.

1 Preface

During a chance visit to a small yet unique restaurant at my aunt's house, I was deeply inspired by their strict standards for ingredients and the impact of food preparation on health. This sparked my desire to learn more about food safety. I aim to apply the knowledge I've gained in school, such as food hygiene, safety, and

biochemistry, to my internship. The health bureau and SGS implement systematic food analysis and safety monitoring, providing consumers with quality assurance and health protection. This internship will enhance my professional skills and give me insight into the industry's future trends. Although I may not be the best among hundreds or thousands of competitors, I will certainly give my best. Singapore's Prime Minister once said: "If an egg is broken from within, it is life; if broken from outside, it becomes food. Learning broken from within is growth; broken from outside is pressure." This quote will serve as my motto during the internship.

With this background, the following internship objective was proposed:

"Being brave in trying, disciplined, courageous in admitting mistakes, and unwavering in determination, along with being skilled at reflection, are how I understand my personality traits."

2 Taipei City Department of Health

The internship lasted from June to July and was conducted at the Taipei City Department of Health. The primary tasks during the first two weeks involved reading relevant literature and discussing possible research methods. In the following four weeks, I participated in testing for veterinary drugs, heavy metals, pesticides, microorganisms, and food preservatives in food. In the final two weeks, I conducted an in-depth case study evaluation focused on testing food preservatives.

2.1 Background

- Internship Period: June 1, 2020 – July 31, 2020
- Internship Location: Department of Laboratory Testing, Taipei City Department of Health. (7th Floor, No. 111, Section 2, Shipai Road, Beitou District, Taipei City)
- Supervisor: Director Huang Jing-Yi
- TEL: +886-2-2828-0102 ext. 5911
- Introduction: The Inspection Department, as I see it, plays the role of behind-the-scenes personnel and frontline responders. They conduct various tests on the samples collected by the Food and Drug Department, as well as on samples voluntarily submitted by the public and those related to sudden food poisoning incidents. These tests include veterinary drugs, pesticide residues, peroxide values, microbiological examinations, and more. After completing the tests, the data is analyzed based on the LOD (Limit of Detection) and MRL (Maximum Residue Limit), ultimately providing the most accurate and reliable reports.
- Agency Certifications:

ISO 17025:2017 transition and extension certification by TAF	
Signatory to the ILAC-MRA (International Laboratory Accreditation Cooperation Mutual Recognition Arrangement)	
Ranked first in testing competency by the Taiwan Food and Drug Administration (TFDA)	
Achieved excellent performance in proficiency testing by the UK's Central Science Laboratory (FAPAS)	

2.2 Internship arrangement

Task Name	Start Date	End Date
Reading relevant literature and discussing possible research methods.	2020-06-01	2020-06-14
1. Introduction to Chemical Management. 2. Handling of Heavy Metal Testing. 3. Multi-Residue Testing for Veterinary Drugs	2020-06-15	2020-06-21
Microbiological Analysis, including:	2020-06-22	2020-06-30

1. Testing of hot spring water, swimming pool water, and bath water. 2. Testing of packaged drinking water. 3. Testing of cooling tower water.		
Food additives testing: preservatives, sterilizers, antioxidants, bleaching agents, color retention agents, colorants, etc.	2020-07-01	2020-07-11
Testing for the adulteration of Chinese herbal medicine (food) with western pharmaceuticals.	2020-07-12	2020-07-17
4. Detection of Aflatoxins in Food. 5. Alcohol testing: methanol content, ethanol content, heavy metals such as lead and mercury, sulfur dioxide, etc.	2020-07-18	2020-07-23
Conducted an in-depth case study evaluation focused on testing food preservatives.	2020-07-24	2020-07-31

2.3 Case study

Experiment Case Study: Testing of Food Preservatives

1. Introduction

Preservatives play a critical role in the food industry by inhibiting the growth of microorganisms, preventing spoilage, and extending the shelf life of various products. However, preservatives can pose potential health risks despite their benefits, leading to strict regulations or outright bans in many countries. To ensure consumer safety and comply with regulatory standards, it is crucial to accurately monitor and control the types and amounts of preservatives used in food products.

This experiment aims to assess the presence and concentration of different preservatives in food samples using high-performance liquid chromatography (HPLC) with specific UV detection wavelengths. By analyzing preservatives such as SSA, BA, DHA, P-HBA, and SA, we can evaluate whether their levels fall within safe and permissible limits.

The hypothesis being tested is that HPLC, with its targeted UV detection capabilities, can effectively identify and quantify various preservatives in food samples after extraction, offering precise and reliable data for safety and compliance assessments. This study is significant as it contributes to broader efforts in food safety regulation and helps maintain public health by ensuring that preservative use in food is within safe and acceptable limits.

Objective

Preservatives can inhibit the growth of microorganisms in food, prevent spoilage and deterioration, and extend the shelf life of food. However, due to their potential health risks, they have often been banned or subjected to restrictions on their usage and the types of food they can be applied to. Therefore, strictly regulating the amount and type of preservatives used is necessary.

Hypothesis

High-performance liquid chromatography (HPLC) with specific UV detection wavelengths can accurately identify and quantify different types of preservatives in food samples after extraction, providing reliable results for safety and compliance assessments based on their concentrations and categories.

2. Background Information

Background

Preservatives are widely used in the food industry to inhibit microbial growth, prevent spoilage, and extend the shelf life of perishable products. Typical preservatives include sorbic acid (SA), benzoic acid (BA), and their derivatives, frequently employed due to their antimicrobial properties^[1]. However, while preservatives help maintain food safety and quality, there are growing concerns regarding their potential health effects. Overconsumption or prolonged exposure to certain preservatives has been linked to allergic reactions, hypersensitivity, and even carcinogenic risks. Consequently, many countries have imposed strict limits on their permissible concentrations in food, regulated by standards such as the Maximum Residue Limit (MRL).

Regulatory Framework and Safety Concerns

Due to the potential risks associated with food preservatives, national and international food safety organizations, such as the World Health Organization (WHO)^[2] and the Food and Agriculture Organization (FAO), have established guidelines to regulate their usage. Many countries enforce these guidelines, stipulating specific limits for different preservatives in various food categories. For example, sorbic and benzoic acids are permitted in particular concentrations but are restricted or banned in products like baby food. To comply with these regulations, food manufacturers and safety authorities must regularly monitor and analyze preservative levels in processed foods.^[3]

Analytical Techniques for Preservative Detection

High-performance liquid chromatography (HPLC) is one of the most reliable and widely used techniques for detecting and quantifying preservatives in food samples. HPLC offers high resolution and sensitivity, making it ideal for separating and identifying complex mixtures of substances, such as preservatives. Previous studies have demonstrated the effectiveness of HPLC in accurately determining the presence of benzoic acid, sorbic acid, and other preservatives in various food matrices, such as beverages, processed meats, and bakery products.

Research by Pankaj et al. (2019)^[4] and Wang et al. (2021)^[5] highlights the utility of HPLC in detecting low concentrations of preservatives, even at the parts-per-million (ppm) level. These studies emphasize the role of UV detection wavelengths, which can be optimized for different preservatives. For instance, 230 nm, 256 nm, and 260 nm wavelengths commonly detect preservatives like SSA, BA, DHA, and P-HBA, corresponding to their maximum absorption points. This approach allows for precise identification and quantification, ensuring that preservative levels remain within safe and legal limits.

Significance of the Study

Given the ongoing concerns regarding food safety and the health risks associated with preservatives, this experiment is designed to assess the levels of common preservatives in food samples using HPLC with specific UV detection wavelengths. The goal is to provide accurate and reliable data on preservative concentrations, ensuring compliance with regulatory standards and contributing to broader food safety and consumer protection efforts. By validating the effectiveness of HPLC in this context, the study also aims to highlight its importance as a tool for routine food safety monitoring.

3. Methods

Materials

- Laboratory Instruments
 - a. High-Performance Liquid Chromatograph (HPLC)
 - Detector: Photodiode array detector
 - Chromatography Column: Luna C18, 5 μm , 4.6 mm internal diameter \times 25 cm or equivalent
 - b. Centrifuge: Capable of reaching speeds up to 3500 rpm
 - c. Ultrasonicator
 - d. Distillation Apparatus
 - e. Electronic Balance: Accuracy to 0.01 g
 - f. Electronic Balance: Accuracy to 0.001 g
 - g. Electronic Balance: Accuracy to 0.0001 g
- Experimental Materials and Equipment
 - a. Distillation Flask: 500 mL
 - b. Centrifuge Tube: 50 mL, made of PP material
 - c. Volumetric Flask: 50, 100, 200, 500, and 1000 mL
 - d. Filter Membrane: Pore sizes of 0.45 μm and 0.22 μm , made of PVDF material
 - e. Sample Vial: 2 mL
 - f. Adjustable Micropipette: 10 – 100 μL and 100 – 1000 μL
 - g. Serum Bottle: 50, 100, 200, 500, and 1000 mL
- Reagent Preparation
 - a. Standard Substances
 - (1) Acids: Para-hydroxybenzoic acid (P-HBA), Salicylic acid (SSA), Benzoic acid (BA), Sorbic acid (SA), and Dehydroacetic acid (DHA).
 - (2) Esters: Methyl para-hydroxybenzoate (ME), Ethyl para-hydroxybenzoate (EE), Isopropyl para-hydroxybenzoate (IPE), Propyl para-hydroxybenzoate (PE), Sec-butyl para-hydroxybenzoate (SBE), Isobutyl para-hydroxybenzoate (IBE), Butyl para-hydroxybenzoate (BE).
 - b. Reagents

- (1) Deionized water (resistivity more significant than 18 MΩ · cm at 25° C)
- (2) Methanol and acetonitrile: HPLC grade
- (3) Sodium hydroxide: analytical reagent grade
- (4) Citric acid ($C_6H_8O_7 \cdot H_2O$): analytical reagent grade
- (5) Trisodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$): analytical reagent grade
- (6) Acetic acid: analytical reagent grade

Procedure

- **Preparation of Solution**

- a. Reagent Preparation

- (1) 50% Methanol Solution: Mix methanol and deionized water in a 1:1 (v/v) ratio.
- (2) 0.1N Sodium Hydroxide Solution: Dissolve 4 g of NaOH in deionized water to make 100 mL.
- (3) Citrate Buffer Solution (5 mM, pH 4.0): Dissolve 7.0 g of citric acid and 6.0 g of trisodium citrate in deionized water to make 1000 mL. Before use, dilute the solution ten times with deionized water and filter through a membrane filter.
- (4) 50% Acetic Acid Solution: Mix acetic acid and deionized water in a 1:1 (v/v) ratio.

- b. Preparation of Standard Solutions

- (1) Acids

	STD1	STD2	STD3	STD4	STD5	STD6
Calibration curve concentration (mg/L)	1	20	40	60	80	100
1000ppm mixed standard solution addition amount (mL)	0.1	2	4	6	8	10
Final constant volume (mL)	100					

- (2) Esters

	STD1	STD2	STD3	STD4	STD5	STD6
Calibration curve concentration (mg/L)	0.25	2	4	6	8	10
1000ppm mixed standard solution addition amount (mL)	0.025	0.2	0.4	0.6	0.8	1.0
Final constant volume (mL)	100					

- c. Preparation of Mobile Phase Solutions

- (1) Acidic Solutions

① Mobile Phase Solution A for Acids: Mix methanol, acetonitrile, and five mM citric acid buffer solution in a ratio of 1:2:7 (v/v/v). For every 1000 mL of the mixture, add 1.5 mL of 50% acetic acid solution and filter through a membrane.

② Mobile Phase Solution B for Acids: Methanol.

③ The ratio of Mobile Phase Solution A to B is 90:10 for analysis.

(2) Esters

① Mobile Phase Solution A for Esters: Mix methanol, acetonitrile, and five mM citric acid buffer solution in a 1:2:7 (v/v/v) ratio. For every 1000 mL, filter through a membrane.

② Mobile Phase Solution B for Esters: Methanol.

③ Analysis: Use a ratio of 55:45 for mobile phase solution A for the analysis.

d. Preparation of Test Solution

(1) Extraction Method (Solid Samples): This method was used to prepare the test solution.

1	Analytical balance	After homogenizing the spring roll wrappers, accurately weigh 5g and place them in a 250 mL serum bottle.
2	Ultrasonic oscillator	Add 50 mL of 50% methanol solution, then sonicate for 30 minutes. Afterward, dilute the solution to 100 mL with 50% methanol.
3	Centrifuge	Take an appropriate amount of the extract and centrifuge it at 3500 rpm for 10 minutes.
4	Membrane filtration	Filter the supernatant through a 0.45 µm PVDF membrane filter to obtain the test solution.

(2) Dilution Method (Liquid Samples): This method was used to prepare the test solution.

1	Analytical balance	After homogenizing the sample (oyster sauce), accurately weigh 5g and place it in a 100 mL serum bottle (remove the gas first for samples containing CO ₂).
2	Volumetric flask (for making up to volume)	Add 50% methanol solution and dilute to 100mL.
3	Ultrasonic oscillator	Sonicate the mixture for 30 minutes.
4	Membrane filtration	Centrifuge the sample and filter the supernatant through a 0.45 µm PVDF membrane filter. The filtrate is used as the test solution.

e. Preparation of Quality Control Solutions

(1) Acids

① CC0 and CC1

1	Take similar sample matrices, such as liquid (cranberry juice) and solid (noodles), homogenize them thoroughly, and accurately weigh 5g of each. Then, place them separately in a 100 mL volumetric flask and a 250 mL serum bottle.
2	Take 0.5 mL of 1000 mg/L mixed standard solution (5 times the LOQ).
3	Dilute to 100 mL with 50% methanol solution.
4	Ultrasonically shake for 30 minutes.

(2)SP and SPD

1	Accurately weigh approximately 1000 mg of P-BHA, SSA, BA, SA, and DHA, and place each into a 1000 mL volumetric flask.
2	Dissolve them in 0.1N sodium hydroxide solution
3	Then dilute to volume with deionized water.
4	This serves as a standard preservative solution with a concentration of 1000 mg/L for each acid preservative.
5	Take 10 mL of each of the above standard solutions and combine them in a 100 mL volumetric flask.
6	Fill to volume with deionized water.
7	This produces a 1000 mg/L mixed standard solution.
8	For use, take 5 mL of the 1000 mg/L mixed standard solution and place it in a 100 mL volumetric flask, filling to volume with 50% methanol solution, to prepare a 50 mg/L CC0 and CC1 mixed standard solution.

(3)BK: Blank samples with a similar matrix (undetected cranberry juice/noodles).

(2) Easters**①CC0and CC1**

1	Homogenize similar sample matrices, such as cranberry juice or noodles, and accurately weigh 5g of the sample. Transfer the sample into a 100mL volumetric flask.
2	Add 0.125mL of 1000mg/L mixed standard solution (5 times the LOQ).
3	Dilute to 100 mL with 50% methanol solution.
4	Ultrasonically shake for 30 minutes.

(2)SP and SPD

1	Precisely weigh approximately 1000 mg of ME, EE, IPE, PE, SBE, IBE, and BE, and place them into separate 100 mL volumetric flasks.
2	Dissolve each sample with 50% methanol solution and make up to the mark.
3	This results in a 1000 mg/L standard solution of lipid preservatives.
4	Take 10 mL of each of the above standard solutions and combine them into a single 100 mL volumetric flask.
5	Fill to the mark with 50% methanol solution.
6	This forms a 1000 mg/L mixed standard solution.
7	For use, take 0.5 mL of the 1000 mg/L mixed standard solution and place it into a 100 mL volumetric flask, then dilute to the mark with 50% methanol solution, preparing 5 mg/L solutions of CC0 and CC1.

(3)BK: Blank samples with a similar matrix (undetected cranberry juice/noodles).

• Instrumental Analysis:

Precisely measure ten μL of each sample and standard solution and inject them into the HPLC system. Compare the retention times and absorption spectra of the peaks obtained from the sample solution and the standard solution for identification. Perform the liquid chromatography under the following conditions:

- a. Chromatographic Column: Luna C18, 5 μm , 4.6 mm internal diameter \times 25 cm length.
- b. Mobile Phase Flow Rate: 1 mL/min.
- c. UV Detector Wavelengths: 230 nm (for SSA, BA, DHA), 256 nm (for P-HBA and seven esters), and 260 nm (for SA).
- d. Mobile Phase Solution: Preparation according to specifications (7, 1, (3), 1, 2, mobile phase solution preparation details).
- e. Injection Volume of Sample and Standard Solutions: 10 μL each.

Data Collection

The content of each preservative in the sample (g/kg) = $(C \times V \times F \text{ conversion factor}) / (W \times 1000)$

C: The concentration of each preservative in the sample (mg/mL) is determined from the peak area of the sample based on the calibration curve.

V: The final volume of the sample after dilution (mL).

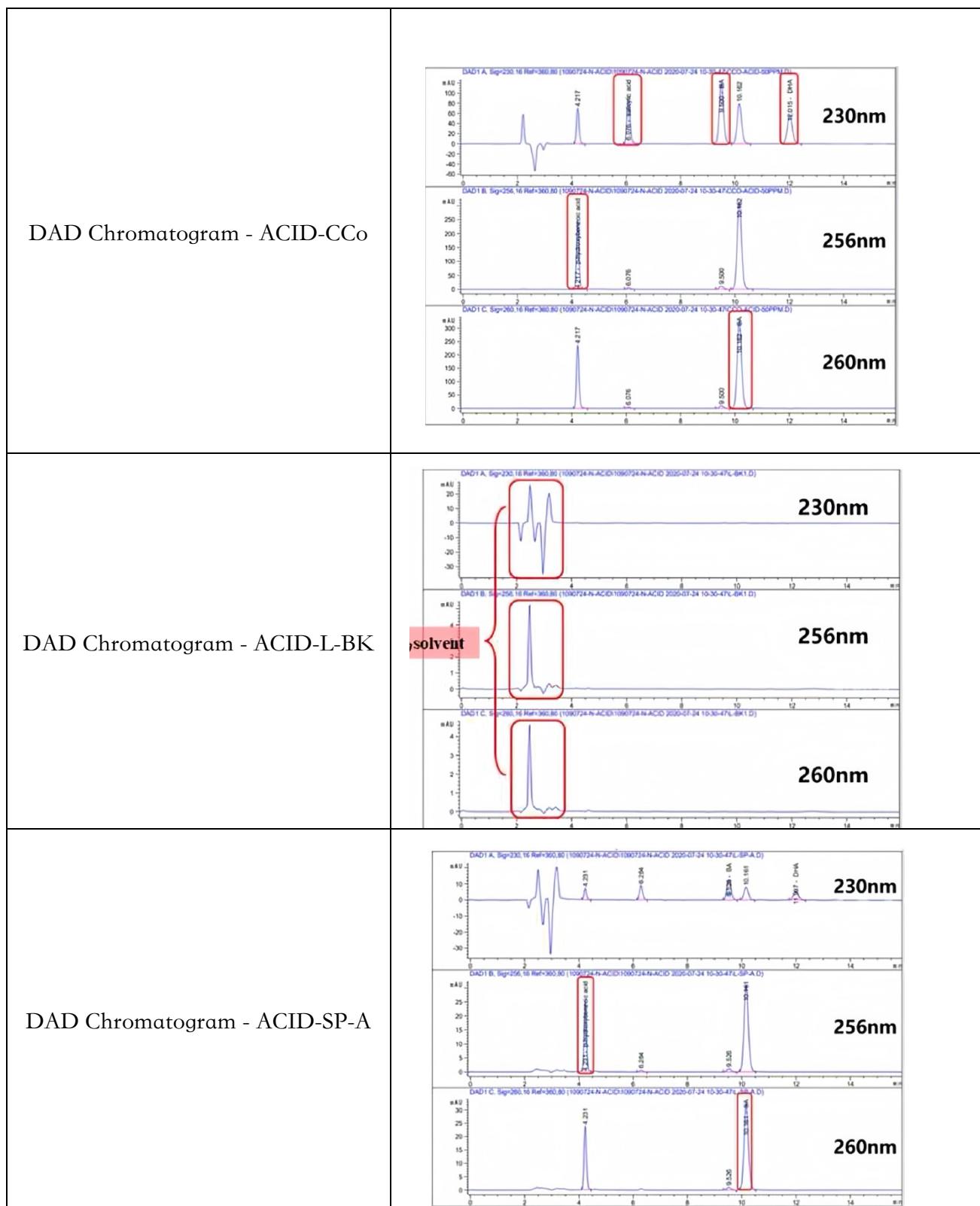
W: The weight of the sample used for analysis (g).

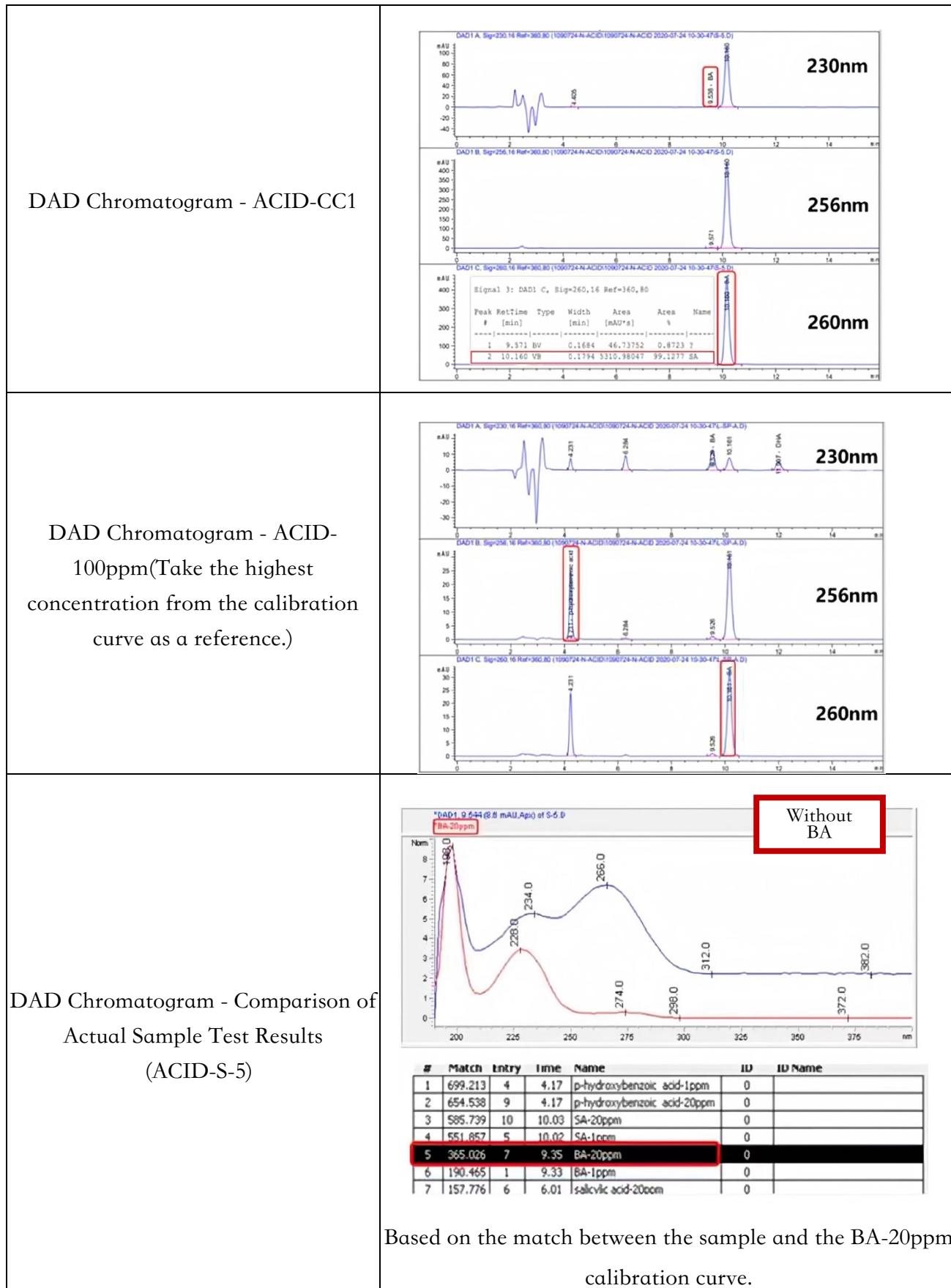
F: Dilution factor.

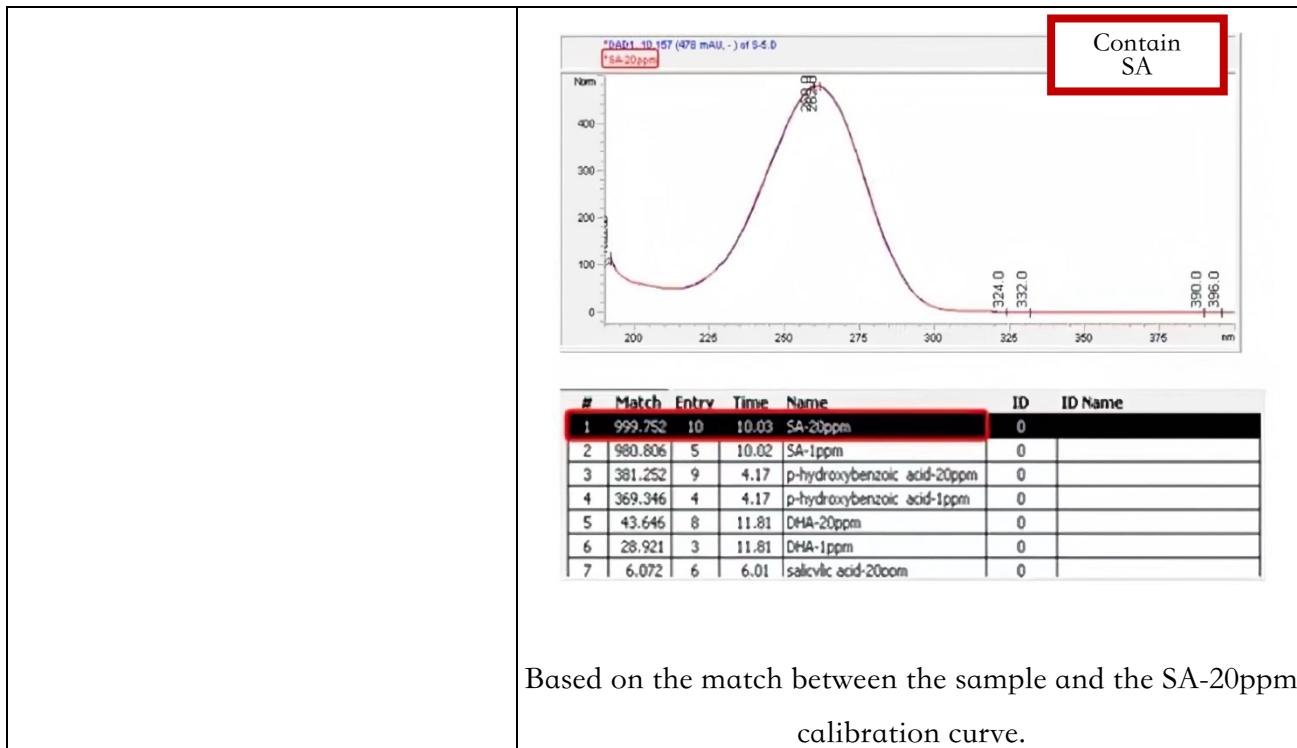
Conversion factor: When esters of preservatives are detected in the sample, the content is calculated as the amount of p-hydroxybenzoic acid using its conversion factor.

Ester Preservatives	Molecular Weight	Conversion value of phydroxybenzoic acid
Para-hydroxybenzoic acid	138.12	1
Methylparaben (methyl para-hydroxybenzoate)	152.15	0.9077
Ethylparaben (ethyl para-hydroxybenzoate)	166.17	0.8311
Isopropylparaben (isopropyl para-hydroxybenzoate)	180.20	0.7664
Propylparaben (propyl para-hydroxybenzoate)	180.20	0.7664
Isobutylparaben (isobutyl para-hydroxybenzoate)	194.23	0.7111
Sec-butylparaben (sec-butyl para-hydroxybenzoate)	194.23	0.7111
Butylparaben (butyl para-hydroxybenzoate)	194.23	0.7111

4. Results







5. Discussion

The results of the experiment confirm that high-performance liquid chromatography (HPLC) with specific UV detection wavelengths is effective in identifying and quantifying preservatives, such as sorbic acid (SA) and benzoic acid (BA), in food samples, supporting the original hypothesis. Most preservative concentrations were within regulatory limits, aligning with previous studies demonstrating HPLC's sensitivity and reliability in detecting preservatives at low concentrations. However, some limitations were noted, including limited sample size, potential matrix interference from certain food types, and solvent selection affecting extraction efficiency. These limitations suggest areas for improvement, but overall, the results validate HPLC as a dependable method for preservative analysis in food safety assessments.

- Why are the methanol and deionized water not mixed using direct dilution when preparing a 50% methanol solution?

Direct dilution is not suitable. Instead, the two liquids should be measured separately in different containers and then combined in a single container. This is because, in addition to the difference in density between the two liquids, the final volume might not exactly match the desired amount due to potential discrepancies. Furthermore, methanol can release heat when mixed, which may cause the final volume to differ from the initially intended volume.

- What is the final judgment sequence for the DAD circular spectrum?

When comparing different wavelengths (250 nm, 256 nm, 260 nm) in the DAD circular spectrum, we first look at their retention times (RT). If the preservative we are looking for appears at the correct RT and the RT of the test sample matches that of the standard sample within ± 0.1

minutes, and the wavelength deviation is within ± 2 nm, we then compare the DAD circular spectra between the sample and the calibration curve. The judgment is primarily based on the AREA, with MATCH as a supplementary criterion.

- Why are SSA, BA, and DHA detected at a wavelength of 230 nm, P-HBA and seven esters at 256 nm, and SA at 260 nm for analysis?

All five acids can be detected at various wavelengths, but some perform better than others at specific wavelengths. Therefore, we choose the wavelength where each acid performs best for optimal measurement.

- What is the difference between preservatives and sterilizers?

Preservatives only inhibit the growth of microorganisms without ultimately killing them, so a specific concentration of preservatives must be maintained in food to prevent the organisms from continuing to grow. Sterilizers, on the other hand, are chemically synthesized substances that kill bacteria and microorganisms.

- Why is the volume of the 50% methanol solution used to prepare the first and second standard solutions different when making preservative standards?

When preparing standard solutions, the SOP requires accurately weighing 1000 mg. Since our electronic balance measures up to four decimal places, there are slight differences in the final volume due to these minor variations in weight. To correct these differences, we use the purity provided in the AOC (Analysis of Certification) test result and the actual weight we measured to calculate the 50% methanol solution needed. This ensures that the final volume is consistent across all standard solutions.

6. Conclusion

The experiment successfully demonstrated that high-performance liquid chromatography (HPLC), with specific UV detection wavelengths, can accurately detect and quantify preservatives such as sorbic acid (SA), benzoic acid (BA), and their derivatives in food samples. The concentrations of these preservatives were found to be within detectable ranges, and the method provided reliable results for safety and compliance assessments. The findings support the hypothesis that HPLC is a highly effective and sensitive technique for analyzing preservatives in various food matrices.

Implications

The results of this study highlight HPLC's critical role in food safety testing. Accurate detection and quantification of preservatives are essential for ensuring that their use in food products complies with regulatory standards, thereby protecting consumer health. Food safety agencies and manufacturers could widely adopt the study's methodology to routinely monitor preservative levels in processed foods.

Recommendations for Future Research

- Expanding Sample Variety: Future research should include a broader range of food products to assess the method's applicability across different food types and matrices.

- Exploring New Preservatives: As food technology evolves, new preservatives are introduced. Future studies should focus on detecting and quantifying emerging preservatives to ensure the continued relevance of HPLC techniques.
- Improving Extraction Methods: Developing more efficient extraction techniques could reduce potential preservative loss during sample preparation and improve the accuracy of results.

Automation and Real-Time Monitoring: Investigating automation of the HPLC process or real-time preservative monitoring systems could enhance the speed and efficiency of food safety testing in industrial settings.

Practical Applications

- Regulatory Compliance: Food safety authorities can implement HPLC-based methods to monitor preservative levels and ensure compliance with legal limits.
- Quality Control in Food Manufacturing: Food manufacturers can use this method to ensure consistent product quality and safety and prevent the overuse or underuse of preservatives.
- Consumer Safety: Routine testing of food preservatives helps maintain high safety standards, thereby reducing the risk of potential health hazards associated with preservatives.

By addressing the current limitations and expanding the scope of future research, the application of HPLC in food safety testing can continue to evolve, ensuring better public health protection.

2.4 Relevant academic theories

Basic Standards - Quality Control

- Qualitative Analysis

1	Chromatography	The retention time (RT) of the test sample and standard should meet the requirement of ± 0.1 min.
2	Chromatography	The wavelength deviation should be within ± 2 nm.
3	Mass Spectrometry	The allowable range for ion intensity should comply with the published method.

- Quantitative Analysis

1	When preparing calibration curves, standard solutions for each concentration of acidic/ester-type preservatives should be included in the calibration curve.
2	The lowest concentration on the calibration curve should correspond to the concentration of the LOQ (Limit of Quantification).
3	The sample concentration should be diluted and reanalyzed if it exceeds the calibration curve. Extrapolation is not allowed.
4	For linear regression, if the R-value is ≥ 0.995 , it is acceptable. If the R-value is lower than the specified value, the model should be recreated or segmented.
5	The optimal concentration of the analyte in the sample should be within 20% to 80% of the highest concentration on the calibration curve.

- Calibration Curve Confirmation (CC0)
 - a. Timing: After the calibration curve is created.
 - b. Standard: A standard different from the one used to create the calibration curve (secondary standard) is used, and the suitability is confirmed using the mid-range concentration of the calibration curve.
 - c. Purpose: To compare the measured value confirmed by the calibration curve with the concentration of the verification standard and to calculate the relative error.
 - d. Range: The control limit is set at $\pm 20\%$. If the result exceeds this range, the calibration curve should be recreated.
 - e. Relative Error (%) = $[(\text{Calculated concentration} - \text{Prepared concentration}) / \text{Prepared concentration}] \times 100\%$
- Calibration Curve Verification (CC1, CC2, etc.)
 - a. Timing: For every batch of up to 20 samples or every 12 hours of analysis time per batch.
 - b. Standards: The same standards used for creating the calibration curve (first standard) should be applied, and the accuracy should be confirmed using the intermediate concentration of the calibration curve.
 - c. Purpose: To calculate the relative error by comparing the measured value obtained from the calibration curve to the actual concentration of the verification standard.
 - d. Range: The control range is within $\pm 20\%$. A new calibration curve must be created if the relative error exceeds this range.
 - e. Relative error (%) = $[(\text{calculated concentration} - \text{prepared concentration}) / \text{prepared concentration}] \times 100\%$
- Calibration Curve Confirmation (CC0)
 - a. Timing: After the calibration curve is completed.
 - b. Standard Solution: A standard solution different from the one used to create the calibration curve (second standard solution) is used, and its suitability is confirmed using the mid-range concentration of the calibration curve.
 - c. Purpose: To compare the measured values of the calibration curve confirmation with the concentration of the check standard solution and calculate the relative error.
 - d. Range: The control range is $\pm 20\%$. If the error exceeds this range, the calibration curve must be re-established.
 - e. RelativeError (%): =
$$\frac{(\text{MeasuredConcentration} - \text{PreparedConcentration}) / \text{PreparedConcentration}}{\times 100} \frac{((\text{MeasuredConcentration} - \text{PreparedConcentration}) / \text{PreparedConcentration}) \times 100}{}$$
- Blank Analysis (BK)
 - a. For each batch, a blank sample with a similar matrix to the test sample should be used. If a blank sample for the specific test item cannot be obtained, a reagent blank may be used as a substitute.

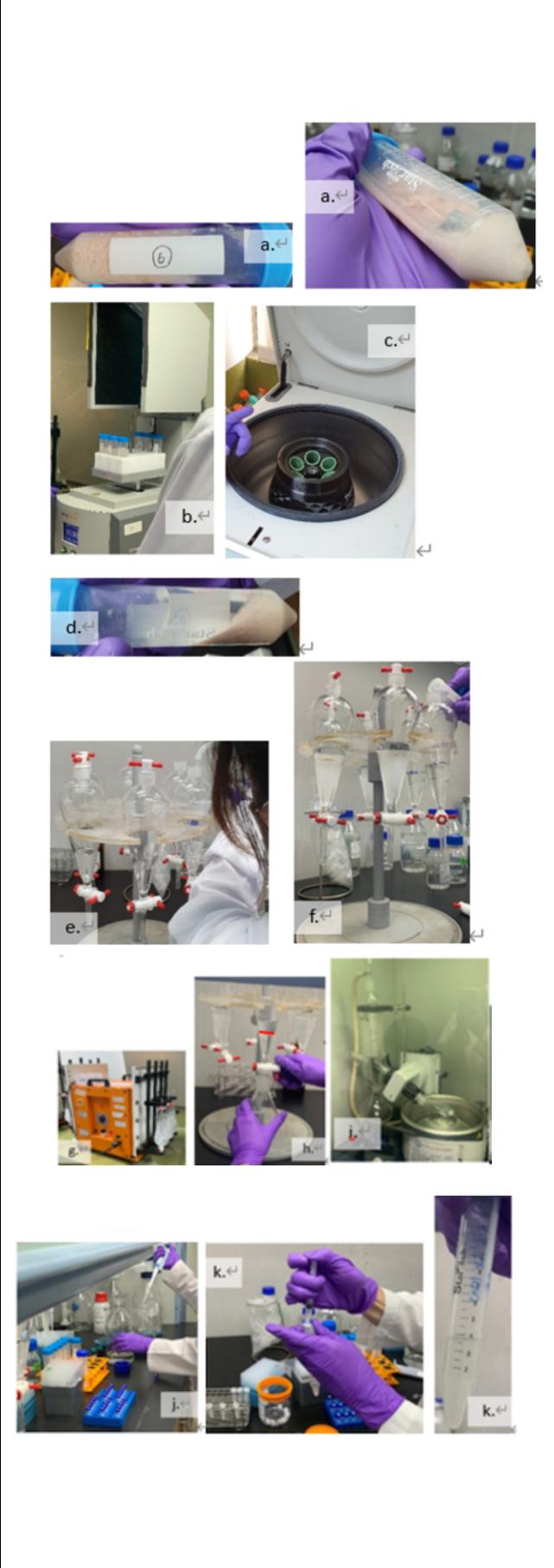
- b. The analysis result should meet $S/N \leq 10$ or be less than one-fifth of the LOQ. If the test method does not provide an S/N ratio, the analysis result must be less than 50% of the LOQ or fall within a stricter control range.
- Analysis of Spiked Blank Samples (SP)
 - a. For each batch of samples, a spiked sample analysis must be performed once for verification. The value should fall within the control limits of the quality control chart and comply with its usage specifications.
 - b. The recovery rate of the spiked sample analysis for this testing method should meet the standard of 80 – 115%.
 - c. Recovery rate (R%): $(\text{Test value of spiked sample for quality control verification} / \text{Prepared value of spiked sample for quality control verification}) \times 100\%$.
- Duplicate Sample Analysis (SPD)
 - a. Each batch of samples must undergo a duplicate analysis.
 - b. Duplicate samples should be processed using the same pre-treatment and analysis procedures simultaneously, and the relative percentage difference (RPD%) should be calculated. The RPD% must fall within the control limits of the quality control chart and comply with its guidelines.
 - c. The relative percentage difference should be within 10%.
 - d. The formula for the relative percentage difference (RPD%) is:

$$[| X_1 - X_2 | / (X_1 + X_2) \times 1 / 2] \times 100\%$$

Experimental Category: Veterinary Drugs

Text Description	Illustration
Preparation of Reagents	
50% Methanol Solution: Measure 50 mL of methanol and add deionized water to make a total volume of 100 mL.	
Acetonitrile containing 5% methanol (to prevent the extract from being decomposed by n-hexane): Measure 50 mL of methanol and add acetonitrile to make up to 1000 mL.	
Preparation of Test Solution	

- (1) Finely chop approximately 5g of the sample and homogenize it in a centrifuge tube.
- (2) Add 25 mL of acetonitrile solution containing 5% methanol.
Add ceramic homogenizer beads (a.)
- (3) Homogenize for 3 minutes (b.)
- (4) Add 10g of anhydrous sodium sulfate (for moisture absorption and reducing interference), and shake for 10 minutes.
- (5) Centrifuge at 3500xg at 4° C for 10 minutes (c.) (This helps to solidify the fats, making centrifugation easier).
- (6) Collect the supernatant.
- (7) Add another 25 mL of acetonitrile solution containing 5% methanol to the precipitate (d.)
- (8) Shake for 10 minutes.
- (9) Centrifuge again at 3500xg at 4° C for 10 minutes (c.).
- (10) Combine the supernatant and transfer it into a separatory funnel (f.).
- (11) Add 30 mL of acetonitrile-saturated n-hexane solution (e.).
- (12) Shake for 3 minutes to perform liquid-liquid partitioning (g.).
- (13) Collect the acetonitrile layer (h.) (n-hexane is on top, acetonitrile is on the bottom due to the lower density of acetonitrile.).
- (14) Concentrate under reduced pressure at 40° C until dry (i.).
- (15) Dissolve the residue in 50% methanol solution (j.).
- (16) +Vortex → transfer to Eppendorf tube → centrifuge (k.).
- (17) Make up to a final volume of 1 mL, filter through a membrane, and use as the test solution.



Matrix-Matched Calibration Curve Preparation					
	1ppm→0.05ppm				
	50ppm	75ppm	100ppm	125ppm	150ppm
Test Solution A(μL)	50	75	100	125	150
Test Solution B(μL)	50	75	100	125	150
MeOH(μL)	900	95	800	750	700
total	1mL				

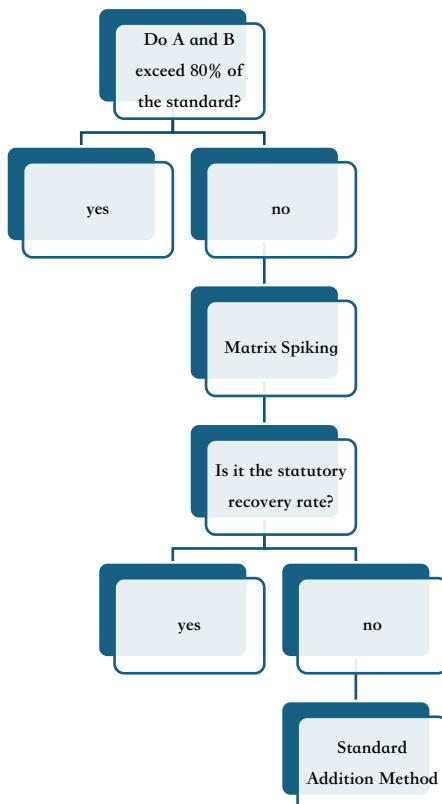
Added an inner tube (l.) → Total volume +5.

	50ppm	75ppm	100ppm	125ppm	150ppm
Test Solution A(μL)	10	25	20	25	30
Test Solution B(μL)	10	25	20	25	30
MeOH(μL)	180	190	160	150	140
total	200μL				



Annotation

- There are two substances, A and B. The concentration of A is five ppm, and B is four ppm. B is tested using the method designed for A...



- Standard Addition Method (Standard Addition is not affected by matrix effects)

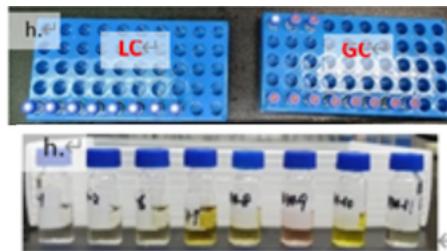
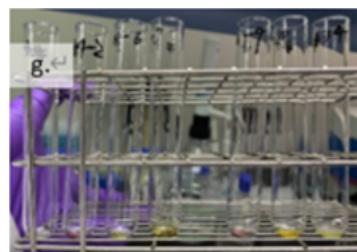
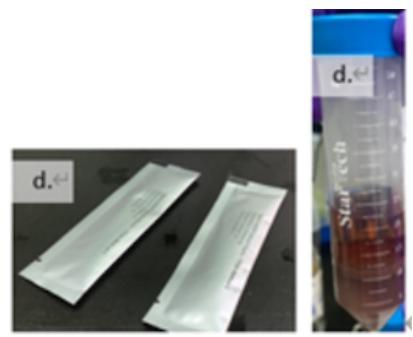
Formula: (Actual concentration of the sample solution × dilution factor) ÷ sample weight

	BL	Standard Solution 1	Standard Solution 2	Standard Solution 3	Standard Solution 4
matrix		1.0 mL	1.0 mL	1.0 mL	1.0 mL
STD		-----	1.0 mL	2.0 mL	4.0 mL
Solvent		10mL	9.0 mL	8.0 mL	7.0 mL
	10 mL	10 mL	10 mL	10 mL	10 mL

Experimental Category: Pesticide Residues

Text Description	Illustration
<p>Fruits, Vegetables, Spices, and Other Herbal Substances (Fresh):</p> <p>(1) Take approximately 10g of homogenized sample and place it in a centrifuge tube. Add one ceramic homogenization stone (a).</p> <p>(2) After freezing, add 10 mL of acetonitrile solution containing 1% acetic acid and 10 mL of 50 µg/mL internal standard solution (TPP) (b).</p> <p>(3) Shake vigorously using a high-speed disperser at 1000 rpm or manually shake for 1 minute (c).</p>	  

- (4) Add extraction powder (4g anhydrous magnesium sulfate and 1g anhydrous sodium acetate), cover the centrifuge tube, and shake vigorously several times to prevent salt crystallization (d).
- (5) Shake again using a high-speed disperser at 1000 rpm or manually shake for 1 minute.
- (6) Centrifuge at 3000xg for 1 minute at 15° C.
- (7) Take 6mL of the supernatant and place it into a clean-up centrifuge tube (containing 300 mg PSA and 900 mg anhydrous magnesium sulfate, suitable for high-water-content fruits and vegetables), shake using a high-speed disperser at 1000 rpm or manually shake for 1 minute (e).
- (8) Centrifuge at 3000xg for 2 minutes at 15° C (f).
- (9) Take 1mL of the supernatant, evaporate to dryness under a nitrogen stream (g).
- (10) Dissolve the residue in 1mL of methanol, mix thoroughly, filter through a membrane, and prepare sample solution I for LC/MS/MS analysis (h).
- (11) Take another 1mL of the supernatant, evaporate to dryness under a nitrogen stream (g) (until no liquid drops).
- (12) Dissolve the residue in 1mL of acetone solution (1:1, v/v) (900 µL of acetone (1:1, v/v)), mix thoroughly, filter through a membrane to prepare sample solution II, add 100 µL of formic acid for GC/MS/MS analysis. For items where detection sensitivity may be affected (such as tetrachloronitrile), add 10 µL of 5% acetic acid in acetonitrile solution to confirm the accuracy of the detected concentration (h).



- QuEChERS

(Quick, Easy, Cheap, Effective, Rugged, Safe, pronounced "catchers")

The method consists of three steps: homogenization, extraction, and purification.

a. Sample Homogenization

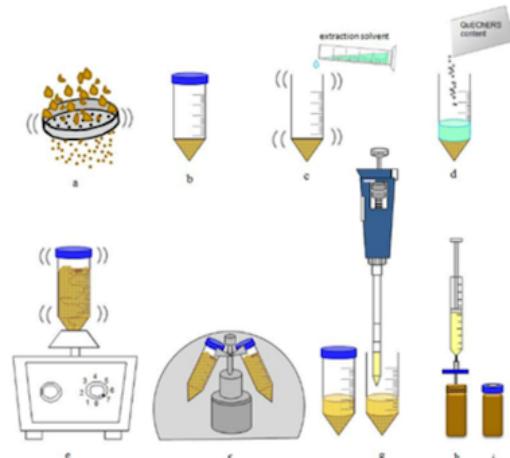
To increase the contact surface area, the sample is broken into fine particles using a grinder(a.). Weigh the sample (fruits, vegetables, grains, tobacco, or tea, etc.) (b.) and add the solvent (an acetonitrile solution containing 1% acetic acid) along with an internal standard (triphenyl phosphate, TPP) to homogenize.

b. Liquid-Liquid Extraction

Extraction powder (containing anhydrous magnesium sulfate and anhydrous sodium acetate)(c) is added during extraction. Modifiers may be added to enhance extraction efficiency, or salts can be added to improve separation. After homogenization, the sample particles become smaller, increasing the surface contact with the solvent, thereby enhancing extraction efficiency.

c. Dispersive Solid-Phase Extraction (DSPE)

A solid-phase sorbent or purification reagent (d)(containing anhydrous magnesium sulfate, PSA, and C18; the amount depends on the sample type) is added to the extraction solution. The mixture is then shaken manually or mixed vigorously to remove co-extracted substances from the extraction solution. (e.f.) This step efficiently removes water and impurities, achieving effective purification. The sorbent primarily adsorbs impurities, and when background noise is significantly reduced, the signal-to-noise ratio (S/N) during instrumental analysis improves. The resulting supernatant is centrifuged and then subjected to further analysis using gas or liquid chromatography.



3 Société Générale de Surveillance (General Surveillance Society)

Your internship lasted from August to September at the company of the General Surveillance Society. The primary tasks during the first two weeks involved reading relevant literature and discussing possible research methods. You participated in the company's "one-stop service" work in the following four weeks. In the final two weeks, you conducted an in-depth case study evaluation.

3.1 Background

- Internship Period: August 1, 2020 – September 31, 2020
- Internship Location: SGS Taiwan Limited, Food Department,(4th Floor, No. 125 Wugong Road, Wugu District, New Taipei City.)
- Supervisor: Manager Zhongwei Cai
- TEL: +886-2-2299-3279 ext. 6657

- Introduction: The food and specialty product testing services began in 1997, with teams spread across Taiwan and offices and laboratories located in Taipei, Taichung, and Kaohsiung, continually improving analytical capabilities. The service targets include food manufacturers, agricultural product producers, processors, wholesalers, and chain food businesses. Additionally, in the field of food hygiene and safety testing and sanitation audits, their extensive execution experience has made them the top brand in the industry.
- Agency Certifications:

<p>Certified by TFDA Laboratory (Certification No.: F002)</p>	
<p>TFA Certified Laboratory</p>	
<p>ISO 17025 Accredited</p>	

Overseas Certified Laboratory by the Ministry of Health, Labor and Welfare of Japan.	
Recognized Laboratory by the Ministry of Agriculture of Indonesia.	

3.2 Internship arrangement

Task Name	Start Date	End Date
Reading relevant literature and discussing possible research methods.	2020-08-01	2020-08-14
"One-stop service" – research and consultation related to food regulations.	2020-08-15	2020-08-22
"One-stop service" – Developing a search engine for relevant food regulations.	2020-08-23	2020-08-26
"One-stop service" – Practical work in the sample collection room and photo room.	2020-08-27	2020-09-01
"One-stop service" – Simulating and implementing customer service department processes.	2020-09-02	2020-09-06
Laboratory operations, such as: multi-residue detection of veterinary drugs, plasticizers, microorganisms, microorganisms (general water quality), artificial additives, nutrition labeling, heavy metals, alcohol testing, edible oils, etc.	2020-09-07	2020-09-12
"One-stop service" – Risk management	2020-09-13	2020-09-17
"One-stop service" – sample submission (potatoes) and completion of testing items.	2020-09-18	2020-09-23
Conducted a risk analysis and evaluation of the hazards detected in potatoes.	2020-09-24	2020-09-31

3.3 Case study

Experiment Case Study: Risk analysis and assessment of hazards detected in potatoes.

1. Introduction

Potatoes are one of the most important food crops globally, but they're naturally occurring glycoalkaloids (such as solanine and chaconine) can reach harmful levels under certain conditions, leading to food poisoning. Glycoalkaloids are primarily concentrated in the potato's skin, sprouts, and greened parts. Consumption of high levels of glycoalkaloids can cause a range of acute symptoms, including nausea, vomiting, abdominal pain, diarrhea, and even neurological disturbances. In 2013 and 2019, Taiwan experienced food safety incidents related to excessive glycoalkaloid levels, involving a local fast-food chain and imported potato products. These incidents raised significant concerns about the potato supply chain, food safety management, and the regulation of glycoalkaloid content.

This study aims to analyze recent food safety incidents in Taiwan related to total glycoalkaloids in potatoes, explore the potential risks posed by glycoalkaloids in potatoes to food safety, and examine the enforcement of existing food safety regulations, particularly the regulatory measures implemented by the Taiwan Food and Drug Administration (TFDA) to ensure food safety.

2. Objectives

- **Investigate the occurrence of foodborne illnesses linked to solanine contamination in potatoes:** To analyze the health impacts, symptoms, and potential causes of food poisoning incidents related to solanine levels in potato-based foods in Taiwan.
- **Examine compliance with food safety regulations:** To assess how the incidents align with Taiwan's food safety laws, particularly the enforcement of the "Food Safety and Sanitation Act" and the 2019 implementation of "Contaminants and Toxins Standards in Food."
- **Evaluate the role of supply chain and quality control:** To explore the importance of raw material quality control in the potato supply chain, particularly the responsibilities of suppliers and manufacturers in preventing contamination.
- **Analyze regulatory actions and preventive measures:** To evaluate the effectiveness of regulatory measures taken by the Taiwan Food and Drug Administration (TFDA) in ensuring food safety, including the rejection and destruction of contaminated imports.
- **Compare domestic and imported potato safety standards:** To examine differences in contamination levels between locally sourced and imported potatoes, focusing on solanine content and adherence to the established safety limits.

3. Methodology

Sample Collection

- **Objective:** To gather representative samples for testing from both domestic and imported potato sources, including raw potatoes, processed potato products (such as fries and chips), and products stored under various conditions.

- **Procedure:**
 - a. Random sampling from different supply chains, including retail outlets, warehouses, and processing plants.
 - b. Ensuring that samples are collected from various regions to account for geographic variation.
 - c. Use of sterile equipment and containers to avoid contamination during collection.

Laboratory Testing and Analysis

- **Objective:** To measure the concentration of harmful substances such as glycoalkaloids, heavy metals, pesticide residues, preservatives, and microbial contamination.

- **Procedure:**

- a. Glycoalkaloid Testing

- (1) Extraction and quantification of solanine and chaconine using high-performance liquid chromatography (HPLC) or mass spectrometry techniques.
 - (2) Results compared against the regulatory limit of 200 mg/kg.

- b. Heavy Metals

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) or Atomic Absorption Spectroscopy (AAS) is used to quantify lead, cadmium, arsenic, and mercury levels.

Ensure that results are compared to permissible limits set by food safety regulations.

- c. Pesticide Residue:

- (1) Use Gas Chromatography-Mass Spectrometry (GC-MS) to detect and quantify pesticide residues.
 - (2) Adherence to national or international maximum residue levels (MRLs).

- d. Microbial Testing

- (1) Microbiological culture methods and Polymerase Chain Reaction (PCR) testing for pathogens such as *Salmonella*, *E. coli*, and *Listeria*.
 - (2) Food safety standards do test for microbial limits.

- e. Preservative Testing

- (1) Determination of preservative levels (e.g., benzoates and sorbates) using spectrophotometry or chromatographic methods.
 - (2) Cross-checking with regulatory guidelines on acceptable preservative concentrations.

- f. Veterinary Drug Residue

Screening for residues using liquid chromatography-mass spectrometry (LC-MS) and comparing results with established limits for food safety.

Data Analysis

- Objective: To interpret the laboratory results and assess compliance with food safety standards.
- Procedure:
 - a. Statistical analysis of the concentration levels of contaminants about the regulatory limits.

- b. Identification of trends based on geographic origin, supplier, or product type.
- c. Assessment of potential risks posed by contaminants that exceed permissible levels.

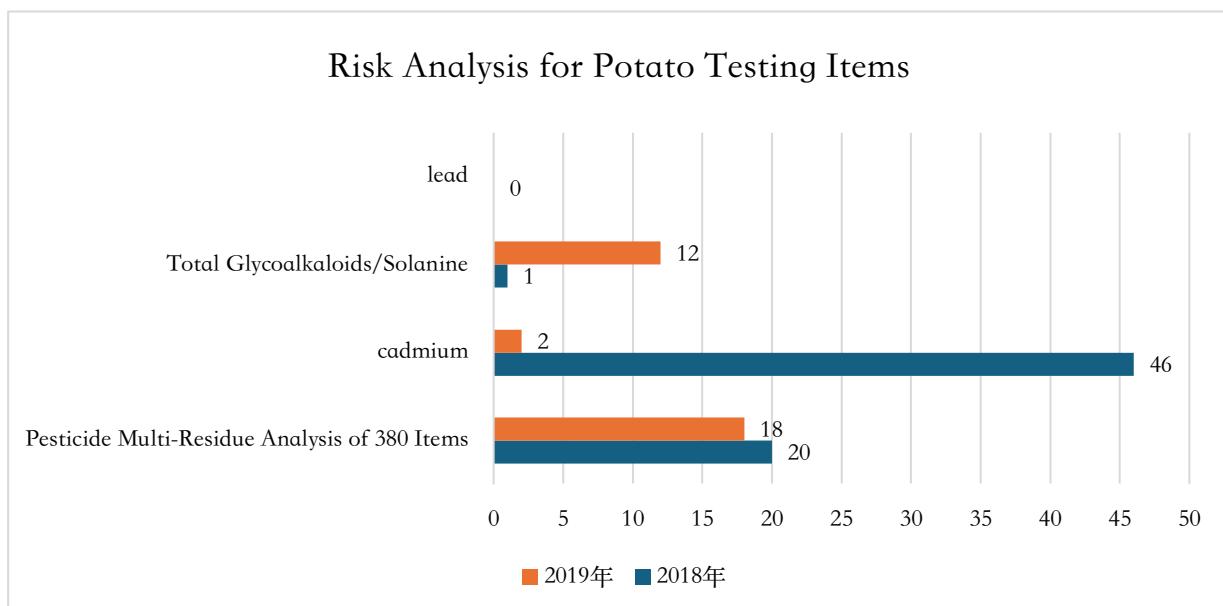
Risk Evaluation

- Objective: To evaluate the severity and likelihood of health risks based on the contaminant levels detected.
- Procedure:
 - a. Categorizing the risks (low, medium, high) based on the magnitude of contamination and its potential impact on public health.
 - b. Estimating the exposure risk to consumers based on consumption patterns and contaminant concentration levels.
 - c. Identifying any critical control points where contamination is more likely to occur.

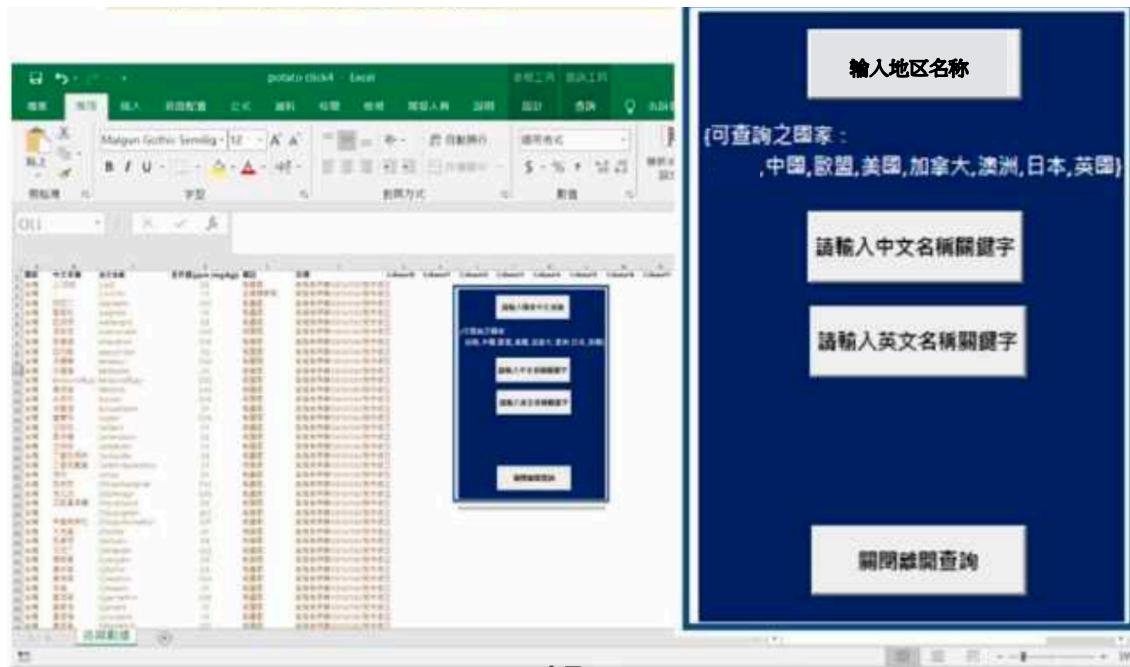
4. Analysis

- Risk Analysis for Test Items

The data shows that the selection of potato test items initially focused on the multiple pesticide residue analysis (380 items) and total glycoalkaloid testing, followed by other tests. The sudden increase in the detection rate of total glycoalkaloids is attributed to the implementation of the "Standards for Contaminants and Toxins in Food" by Taiwan's Food and Drug Administration (TFDA) on January 1, 2019, which set the total glycoalkaloid limit for potato tubers at 200 mg/kg. Before 2019, there were no regulatory limits.



- Test Item Query Creation



- Test Report





食品實驗室-台北
FOOD LAB-TAIPEI
測試報告
Test Report

頁數：3 of 3

以下為申請廠商委託測試項目、測試方法、定量/檢測極限：
FO/2020/80026

Page 2

測試項目名稱旁有加★者，為本實驗室通過衛生福利部認證項目
而此項為必檢項目。未標註★之項目為非必檢項目。

測試項目名稱旁有加○者，為經委託實驗室通過衛生福利部認證項目

測試方法皆遵循衛生福利部公告方法分析

SOG Taiwan Ltd.
台灣矽膠科技股份有限公司
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Member of SOG Group

5. Annotation

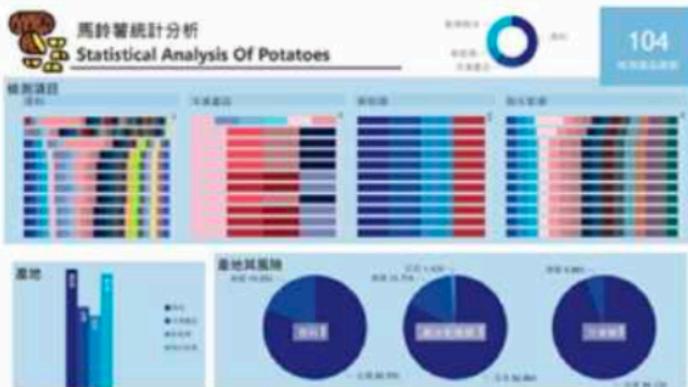
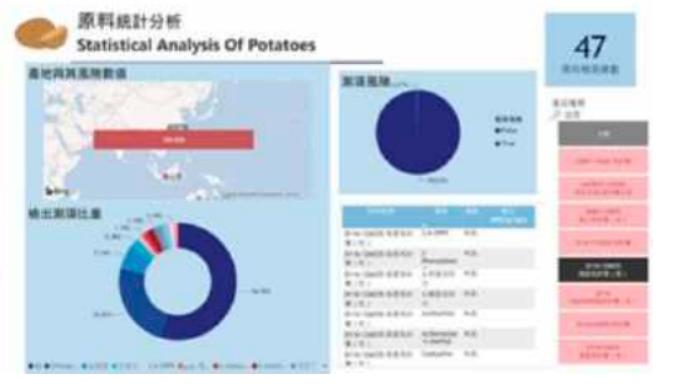
- potato by-products

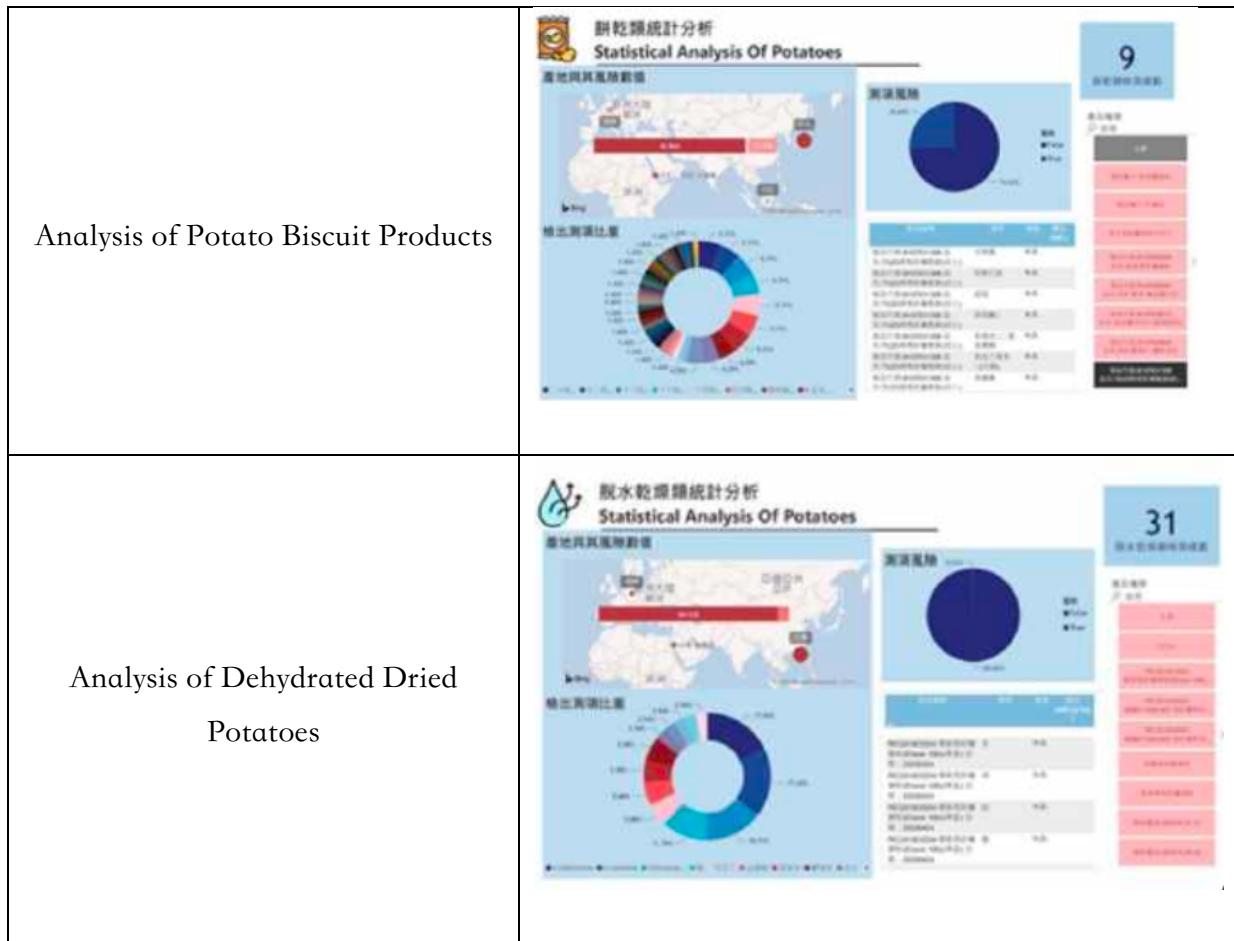
Ingredients (Potato)	Frozen Products	Frozen French Fries
		Frozen Potato Balls
		Frozen Potato Pancakes
		Frozen Potato Cubes
		Frozen Diced Potatoes
	Biscuits and Snacks	Potato Chips
		Salt Crackers
		Crispy Crackers
	Dehydrated and Dried Products:	Cooked Potato Flour
		Potato Starch
		Whole Potato Powder
		Potato Protein Powder
		Raw Potato Flour

I selected potatoes as the focus, initially pursuing vertical development by deeply researching Taiwanese regulations and historical food safety incidents through a search engine, while also

integrating regulations from various countries. In the horizontal development, I considered potato-related products such as frozen items, biscuits, and dehydrated dried goods.

- Create Power BI data analysis

Comprehensive Analysis of Potatoes	
Potato Ingredient Analysis	
Analysis of Frozen Potato Products	



3.4 Relevant academic theories

One-stop service

- Background of "One-stop Service": With the rapid development of electronic network technology in the 21st century, e-government, e-commerce, and enterprise information services have flourished. In this context, the "one-stop service" emerged.
- Advantages and Disadvantages of "One-stop Service":
 - Advantages:
 - It simplifies the operational process: one person handles the request, and internal operations manage the workflow, making it convenient for customers and improving efficiency.
 - It is easy to promote, low-cost and can achieve the maximum marginal benefit (achieving the greatest economic profit with the lowest cost).
 - Disadvantages:
 - Increases the risk of corruption.
 - It is not conducive to personalized service. Some people prefer to handle every step themselves to improve participation and monitor progress. If managed internally, it may lead to a lack of transparency.

- In simple terms, SGS provides a wide range of services allowing customers to choose almost all they need on one platform. This includes a "one-stop service" that covers everything from front-end professional inspection consulting, catering hygiene auditing services, test applications, product quality control (factory audits, product specification formulation, product specification manuals, routine sampling), food hygiene and safety training courses, to risk management and providing solutions through food safety monitoring programs.
- The "one-stop service" improves both service quality and efficiency, while also increasing customer satisfaction.

Quality Assurance x ISO

- Quality Meets Certain Standards

Various quality standards (ISO, work instructions, operational procedures)

- Prevention Over Post-Inspection

Quality assurance (QA) is the broader company quality management work. Externally, it involves third-party vendors and customers, and internally, it includes designing and developing new products, production, shipping, and after-sales service. A comprehensive quality management system must ensure the product meets customer needs. QA focuses on preventive measures, aiming to "get it right the first time," whereas quality control (QC) is more about post-issue inspection and remediation.

- Zero Defects

Eliminating factors that cause errors.

- The High Cost of Non-Compliance

Understanding the cost of quality.

Certification vs. Verification vs. Testing vs. Inspection vs. Examination

- Certification vs. Verification

It can be divided into three levels: the enterprise, the verification body (CB), and the accreditation body (AB). The "verification body" is the referee, conducting assessments and issuing ISO certificates to companies. It refers to an impartial and independent third-party verification body (such as SGS). To ensure fairness, the verification body must be "accredited" by an accreditation body according to ISO 17021 standards and supervised through audits (such as TAF and the Taiwan Accreditation Foundation). The "accreditation body" serves as the inspector, specializing in supervising and assessing verification bodies. Only through this process can the ISO certificates issued by verification bodies gain the public's trust.

- Testing

Testing refers to analyzing samples in a laboratory according to standard procedures. For example, a manufacturer sends product samples to SGS for testing. SGS will follow relevant testing regulations and issue an official test report to inform the client of the results. Since the test report only applies to the specific samples provided by the client, if SGS did not participate in the sampling process, the test report cannot represent the quality of the entire batch of goods. Testing refers to laboratories conducting tests on samples to obtain data from them, but it does not judge the sample's qualification, quality, or compliance.

- Inspection

Inspection usually refers to the visual examination and professional judgment of products at the factory or warehouse based on the buyer's requirements to determine whether the goods are ready for shipment. Due to equipment and time constraints, precise instrument analysis and judgment are often not feasible. Inspections involve making compliance judgments on the products being inspected, and the data used in the judgment process may come from testing conducted by SGS or similar bodies.

4. Reflection

As the weeks progressed, I gradually realized that preparing for quality assurance is not only about understanding all the operational processes but also about becoming familiar with the relevant regulations for the product. This includes both domestic regulations and incidents related to food safety, as well as international limits. I began to develop a preliminary concept of starting with a single product for vertical development, then expanding horizontally. For example, I chose potatoes as my focus. Initially, I pursued vertical development by researching Taiwanese regulations and historical food safety incidents through a search engine and integrating international regulations. In the horizontal expansion, I considered potato-related products, such as frozen items, biscuits, and dehydrated dried goods. By analyzing layers of relationships, I worked from the outermost circle of related products and the test items detected in them back to the raw materials in the innermost circle, noticing details that were previously overlooked. I highlighted testing items that had a high possibility of being detected but had not been paid attention to before, thus preventing violations. This is the essence of quality assurance—proactive prevention, with the goal of "getting it right the first time" to avoid potential mistakes.

After observing the entire process, from the submission of the test request form to the final report production, I found that it is all about division of labor and collaboration, with each task meticulously carried out. Each stage carefully handles its responsibilities, much like opening a card. Whether it's front-end customer service or back-end administration, everyone has their own permissions and completes each task with caution.

This internship opportunity has given me another path to consider for my future career. Last month's internship was focused on testing, while this month's internship was more aligned with administrative management, which is a subject I need to study in my health management program. Through this, I not only learned about SGS's process from sample receipt to report production but also gained insight into the skills I will need in the future. These include having more capabilities than others, a broader perspective, and standing at a higher level. I believe these will be crucial experiences in my life. As a result, I plan to choose more courses related to food safety and hygiene in the future, including food safety management systems, regulations, risk assessment, and the writing of food safety management system documentation.

Thanks to this internship opportunity, I was able to work diligently, learn actively, and seek advice, allowing myself to improve and realize my value.

5 Reference

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