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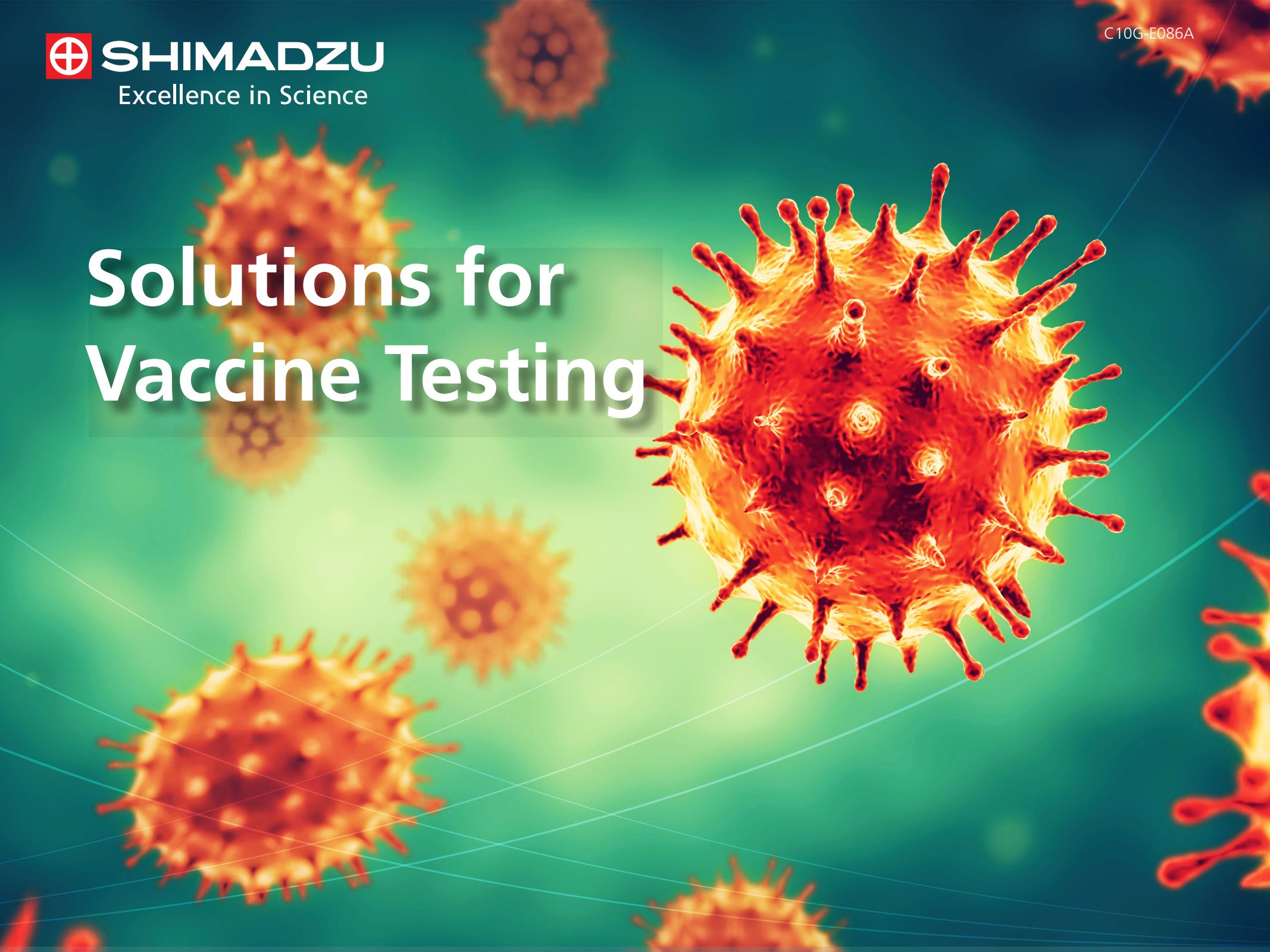
C10G-E086A

Solutions for Vaccine Testing



SHIMADZU
Excellence in Science

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Preface

The father of the vaccine, Edward Jenner

In the 18th Century Europe, smallpox killed approximately 400,000 people every year. Despite various inhumane methods used in attempt to contain the disease, such as burning the wound using soldering iron, deaths associated with smallpox continued until Edward Jenner finally discovered the immune function of cowpox scabs.

Since that time, one vaccine after another have emerged in the fight against diseases. As the result of increase in vaccination rates and the variety of vaccines available, the death rate among children under 5 years old has halved since 1990. However, total eradication of a disease by vaccination has only realized for smallpox, and the threat of deadly pathogens remains persistently at the global scale. For instance, according to the 2016 statistics published in The Lancet, pneumonia was the most significant cause of child deaths in Angola, the Congo, Ethiopia, and Tanzania. The incidence of pneumonia in different regions was found to be negatively correlated with vaccination rate, clearly indicating the demand for vaccine deliveries to these regions. On the other hand, in the developed countries that already undergo national immunization programs recommended by the World Health Organization, there are growing concerns for the quality of vaccines to reduce hazardous side effects.

Shimadzu's corporate philosophy is "Contributing to Society through Science and Technology" and our management principle is "Realizing Our Wishes for the Well-being of Mankind and the Earth". Since the establishment in 1875, Shimadzu has been developing advanced instruments and gathering industry elites to provide solutions for various difficulties. Now Shimadzu is striving to apply modern analytical techniques to ensure high quality, safety and reliability of vaccine products as described herein, and, in light of social impact, it is our honor to deliver solutions to this industry.

About Vaccines

Vaccines are biological products used for the prevention or treatment of human diseases and made from biological materials rich in immunogenicity, such as microorganisms, proteins, polysaccharides, or nucleic acids. Vaccines are produced using either traditional methods or genetic engineering and other biological technologies. The effects of disease prevention inherent in vaccines have been widely recognized, such as vaccinia, which eradicated smallpox globally. However, in recent years, some emerging and re-emerging infectious diseases have posed new threats to human health. Meanwhile, the development of modern biotechnology and analytical technology has laid a foundation and created conditions for both the development of new vaccines and the improvement of existing vaccines.

About Vaccines

Figure 1. The history of vaccine development by mankind

1. Development of vaccinia

As early as the 10th Century, China adopted the method of variolation (smallpox pathogen) to prevent smallpox. "Ningguo House Taiping vaccinia" appeared during the Longqing period in the 16th Century. Thereafter, smallpox vaccination was widely used in China. By the 17th Century, these methods had spread to Russia, Japan, Korea, and other countries. Western European countries also began to use variolation in the 18th Century, but it was not until the late 18th Century that the British physician Edward Jenner proved that the cowpox vaccination could prevent smallpox, ushering in the era of vaccines.

Legend

- Attenuated Live Vaccine
- Inactivated Vaccine
- Protein and Polysaccharide Vaccine
- Genetic Engineering Vaccine

2. Technical route for vaccine development

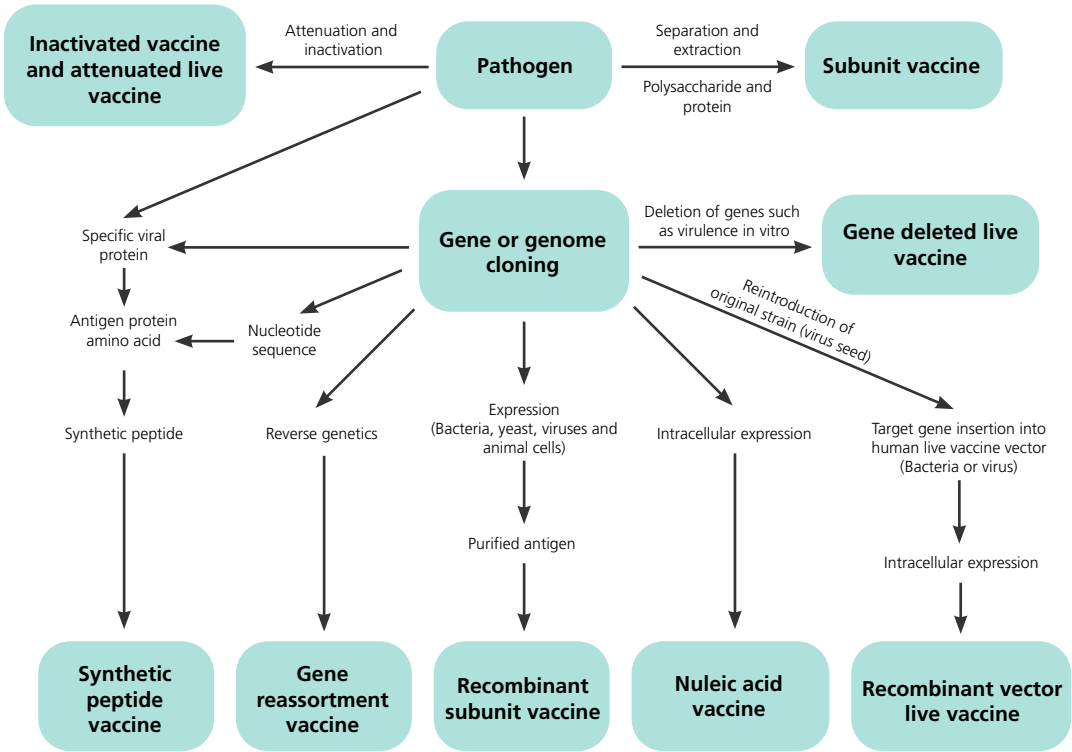


Figure 2. Technical route for vaccine development

3. Classification of vaccines

According to the physical and chemical compositions of immune substances and vaccine technology, vaccines can be approximately divided into bacterial inactivated vaccines (such as the whole-cell pertussis vaccine and the leptospirosis vaccine), bacterial attenuated live vaccines (such as BCG and oral live typhoid vaccines), viral inactivated vaccines (such as inactivated rabies vaccines), viral attenuated vaccines, bacterial polysaccharide vaccines, proteoglycan protein conjugate vaccines (such as the pneumococcal polysaccharide vaccine), protein vaccines (such as acellular pertussis vaccine), and combined vaccines (such as the DPT vaccine).

About Vaccines

4. Global vaccine dynamics

Statistics from July 2015 to June 2016 suggest that, when the amount of vaccine sales is divided by geographic regions, North America has the highest vaccine coverage (more than 60%), and Asia, Africa, and Australia have less than 20%. In Asia, Japan has the highest vaccine coverage and four of the world's top 10 vaccine companies. China ranks sixth in Asia for vaccine coverage. Compared with the United States, where vaccination is most advanced, China still has a lot of room for growth.

Vaccination rates are strongly correlated with child survival worldwide. North America and Europe, where vaccination rates are high, have the highest child survival rates, whereas Africa and Latin America have the lowest.

Virus and protein vaccines account for the largest market size (about 50%) by variety and are also the most diverse vaccine types. Therefore, the establishment of quality evaluation method for virus protein and protein vaccines is conducive to the improvement of quality evaluation levels in the vaccine industry. This booklet describes the subunit evaluation method using MALDI-TOF MS for virus and protein vaccines, the vaccine aggregate analysis using Aggregates Sizer, and the vaccine adjuvant content determination method using ICP-MS/OES.

Table 2 shows the top 10 vaccines in terms of global market share. According to these data, pneumonia is still the most prevalent disease in the world, consistent with the World Health Organization's (WHO) statistics on the causes of child deaths (pneumonia is one of the most deadly diseases for children). Pneumonia is especially widespread in Africa, where the vaccination rates are lowest. In this collection, LC-MS/MS technology is used to develop a method for determining the content of 23-valent pneumococcal polysaccharide vaccines, the inactivator deoxysodium cholate, and the process residual reagent cetyl trimethyl ammonium bromide (CTAB).

Table 1: Top 10 vaccines in global market share

Ranking	Product Name (Trade Name)	Vaccine Type (Chemical)
1	Pneumococcal conjugate vaccine (PREVNAR®)	Bacterial polysaccharide (glycoprotein)
2	Varicella zoster vaccine (VARIVAX®)	Virus (protein + nucleic acid)
3	Influenza vaccine (VAXIGRIP®)	Virus (protein + nucleic acid)
4	Human papillomavirus vaccines 6, 11, 16, 18, 31, 33, 45, 52, and 58	Virus (protein + nucleic acid)
5	Human papillomavirus vaccines 6, 11, 16, and 18	Virus (protein + nucleic acid)
6	Pneumococcus vaccine (PNEUMOVAX®)	Bacterial polysaccharide (polysaccharide)
7	Varicella zoster vaccine + measles, mumps, and rubella vaccine	Virus (protein + nucleic acid)
8	Pentacellular pertussis vaccine (TRIPACEL®)	Protein + glycoprotein + virus
9	Meningococcal conjugate vaccine (MENACTRA®)	Bacterial polysaccharide (glycoprotein)
10	Rotavirus vaccine (RotaTeq®)	Virus (protein + nucleic acid)

5. Examples of vaccine production processes

The production of vaccines is very complicated. Taking the pneumococcal polysaccharide vaccine as an example, its production requires several major steps, namely, bacterial culture, centrifugation to remove thallus, polysaccharide purification, and polysaccharide stock solution, to produce a semi-finished product that is refined into the finished product. Polysaccharide purification alone requires 12 major steps.

Every stage in the production of vaccines must be strictly monitored and checked step by step. From culture, purification, adjuvant (excipient) addition, to the finished product (preservation), the negligence of any link may have extremely negative consequences.

12 major steps for Polysaccharide Purification

1	Termination of liquid culture (sodium deoxycholate inactivation)
2	Continuous flow centrifugation and collection of supernatant (add CTAB)
3	Continuous flow centrifugation and collection of precipitated compound polysaccharide
4	Depolymerization of compound polysaccharide (add $CaCl_2$ or NaCl)
5	Addition of cold ethanol to 25% and removal of precipitated nucleic acid and impurities
6	Obtainment of the centrifugal supernatant, addition of cold ethanol to 70%-80%, and acquisition of crude polysaccharide
7	Crude polysaccharide acquired by centrifugation (add sodium acetate solution to dissolve)
8	Addition of twice the volume of pre-cooled phenol solution and removal of protein by extraction
9	Absorbing the polysaccharide-sodium acetate solution phase and repeating the phenol extraction 2-4times
10	Addition of cold ethanol to 70%-80% with the last polysaccharide-sodium acetate solution phase
11	Collection of precipitation by centrifugation and dissolution in water for injection
12	Sterilization and filtering using a membrane to refine the polysaccharide stock solution

6. Application of testing technology in vaccine industry

There are global standards for vaccine quality control, such as the European Pharmacopoeia, United States Pharmacopoeia, British Pharmacopoeia, Japanese Pharmacopoeia and the Chinese Pharmacopoeia. Methods of vaccine quality evaluation in these pharmacopoeias are generally very classical. For example, UV spectrophotometry is the main technology for vaccine quality control; the content of a saccharide unit, deoxycholic acid, formaldehyde, and the O-acetyl group of pneumococcal polysaccharide vaccines are detected using UV spectrophotometry. Some vaccine enterprises and quality supervision departments have promoted the wider use of analytical techniques for vaccine quality evaluation, and such approaches gradually become the standard methods of vaccine quality control, such as for the evaluation of lactose purity using HPLC-RI and the determination of residual thiomersalate content using atomic absorption spectroscopy.

More recently, according to research published by several vaccine enterprises and research units in the past five years, advanced analytical techniques are being applied in the quality evaluation of vaccines. These techniques include LC-MS technology in the quality evaluation of recombinant proteins, virus-like vaccines, inactivated virus particles, and polysaccharide- and polyglycoprotein-binding vaccines. These data provide strong support for evaluating differences in vaccine quality and monitoring and optimizing vaccination processes. Shimadzu is keeping up with the times and working with vaccine companies and regulatory authorities to customize new testing technologies for vaccines, including LC-MS, MALDI-TOF, biomedical aggregate analysis, and ICP-MS/ICP-OES. These new methods can be used to achieve quality evaluation and production monitoring which could not be attained in the past.

Data Integrity Management

LC, GC and other instruments are widely used in the field of quality management and R&D by chemical and food safety manufacturers and other enterprises mainly engaged in pharmaceuticals. Centered on the pharmaceutical industry, in order to support the data reliability guidelines, FDA21 CFR Part11, GMP and other regulations and guidelines, it is necessary to provide more accurate and efficient maintenance and management for devices and analytical data. LabSolutions™ DB/CS workstation software is exactly the analytical data system that comes into being under these requirements, which has the integrity, innovation and security of data management, and meets the requirements of modern laboratories.

LabSolutions DB/CS software can control a wide variety of instruments, including LC, LC-MS/MS, ICP-MS, Aggregates Sizer, GC and GC-MS/MS for effective component quality evaluation and undesirable substance monitoring of vaccine products, as well as spectral class, dissolution tester and balance. It perfectly achieves integrated management of all data in the database.

The data integrity of LabSolutions DB/CS software includes audit trail and data security.

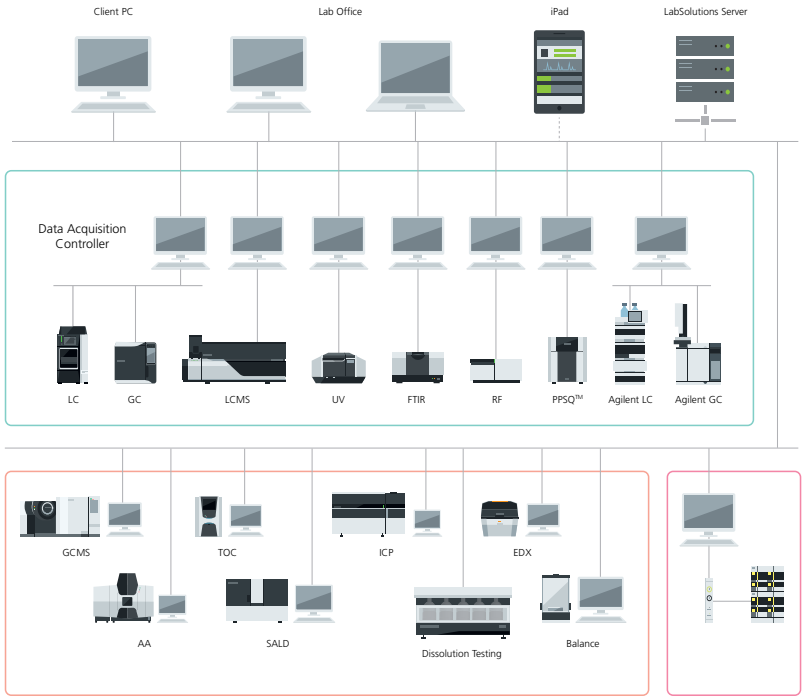


Figure 1. LabSolutions CS system architecture

Data Integrity Management

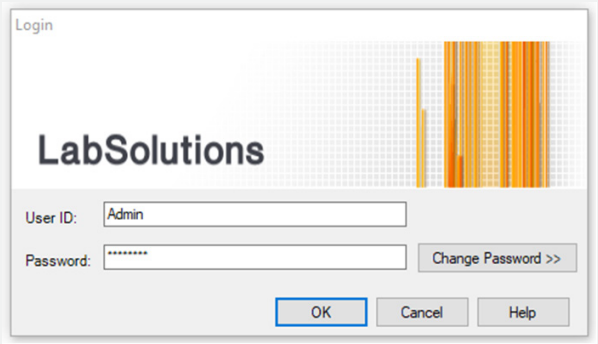


Figure 2. Strict user management mode of LabSolutions™ DB/CS

1. Data security

LabSolutions DB/CS software realizes the closed management of data through the database mode, effectively blocks the unreasonable manipulation of data, prevents the deletion or tampering of data, and thereby better complies with the regulatory requirements. Furthermore, LabSolutions DB/CS implements strict user management principles and can only be entered and used by licensed personnel.

Strict User Management Principles

Management principle of user account: account cannot be repeated and cannot be deleted by the user, illegal login prompt, display login time.

Basic principles of user rights management: separation of duties, prohibition without explicit permission, requirement orientation and minimum authorization

Scope of user rights management: access rights and separation of duties

User password management principles: password length and expiration, password complexity (a mixture of numbers, symbols, and upper and lower case English letters), old passwords cannot be reused

Data Integrity Management

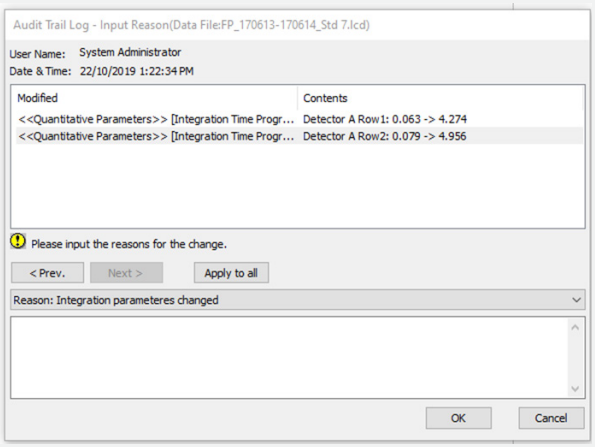


Figure 3. LabSolutions DB/CS data file audit trial example

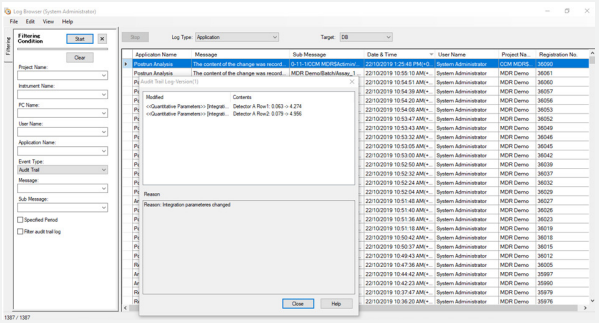


Figure 4. LabSolutions DB/CS log audit trial example

2. Audit trail

Audit trail stipulated in GMP is a series of records of events related to computer operating system, application program and user operation to help track from original data to relevant records, reports or events, or trace back to original data from records, reports or events. Audit trail of LabSolutions DB/CS is mainly divided into four categories: instrument, data, personnel and settings. The reasons for change can also be reset. No manual input is required, and only select with the mouse, meeting the regulatory requirements and reducing the burden of operators.

For data files, version number is adopted to manage data, and data and reports before and after each data modification are saved and can be recovered as the latest data. Meanwhile, data coverage is prevented and data traceability is realized.

	Data File Name	Version	Data No.	Date Registered	Registered by	Date Acquired	Acquired by
	Tutorial_Std002.lcd	4	0-47-4	26/3/2018 11:02:46 AM(+08:00	System Administrator	12/5/2009 6:30:0	System Administr
	├Tutorial_Std002.lcd	3	0-47-3	26/3/2018 10:57:12 AM(+08:00	System Administrator	12/5/2009 6:30:0	System Administr
	├Tutorial_Std002.lcd	2	0-47-2	26/3/2018 10:51:24 AM(+08:00	System Administrator	12/5/2009 6:30:0	System Administr
	└Tutorial_Std002.lcd	1	0-47-1	4/4/2017 12:10:15 PM(+08:00)	System Administrator	12/5/2009 6:30:0	System Administr

Figure 5. LabSolutions DB/CS data file information example

Data Integrity Management

3. Backup and recovery

Make regular backup of the whole or partial data/logs of the laboratory by project. They can be backed up on CD/DVD, removable media, tape, etc to achieve remote backup.

Thus, LabSolutions DB/CS software provides pharmaceutical enterprises with perfect data integrity solutions to meet regulatory requirements. Shimadzu, as a world-renowned analytical instrument manufacturer, owes responsibility on data integrity to ensure that all the data generated by Shimadzu's instruments are for the benefit of people waiting for effective drugs and vaccines to be delivered.

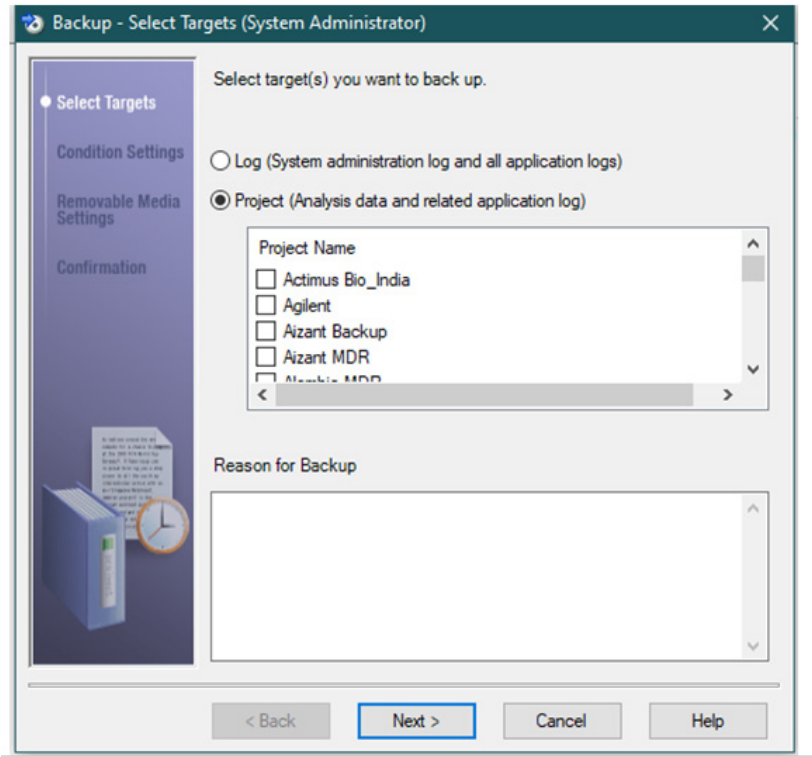


Figure 6. LabSolutions DB/CS backup example

Cell Culture Supernatant Component Analysis

Herein, we describe the analyses of cell culture supernatants that provided a reference method for feasibility studies aiming to identify key factors affecting yields and qualities of biological products. The method simultaneously determined 95 components in cell culture supernatants in 17 minutes using Nexera™ UHPLC coupled to LCMS-8050 triple quadrupole mass spectrometer. Target components including carbohydrates, amino acids, nucleosides, and vitamins were detected with good repeatability and sensitivity. Time-course changes in the cell culture supernatants were elucidated for four different culture conditions, giving valuable information for an efficient process optimization to meet requirements

Keywords:

Liquid Chromatography Mass Spectrometry (LC-MS/MS), Process Development, Process Optimization, Culture Medium Analysis, Metabolomics, Amino Acids, Carbohydrates, Nucleotides, Carbohydrates

1. Introduction

Culture media and processes for linear amplification of products need to be optimized so that vaccine quality and yield can be monitored in real time during vaccine production. To optimize culture media and processes, it is necessary to correlate changes in the contents of cell culture supernatants with favorable outcome, such as high yield.

We developed a cell culture analysis package to rapidly and comprehensively analyze components of cell culture supernatants, and to detect and analyze basic carbon sources, nitrogen sources, nucleotides, vitamins, and other major metabolites so that biological processes can be described in detail. Using LC-MS/MS technology platform, relative changes in quantities of 95 nutrients and metabolites in cell culture supernatants were monitored and analyzed simultaneously in 17 min. The present cell culture analysis package can be used with the Shimadzu Nexera UHPLC with LCMS-8050 triple quadrupole mass spectrometer.

Cell Culture Supernatant Component Analysis

2. Experimental

Sample pretreatment method: 500 µL of cell culture medium was centrifuged at room temperature for 1 min (3000 rpm) and 100 µL aliquots of supernatant were transferred to new centrifuge tubes containing 20 µL of 2-isopropylmolic acid internal standard solution (0.5 mmol/L) and 200 µL of acetonitrile. Mixtures were mixed thoroughly by vortexing and were then centrifuged at room temperature for 15 min (15,000 rpm). Subsequently, 100 µL aliquots of supernatant were added to 900 µL aliquots of water, and were mixed by vortexing and then diluted 100 times with pure water for assays.

Four different culture processes were assembled and samples of fresh culture medium samples were taken before culture on day 0 (D0) and then on D1–5 as follows:

Culture process 1: F1-D0, F1-D1, F1-D2, F1-D3, F1-D4, F1-D5
Culture process 2: F2-D0, F2-D1, F2-D2, F2-D3, F2-D4, F2-D5
Culture process 3: F3-D0, F3-D1, F3-D2, F3-D3, F3-D4, F3-D5
Culture process 4: F4-D0, F4-D1, F4-D2, F4-D3, F4-D4, F4-D5

3. Results

MRM chromatograms of cell culture supernatants

Compounds in cell culture supernatants from different culture processes were analyzed using the cell culture analysis package, and relative quantities of target components were determined using the chromatogram shown in Figure 1.

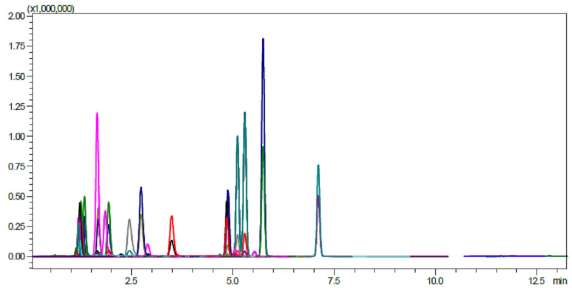


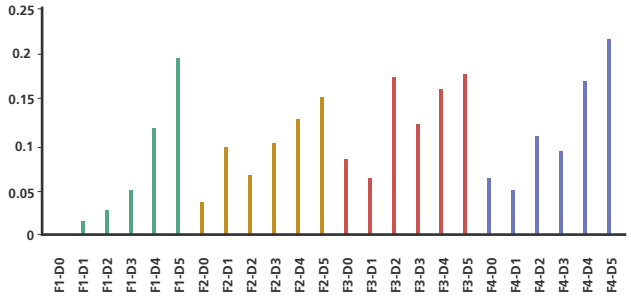
Figure 1. Overlaid MRM chromatograms of 95 compounds in F1-D3 samples

Changes in carbohydrate, amino acid, nucleoside, and vitamin compounds under four differing cell culture conditions

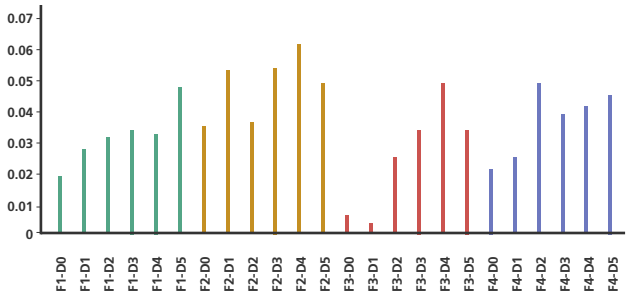
The following bar charts were all plotted with culture time as the abscissa and the peak area ratio of the target compound to the internal standard compound as the ordinate.

Cell Culture Supernatant Component Analysis

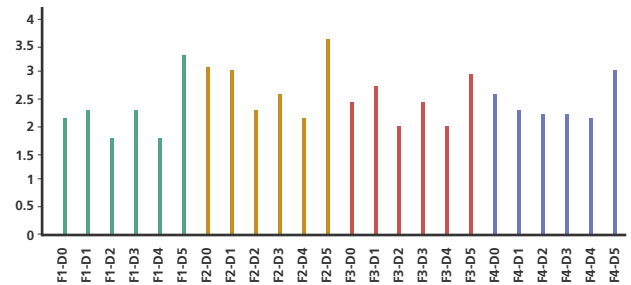
Threonic Acid



Gluconic Acid



Hexose (Glucose)



Carbohydrate (Figure 2)

Glucose concentrations varied little between culture processes and time points. In comparison, gluconic and threonic acids were present at relatively low levels, and threonic acid in the F1 culture process exhibited the most significant increase with culture time.

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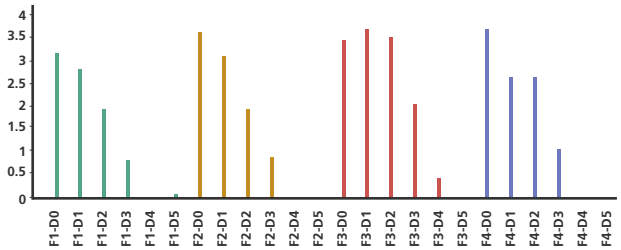
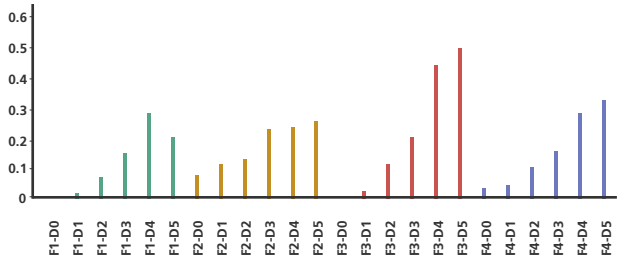
Cell Culture Supernatant Component Analysis

Amino acids (Figure 3)

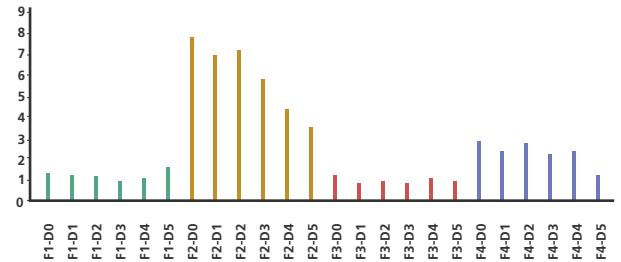
In all four cell culture processes, glutamine was consumed in large quantities and was almost exhausted by Day 4 of culture. Thus, supplementation with this amino acid would likely impact the culture outcome. In contrast, relative concentrations of alanine exhibited an inverse trend to glutamine, suggesting that alanine was the by-product or end-product of glutamine metabolism. Ornithine concentrations varied only slightly with time in all culture processes, but were specifically greater under the F2 process, suggesting that ornithine might be the key factor reflecting the process condition.



Glutamine



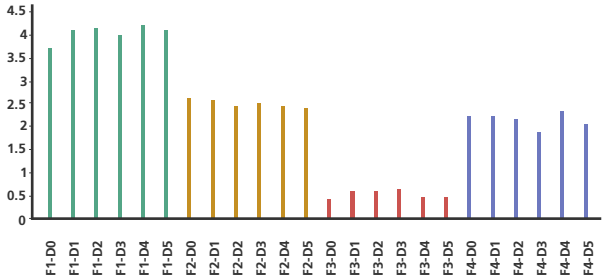
Alanine



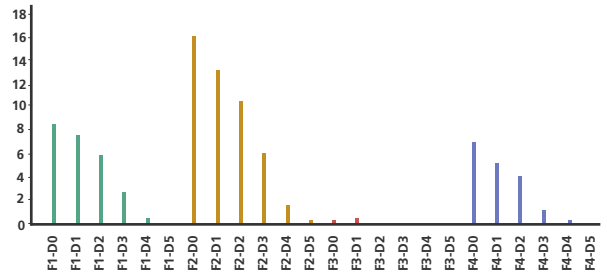
Ornithine

Cell Culture Supernatant Component Analysis

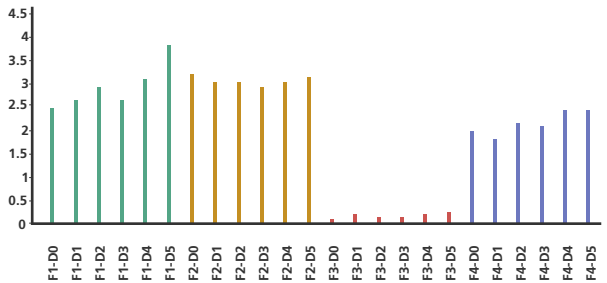
Hexose (Glucose)



Gluconic Acid



Threonic Acid



Nucleoside compounds (Figure 4)

In all four different culture processes, nucleoside concentrations increased over time, even though the medium did not contain these four nucleoside compounds at the beginning.

LCMS-8050

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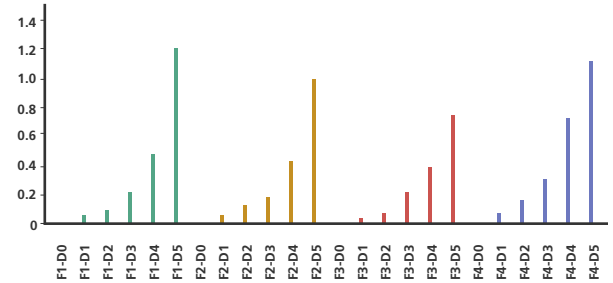
Cell Culture Supernatant Component Analysis

Vitamin compounds
(Figure 5)

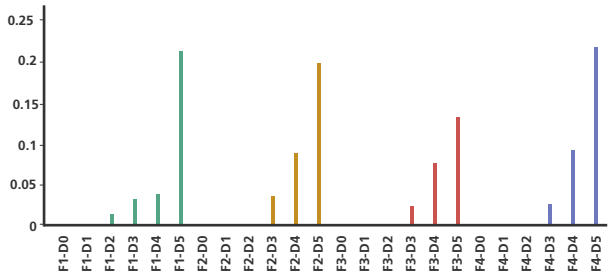
In the four different culture processes, relative concentrations of biotin and folic acid were maintained over the 5 days of culture, yet relative concentrations of vitamin B6 (pyridoxine) decreased with culture time. Hence, vitamin B6 concentrations could be optimized in favor of meeting the target requirements of final qualities and yields.

4. Conclusion

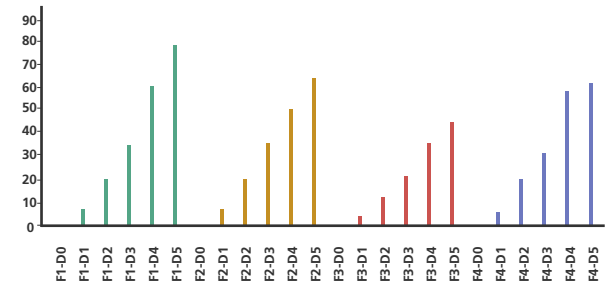
The relative abundance of 95 compounds in four culture processes were rapidly analyzed using the cell culture profiling method package and the LCMS-8050 triple quadrupole mass spectrometer. These experiments showed changes of some carbohydrates, amino acids, nucleosides, and vitamins with time in the four cell culture processes. To accelerate process optimization, the key factors that might be affecting yields and qualities of biological products were identified. This method could be used as a reference for the selection and optimization of cell culture processes.



Glutamine



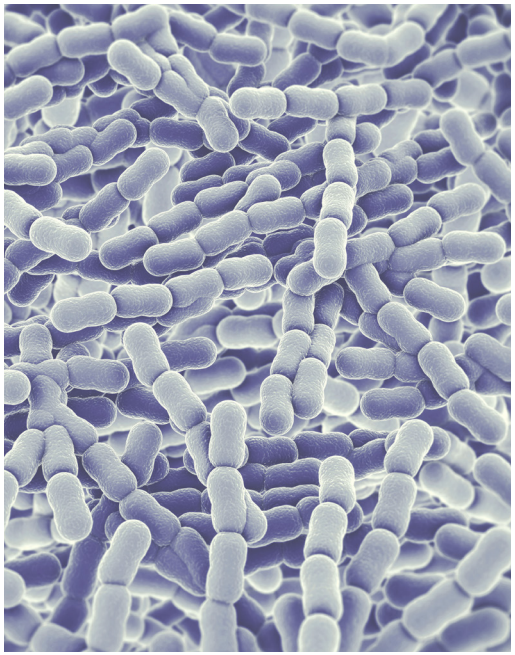
Alanine



Ornithine

4.1 Determination of saccharide units of pneumococcal polysaccharide vaccine using LC-MS/MS

There are 23 serotypes present in the pneumococcal polysaccharide vaccine (PPSV-23), and the quality control of PPSV-23 requires determination of relative contents of saccharide units in each serotype. The analytical method recommended by the European Pharmacopoeia may not be the most efficient due to poor analytical throughput and vulnerability to matrix interference. To address this limitation, we developed the LC-MS/MS method for simultaneously determining uronic acid, hexosamine, hexose, and methylpentose contents in PPSVs that tolerates matrix interference and deliver results at the throughput of 10 minutes per analysis. The method demonstrated high sensitivity and showed excellent quantitative performance as evaluated by linearity, precision, and accuracy. Herein, we demonstrate the use of this method to determine saccharide units in the 23 serotypes of PPSV-23 and compared our measurements of hexosamine with the reference method of the European Pharmacopoeia.



Keywords:

liquid chromatography mass spectrometry (LC-MS/MS); pneumococcal polysaccharide vaccine (PPSV); quality control; methylpentose; hexose; hexosamine; uronic acid.

Vaccine Active Ingredient Testing

1. Introduction

The development of pneumococcal polysaccharide vaccine (PPSV) in 1983 was a milestone in the history of medicine, which has contributed to the decline in the mortality rate of pneumonia. PPSV is now a mixture of 23 serotypes (PPSV-23) and is mainly used to immunize children of over 2 years-of-age and the elderly.

Relative contents of saccharide units are key quality control criteria for PPSV-23. In the European Pharmacopoeia (EP), hexose, hexosamine, uronic acid, and methylpentose contents in each serotype PPSV are tested after hydrolyzing into individual monosaccharide units that consist of isomeric monosaccharide species as displayed in Figure 1. Detection of monosaccharides by UV spectrophotometry further requires chemical derivatization and purification. These processes are time consuming and unreliable as the efficiency of chemical derivatization is affected by matrix composition.

Alternative detectors that can detect underivatized monosaccharides, such as differential refraction detectors (RI), evaporative light-scattering detectors, and charged aerosol detectors, are not selective and fail to demonstrate accuracy in the presence of vaccine matrices. Moreover, whereas ion chromatography-electro-chemical detectors (IC-ECD) have improved the selectivity of detection without derivatization, their quantitative accuracies are in considerable trade-off for analytical throughput.

This study was aimed at overcoming these drawbacks by developing a method using the LC-MS/MS with high selectivity, high throughput and robustness. The developed method for simultaneous determinations of four saccharide units (uronic acid, hexosamine, hexose, and methylpentose) in PPSV-23 was validated and evaluated against the results given by the EP reference method.

Vaccine Active Ingredient Testing

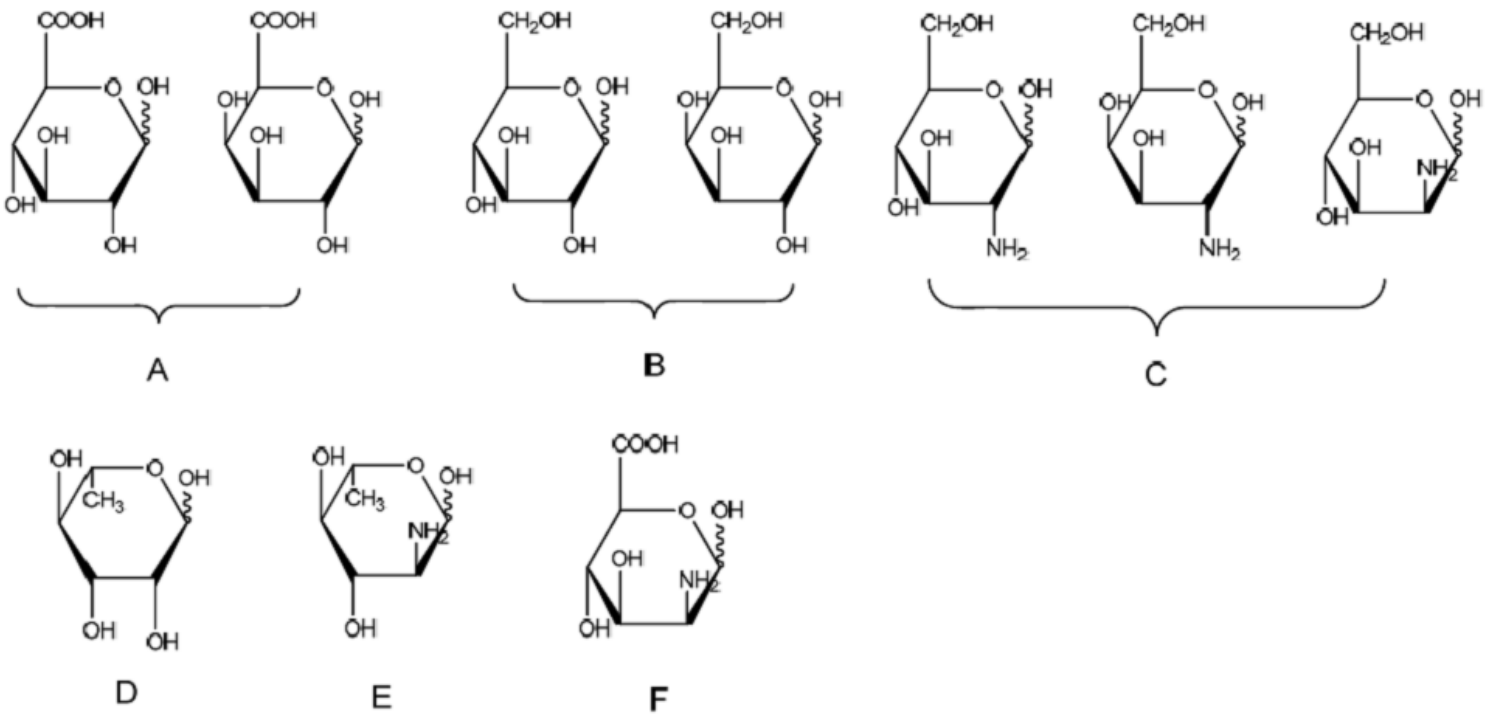


Figure 1. Common saccharide unit types in PPSV hydolysates; compounds A-F are uronic acid, hexose, hexosamine, methylpentose, methyl-pentosamine, and 5-amino-2 carboxyhexose, respectively.

2. Experimental

In accordance with the EP method for hexosamine determination, PPSV samples were hydrolyzed for 1 hour in 8 M hydrochloric acid at 100 C and diluted 2,000 times with water prior to LC-MS/MS injection. Hydrolysis condition was the same for all serotypes except for serotypes 19A and 19F, which were hydrolyzed for 2 hours in 10 M hydrochloric acid at 100 C. The LC-MS/MS conditions were optimized, and samples were ran under conditions summarized in Table 1. The reference data for comparison were obtained by derivatizing the hydrolyzed samples before they were measured by UV/Vis spectrophotometer, as prescribed in the EP method.

This study was aimed at overcoming these drawbacks by developing a method using the LC-MS/MS with high selectivity, high throughput and robustness. The developed method for simultaneous determinations of four saccharide units (uronic acid, hexosamine, hexose, and methylpentose)

Table 1. LC-MS/MS Conditions

Nexera XR UHPLC			
Pump System	:	LC-20ADXR binary gradient system	
Analytical Column	:	HILIC column, 2.1 x 150 mm, 5 µm	
Mobile Phase	:	Composed of ultrapure water, ammonium formate and acetonitrile	
LC Program	:	6 min gradient, 10 min runtime, 0.3 mL/min flow rate	
Injection Volume	:	10 µL	
LCMS-8045			
Ionization Source	:	Heated ESI	
Acquisition Mode	:	Multiple Reaction Monitoring (MRM)	
Uronic acid [M-H]$^-$	:	192.9 > 113.0	192.9 > 103.1
Hexose [M+HCOO]$^-$	:	225.3 > 89.0	225.3 > 59.1
Methylpentose [M+HCOO]$^-$	:	209.3 > 89.1	209.3 > 59.0
Hexosamine [M+H]$^+$	:	180.2 > 162.1	180.2 > 84.2
Loop Time	:	0.23 sec	

3. Results

LC-MS/MS Method Development

Given the presence of isomeric species within uronic acid, hexose and hexosamine, we first developed the quantitative methods using the authentic standards for each isomer, as shown in Table 2. It was found that the elution patterns on this HILIC chromatography were identical between isomers, and the same MRM parameters could be used to detect the isomers. Moreover, the calibration curves of isomers resulted in near-identical regression equations with MRM responses varying by no more than 10%. These findings provided the basis to assume that a calibration curve for one isomer can represent all isomers and that the result of quantitation would equal the sum of all isomers present. Thus, in this investigation, we used the glucuronic acid standard for quantitating the total uronic acid, and likewise used glucose, glucosamine and rhamnose to quantitate the total hexose, hexosamine and methylpentose, respectively. This approach effectively reduced the number of authentic standards required for the present purpose, while also mitigating the cumbersome data processing for giving the summed quantity of individual isomers.

Table 2. Comparison of calibration curves acquired for the isomers of uronic acid (A), hexose (B), hexosamine (C), and methylpentose (D)

Compound	Calibration curve [a]	R^2
A. Glucuronic acid	$A = -11.2C^2 + 16686C + 51493$	0.9999
A. Galacturonic acid	$A = -12.1C^2 + 15029C + 10597$	0.9996
B. Glucose	$A = 2229.4C + 489.4$	0.9999
B. Galactose	$A = 2198.9C + 376.5$	0.9994
C. Glucosamine	$A = 6634.0C + 8907.8$	0.9998
C. Galactosamine	$A = 6237.9C + 3118.9$	0.9996
C. Mannosamine	$A + 6214.5C + 1663.1$	0.9998
D.Rhamnose	$A = 4287.7C + 2102$	0.9996

[a] A, Peak area; C, Concentration (ng/mL)

Vaccine Active Ingredient Testing

As shown in Figure 2, the method successfully separated the four monosaccharide types within 6 minutes of HILIC chromatography and selectively detected each by MRM mass spectrometry. Moreover, when applied to unhydrolyzed PPSV-23, no target peaks were found, indicating that there were no degraded monosaccharides present in the original composition.

To determine the degree of sample carryover, we injected high-concentration samples followed by a blank sample (Figure 2C) and did not detect any residual monosaccharides. Method stability is very important for vaccine quality evaluations. Thus, we determined retention times (RSD%) of methylpentose, hexose, hexosamine, and uronic acid and showed that they were 1.16%, 0.34%, 1.46%, and 1.64%, respectively, after more than 3000 injection tests over 3 months. Other metrics of quantitative performance are summarized in Table 3.

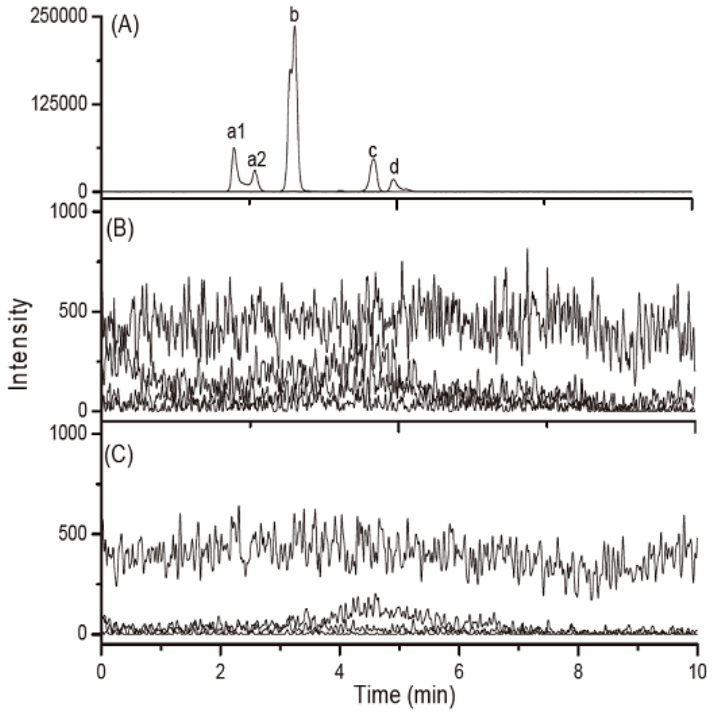


Figure 2. MRM chromatogram of 4 vaccine saccharide units (A); MRM chromatogram of unhydrolyzed PPSV-23 (B); blank sample injection chromatogram after high-concentration sample test (C); a1 and a2, rhamnose; b, hexose; c, uronic acid; d, hexosamine

Table 3. Quantitative performance of uronic acid, hexose, hexosamine, and methylpentose

Saccharide Unit	LOD (ng/mL)	Quantitative Range (mg/mL)	Precision	Accuracy
Uronic Acid	0.98 (S/N=5.6)	1.56 - 25 ng/mL	1.14%	92.9 - 104.2 %
Hexose	3.32 (S/N = 5.6)	13.3 - 425 ng/mL	1.67%	97.6 - 102.8%
Hexosamine	3.96 (S/N = 5.1)	3.15 - 50.0 ng/mL	0.98%	97.2 - 101.3%
Methylpentose	8.20 (S/N = 5.2)	8.2 - 131.3 ng/mL	0.80%	98.3 - 102.6 %

[b] Defined as signal to noise ratio (S/N) greater than 3.

[c] At least five concentration levels were selected to cover each of the shown ranges.

[d] Evaluated by making six repeat measurements for 25 ng/mL standard.

[e] Accuracy % = $\frac{\text{Calculated C}}{\text{True C}}$ x 100%, calculated for all calibration points

Vaccine Active Ingredient Testing

The developed LC-MS/MS method was used to determine the concentrations of four types of monosaccharide in hydrolysates of the 23 serotypes of PPSV-23. To illustrate monosaccharide detection in real samples, MRM chromatograms acquired for serotypes 5 and 7F have been shown in Figure 3. The polysaccharide structure antigen of serotype 5 comprises glucose, glucuronic acid, N-acetyl-L-fucosamine, 2-acetamido-2,6-dideoxy-l-talose and 2-acetamido-2,6-dideoxy-d-xylo-hexos-4-ulose. The latter three structures contain the N-acetyl group and all hydrolyze to methylpentosamine during sample preparation, which is not targeted in this analysis. Thus, the analysis of serotype 5 resulted in the detection of hexose and uronic acid. Similarly, the polysaccharide structure of serotype 7F is known to contain galactose, glucose, rhamnose, N-acetylgalactosamine, and N-acetylglucosamine, which hydrolyze to generate a mixture of hexose, methylpentose and hexosamine as observed in the MRM chromatogram.

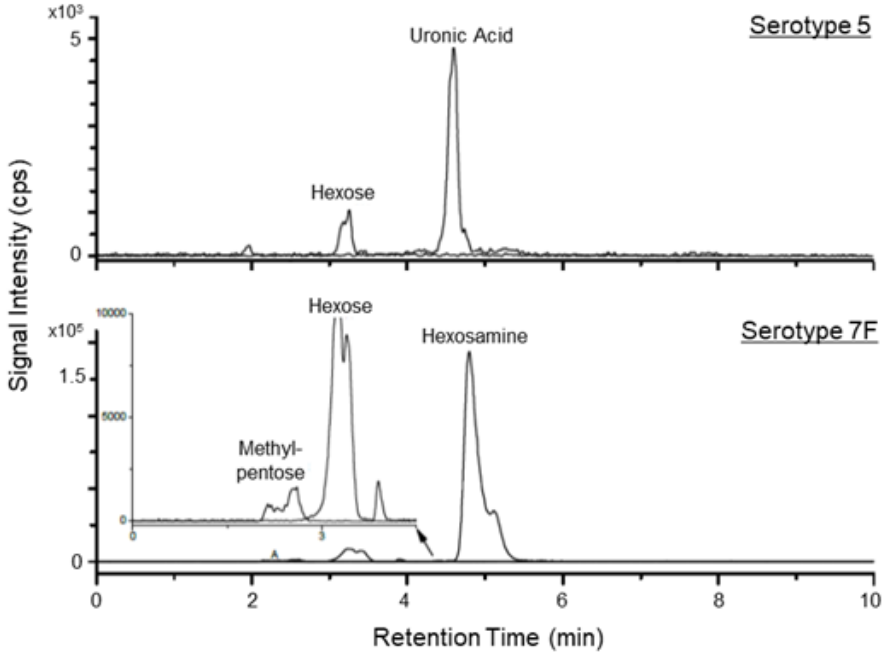


Figure 3. LC-MS/MS analysis of hydrolysis products of serotypes 5 (Upper) and 7F (Lower).

Comparison of LC-MS/MS results with the EP method

Table 4 summarizes all results acquired by LC-MS/MS with the results of hexosamine quantitation by the EP method. All results were in agreement with the expected compositions of the hydrolysates without false positive and false negative detection. However, discrepancy was observed for serotypes 5 and 7F with regard to hexosamine contents measured by LC-MS/MS and the EP method.

The false positive result observed for serotype 5 was likely attributed to the presence of methylpentosamine residues as the derivatizing agent employed in the EP method reacts with all amino sugars. Indeed, using Shimadzu LCMS-IT-TOF™ for acquiring MSn spectra at high mass measurement accuracy, we confirmed in a separate experiment that methylpentosamine was present in the hydrolysate at m/z 164.0996 ([M+H]+).

The other discrepancy observed was for serotype 7F, where the EP method failed to detect hexosamine which was detected by LC-MS/MS and was expected to be detected according to the structure of type 7F antigen. The reason why the EP method failed was unclear, but we speculate that the vaccine matrix interfered with the derivatization step. Consequently, this result demonstrated the robustness of derivatization-free methodology.

Although we could not include methylpentosamine and other putative sacchrides as the target compound due to unavailability of authentic standard, our LC-MS/MS method should, in principle, be capable of simultaneous measurement of additional monosaccharide units comprising the polysaccharides of PPSV-23 antigen.

Vaccine Active Ingredient Testing

Table 4. Concentrations of monosaccharides in each serotype determined by LC-MS/MS (hexose, uronic acid, methylpentose and hexosamine) and the EP method (hexosamine)

LC-MS/MS					EP Method
Serotype	**Hexose**	**Uronic Acid**	**Methylpentose**	**Hexosamine**	**Hexosamine**
1	-	122.5	-	-	-
2	1255.6	388.1	1973.1	-	-
3	755.6	488.8	-	-	-
4	-	-	-	642.1	465.0
5	152.0	47.5	-	-	606.0
6A	1457.9	-	680.4	-	-
7F	1103.8	-	561.0	958.7	-
8	2095.0	366.7	-	-	-
9N	1203.3	314.4	-	4069.6	1566.0
9V	797.8	214.1	-	1279.2	432.0
10A	1876.6	-	-	658.9	480.0
11A	2524.9	-	-	-	-
12F	1392.4	-	-	692.0	1111.5
14	1655.7	-	-	659.2	699.0
15B	2147.0	-	-	734.6	592.5
17F	463.4	-	519.5	-	-
18C	973.7	-	313.2	-	-
19A	235.2	-	20.6	703.8	646.8
19F	208.5	-	30.3	926.1	742.0
20	2255.4	-	-	596.1	651.0
22F	2057.0	334.6	1089.1	-	-
23F	1635.6	-	1263.4	-	-
33F	3602.7	-	-	-	-

N.E. all concentrations are given in ng/mL
"-" indicates no detection.

5. Conclusion

The present LC-MS/MS method can be used to simultaneously determine the concentrations of the four monosaccharide units in hydrolysates of PPSV-23. This analysis is superior in many respects to the conventional EP method prescribed for quality control analysis of PPSV-23 products. The LC-MS/MS method demonstrated high sensitivity (LOD of 0.98 ng/mL for uronic acid), good precision (peak area RSD% less than 2%), high quantitative accuracy and short analysis time. Most importantly, the method uses no derivatization, making the total process much simpler and more robust, as demonstrated in the comparison experiment where LC-MS/MS circumvented the challenges previously faced by the EP method.

Note: This method has been submitted for a Chinese patent in cooperation with vaccine enterprises.

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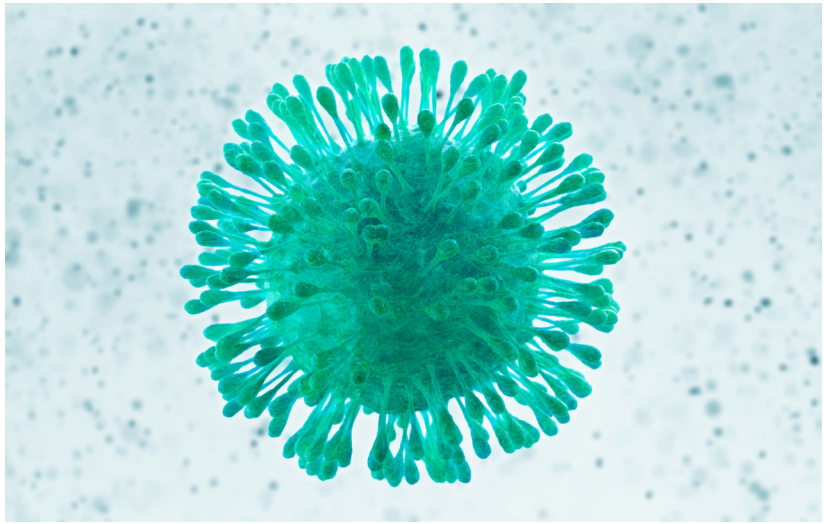
4.2 Rapid Measurement of Viral Protein Vaccine Subunits using MALDI-TOF MS

In this paper, Shimadzu matrix-assisted laser desorption ionization time-of-flight mass spectrometer (MALDI-TOF MS) was used to directly detect subunit components of a vaccine protein. Structural integrity of vaccine products was deduced by elucidating the molecular weights and abundance ratio of each subunit. As a result, the subunit composition observed in the standard sample reproduced well in the vaccine sample, indicating that the vaccine protein kept good subunit integrity in formulation. This appeared to correlate with the immunogenic activity of the vaccine product. Thus, we demonstrated that this rapid and simple method could provide a powerful reference for analyses of vaccine subunit compositions without sample pretreatments.

Keywords:

Matrix-assisted laser desorption ionization time-of-flight mass spectrometer (MALDI-TOF); vaccine; subunit



Vaccine Active Ingredient Testing

1. Introduction

Amongst various types of vaccines, protein vaccines are the most widely available and have the highest vaccination rates. Viral proteins contained in a vaccine formulation must retain the same three-dimensional structure as the original antigens for immunization to be effective against real viral infections. It is commonly known that viral proteins have complex quaternary structures generated by multiple protein subunits assembling in pre-determined conformations and stoichiometry. Therefore, the contents of vaccine protein subunits are an important quality criterion of vaccine formulation. To analyze protein subunits in vaccine formulations, we employed matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS), which is an established technique suitable for quick analysis of proteins and other macromolecules.

2. Experimental

Standard and vaccine samples were applied to MALDI plate by the "sandwich method". The matrix solution (10 mg/mL sinapinic acid) and the sample solution (20 mg/mL protein) were applied in the matrix-sample-matrix sequence with drying up in between, thereby sandwiching the sample with layers of matrix. AXIMA-Performance™ was used to measure the standard sample in the Reflectron positive mode and MALDI-8020 was used to measure the vaccine sample in the Linear positive mode.

Vaccine Active Ingredient Testing

3. Results

Measurement of a pure protein sample

MALDI-TOF MS analysis was performed first on the standard sample of a pure viral protein that consisted of five types of subunits, denoted A-E. Peaks detected have been shown in figures 1-3. It was found that subunits A and B differed by approximately 84 Da and their signal intensities were in 2:1 ratio, indicating subunit stoichiometry. Subunits C and D were found to differ by approximately 47 Da and were found in 1:1 ratio. Subunit E was found to have molecular weight around 26,226 Da. These findings agreed with theoretical calculations

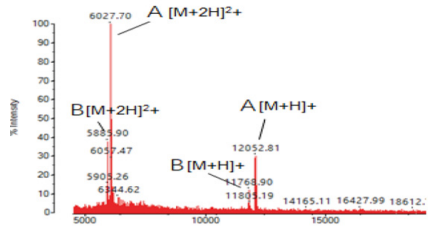


Figure 1. Mass spectrum of the standard sample, magnified for subunits A and B

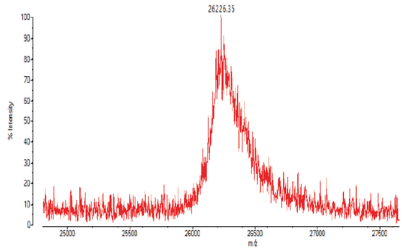


Figure 2. Mass Spectrum of the standard sample, magnified for subunits C and D

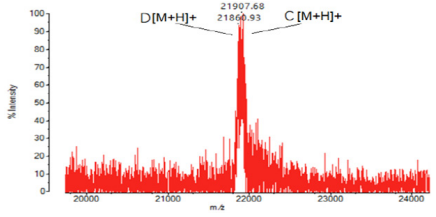


Figure 3. Mass spectrum of the standard sample, magnified for subunit E.

Vaccine Active Ingredient Testing

Measurement of a vaccine formulation

Next, MALDI-TOF MS analysis was performed on a sample of real vaccine formulation. As shown in Figures 4-6, all subunits were detected at the same signal intensity ratios as observed in the standard sample.

Standard sample of a pure viral protein that consisted of five types of subunits, denoted A-E. Peaks detected have been shown in figures 1-

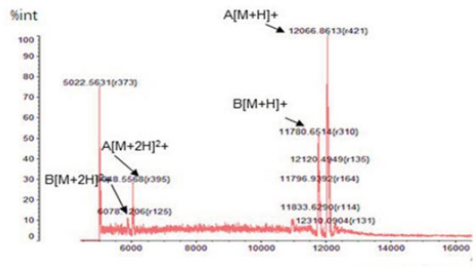


Figure 4. Mass spectrum of the vaccine sample, magnified for subunits A and B

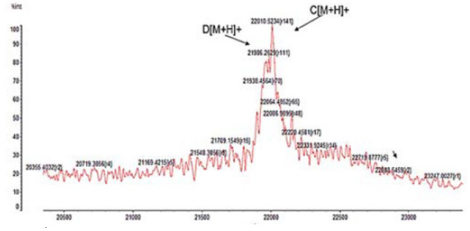


Figure 5. Mass spectrum of the vaccine sample, magnified for subunits C and D

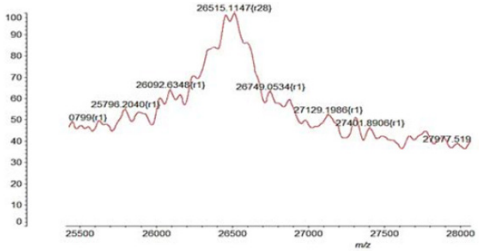


Figure 6. Mass spectrum of the vaccine sample, magnified for subunit E

4. Conclusion

In this paper, we demonstrate the use of a rapid MALDI-TOF based detection method for vaccine subunits. We detected all protein vaccine subunits simultaneously in a simple procedure that only takes minutes, rather than hours. The data shown herein indicate a powerful analytical method for rapid identification of vaccines and protein drugs.

4.3 Determination of Aluminium Contents in Vaccine Adjuvants using ICP-OES and ICP-MS

Aluminum is widely used as a vaccine adjuvant, despite the known health risks. The Chinese Pharmacopoeia (the third part of the 2015 edition) stipulates the range limits for aluminum in vaccines with aluminum adjuvants. Thus, we determined aluminum contents in adjuvants of adsorbed tetanus and DPT samples dissolved in nitric acid using ICP-OES and ICP-MS. A linear correlation coefficient of 0.9999 was observed in the range of 0–10 mg/L. Detection limits were 0.90 (ICP-OES) and 0.17 mg/L (ICP-MS), and spike recovery was 107%-116%. This method can be used for the rapid determination of aluminum contents in the aluminum vaccine adjuvants.

Keywords:
ICP-OES; ICP-MS; vaccine; aluminum adjuvant; aluminum

Vaccine Active Ingredient Testing

1. Introduction

Aluminum salts are contained in many vaccine formulations as adjuvant to allow antigenic proteins to be adsorbed and thereby sufficiently stimulate the host immune response. Due to known health risks of aluminum adjuvants, regulatory authorities require determinations of aluminum contents in vaccine formulations and stipulate limits of aluminum concentrations. In China, the model analytical method for aluminum detection has been set forth by General Principles 3106 (GP 3106). In these procedures, excess EDTA-2Na is used to react with aluminum ions, and the remaining EDTA-2Na is titrated with zinc titrant to calculate aluminum contents. However, these procedures are laborious and more efficient methods are warranted. Thus, we analyzed aluminum contents of aluminum adjuvants in samples dissolved in nitric acid using ICP-OES and ICP-MS. We report a simple and rapid method for detecting aluminum contents in vaccine aluminum adjuvants.

2. Experimental

Aluminum adjuvants in vaccines are mainly present in the form of colloids and 100 µL of samples were directly analyzed using instruments after acid dissolution and dilution to 10 mL. Standard solutions were diluted in 2% nitric acid to standard concentrations of 0, 1, 2, 5, and 10 mg/L. Method detection limits (MDLs) were calculated following 7 successive injections of blank solution. The analytical conditions for ICP-OES and ICP-MS have been summarized in Table 1 and 2, respectively.

Table 1. ICP-OES analysis conditions

Instrument Parameters	Set Value	Instrument Parameters	Set Value
High-Frequency Power	1.20 kW	Plasma Gas Flow Rate	10.0 L/min
Auxillary Gas Flow Rate	0.60 L/min	Carrier Gas Flow Rate	0.70 L/min
Torch type	Mini Torch	Nebulizer Type	Coaxial
Chamber	Cyclonic	High Frequency	27.12 MHz

Table 4. ICP-MS analysis conditions

Instrument Parameters	Set Value	Instrument Parameters	Set Value
High-Frequency Power	1.20 kW	Plasma Gas Flow Rate	8.0 L/min
Auxillary Gas Flow Rate	1.10 L/min	Carrier Gas Flow Rate	0.70 L/min
Torch type	Mini Torch	Nebulizer Type	Coaxial
Chamber	Cyclonic	Chamber Temperature	5°C
Sampling Depth	5.0 mm	High Frequency	27.12 MHz

3. Results

Analysis by ICP-OES

The results of standard sample analysis revealed that the MDL of ICP-OES for aluminum was 0.90 mg/L. The calibration curve (Figure 1) was perfectly linear for 0.90–10 mg/L range. Using this method, two types of commercially available adsorbed vaccines using aluminum adjuvants, namely Tetanus vaccine and Acellular Pertussis-Diphtheria-Tetanus (APDT) vaccine, were analyzed (Figure 2). Determined aluminum concentrations and the corresponding Al(OH)3 contents have been summarized in Table 2. The results demonstrated that the aluminum hydroxide contents in two batches of Tetanus and APDT vaccines were within the limits stipulated in the Chinese Pharmacopoeia. Spike recovery test results are shown in Table 3 and recovery rates of 113%-116% were achieved.

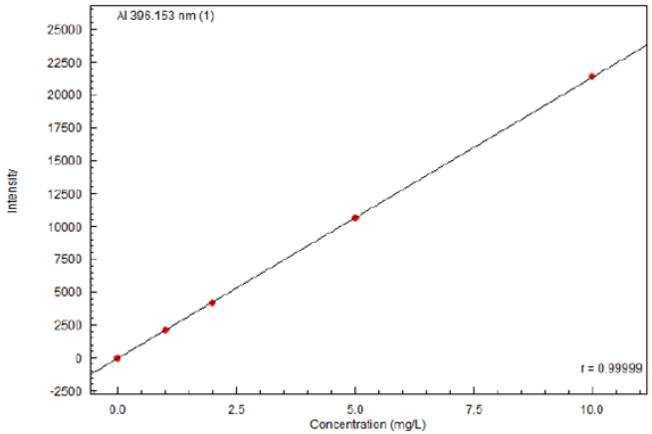


Figure 1. Calibration curve of aluminium standard using ICP-OES

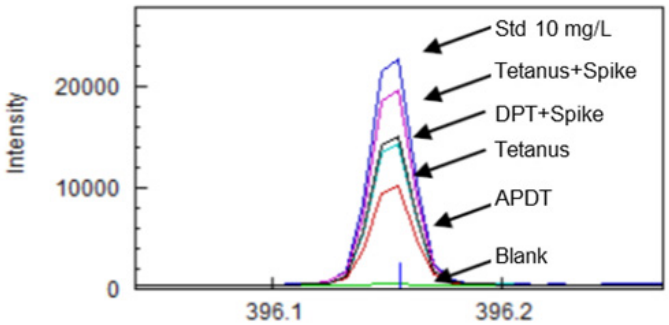


Figure 2. Spectral profiles of aluminium (396.153 nm) in various samples

Vaccine Active Ingredient Testing

Table 2. Determinations of vaccine aluminium adjuvant (ICP-OES)

Sample	Measured Al conc. (mg/L)	Calculated $Al(OH)_3$ conc. (mg/mL)	Mean	RSD%	Regulatory limit of $Al(OH)_3$ (mg/mL)
Tetanus, Batch 1	6.28	1.83	1.86	1.64	< 3.0
Tetanus, Batch 2	6.49	1.89			
APDT, Batch 1	4.37	1.27	1.25	1.86	1.0 - 1.5
APDT, Batch 2	4.21	1.23			

Table 3. Sample spike test results (ICP-OES)

Sample	Sample Result (mg/L)	Spiked Level (mg/L)	Spike Test Result (mg/L)	Recovery %
Tetanus	6.39	2.0	8.65	113
APDT	4.29	2.0	6.61	116

Vaccine Active Ingredient Testing

Analysis by ICP-MS

Measurement by ICP-MS demonstrated superior sensitivity than ICP-OES with MDL 0.17 mg/L. The analysis of same vaccine samples resulted in equivalent concentrations, showing reliability of both methods (Figures 3-4, Table 4). The recovery test results were within the accepted range (Table 5).

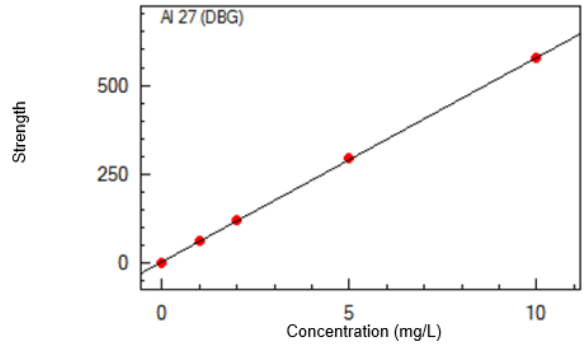


Figure 3. Calibration curve of aluminum standard using ICP-MS

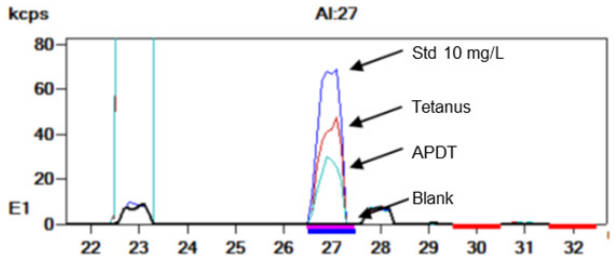


Figure 4. ICP-MS spectrum showing Al peaks in various samples

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Table 4. Determinations of vaccine aluminium adjuvant (ICP-MS)

Sample	Measured Al conc. (mg/L)	Calculated $Al(OH)_3$ conc. (mg/mL)	Mean	RSD%	Regulatory limit of $Al(OH)_3$ (mg/mL)
Tetanus, Batch 1	6.44	1.88	1.86	1.10	< 3.0
Tetanus, Batch 2	6.30	1.84			
APDT, Batch 1	4.29	1.25	1.27	1.83	1.0 - 1.5
APDT, Batch 2	4.45	1.30			

Table 5. Sample spike test results (ICP-MS)

Sample	Sample Result (mg/L)	Spiked Level (mg/L)	Spike Test Result (mg/L)	Recovery %
Tetanus	6.37	2.0	8.50	107
APDT	4.37	2.0	6.68	116

4. Conclusion

Aluminum contents in aluminum vaccine adjuvants were determined using the Shimadzu ICPE-9820 full-spectrum direct-reading ICP-OES and an ICPMS-2030 inductively coupled plasma source mass spectrometer. With characteristics of high sensitivity, satisfactory precision, fast analysis, and simple operation, this method meets the requirements of aluminum determinations for aluminum vaccine adjuvant.

Testing for other Substances in Vaccines

5.1 Simultaneous detection of vaccine inactivator sodium deoxycholate and precipitant cetyl trimethyl ammonium bromide residues using LC-MS/MS

In this study, we developed a LC-MS/MS method for rapid determination of sodium deoxycholate (DOC) and cetyl trimethyl ammonium bromide (CTAB) residues in PPSV for assuring safety of the vaccine formulation. The method demonstrated a linear range within 1.56–50 μg/L, good repeatability (< 2%) and good recovery (90%–103%) for CTAB. After systematic investigation, high-sensitivity analysis was made possible by reducing carryover through extensive washing of the LC autosampler injector needle.

Key words:

LC-MS/MS; PPSV; cetyl trimethyl ammonium bromide; sodium deoxycholate

Testing for other Substances in Vaccines

1. Introduction

Safety of vaccines is a big public concern as vaccines are introduced to healthy individuals, and chemical impurities are one of the major causes of unwanted side effects. As such, sodium deoxycholate (DOC) and cetyl trimethyl ammonium bromide (CTAB) are antiseptic surfactants used for inactivating pathogenic bacteria and subsequent purification of immunizing antigen. Both DOC and CTAB are known to persistently remain in the final vaccine formulation, and their contents must be monitored to ensure that residual levels are kept to a minimum.

As discussed in Section 4.1, the 23-valent pneumococcal polysaccharide vaccine (23-PPSV) is a mixture of 23 serotypes produced by extracting the polysaccharide content of each bacterial culture. Their manufacturing process involves the usage of DOC and CTAB, and an example of a pretreatment procedure is shown in Table 1. The European Pharmacopoeia (EP) describes a method for quantitating the DOC content using UV/Vis spectrophotometry, however, there is currently no method specified for the CTAB content as CTAB is not amenable to UV detection. Moreover, the EP method for DOC involves chemical derivatization that is prone to matrix interferences, and an alternative method for accurate and robust quantitation is warranted. This motivated us to develop a LC-MS/MS method that can simultaneously monitor DOC and CTAB without the need for derivatization. Described herein, the method provided reliable and rapid quantitation of DOC and CTAB that can be used for safety evaluation of PPSV and other vaccines.

Table 1. Pretreatment procedure of PPSV serotypes

Step	Procedure
1	Termination of liquid culture using DOC
2	Continuous flow centrifugation and treatment of supernatants with CTAB
3	Continuous flow centrifugation and collection of the precipitated compound polysaccharide
4	Depolymerization of polysaccharides using $CaCl_2$ or NaCl
5	Addition of cold ethanol to 25% and removal of precipitated nucleic acids and impurities
6	Treatment of centrifugal supernatants with 70%-80% cold ethanol and precipitation of crude polysaccharide
7	Centrifugation of crude polysaccharide solution in sodium acetate
8	Addition of two volumes of pre-cooled phenol solution and removal of protein by extraction
9	Absorption of polysaccharide-sodium acetate solution phase and repeated phenol extraction (2-4 times)
10	Addition of 70% - 80% cold ethanol to the final polysaccharide - sodium acetate solution phase
11	Collection of precipitates by centrifugation and dissolution in water for injection
12	Sterilization and filtration with membrane and refinement of the polysaccharide stock solution
13	Cryopreservation at -20°C

Testing for other Substances in Vaccines

2. Results

Quantitative performance

First, reference solutions with various concentrations of DOC were analyzed (Figure 1). The definition of limit of detection (LOD) set forth by the ASTM was adopted in this study, and the concentration to achieve S/N ratio greater than 3 was estimated to be 1.02 µg/L for DOC (S/N:5.6). However, it was not possible to use the S/N ratio for CTAB due to considerable carryover in the system resulting in peak detection in blank samples. It was found that carryover was considered insignificant only at 1.56 g/L or higher concentrations.

Calibration curve was plotted using the external standard method with 5–6 concentrations ranging around that of the test sample (Figure 2). Linear regression equations and correlation coefficients of each component are shown in Table 2. All components had high linearity within their concentration ranges, with R2 greater than 0.999 and accuracy of all calibration points between 95% and 106%.

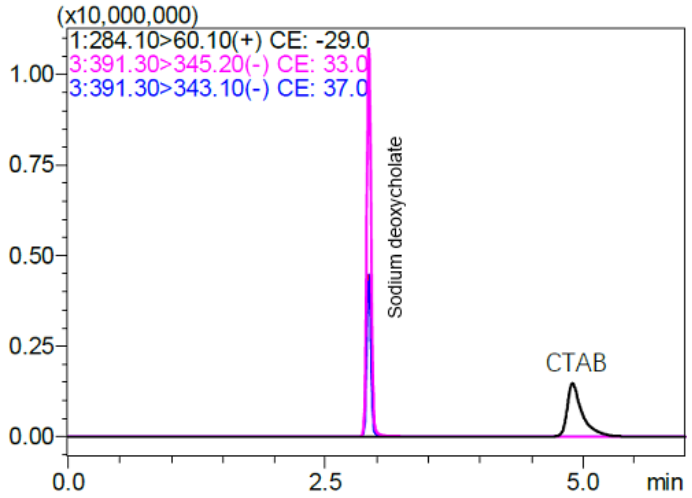

Figure 1. Chromatogram of sodium deoxycholate and CTAB

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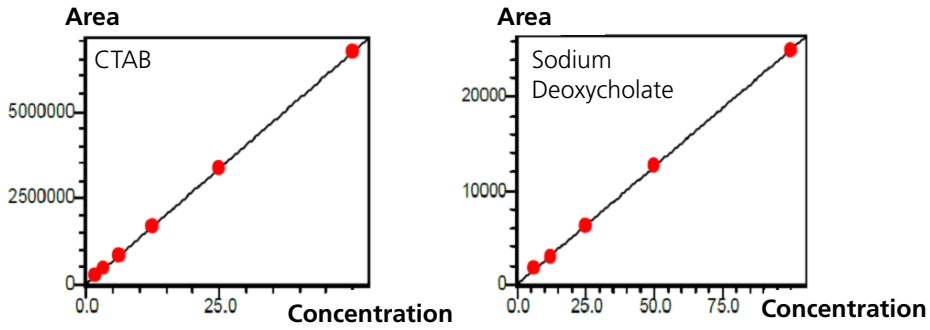

Figure 2. Calibration curve of CTAB and DOC

Table 2. Statistics of the calibration curves of CTAB and DOC

Compound	Linear Range (μg/L)	Curve Equation	R^2	Accuracy %
CTAB	1.56-50	A = 133056C - 29015	0.9999	97.4 - 101.9
DOC	6.25-99	A = 250.5C - 59.1	0.9997	95.4 - 105.6

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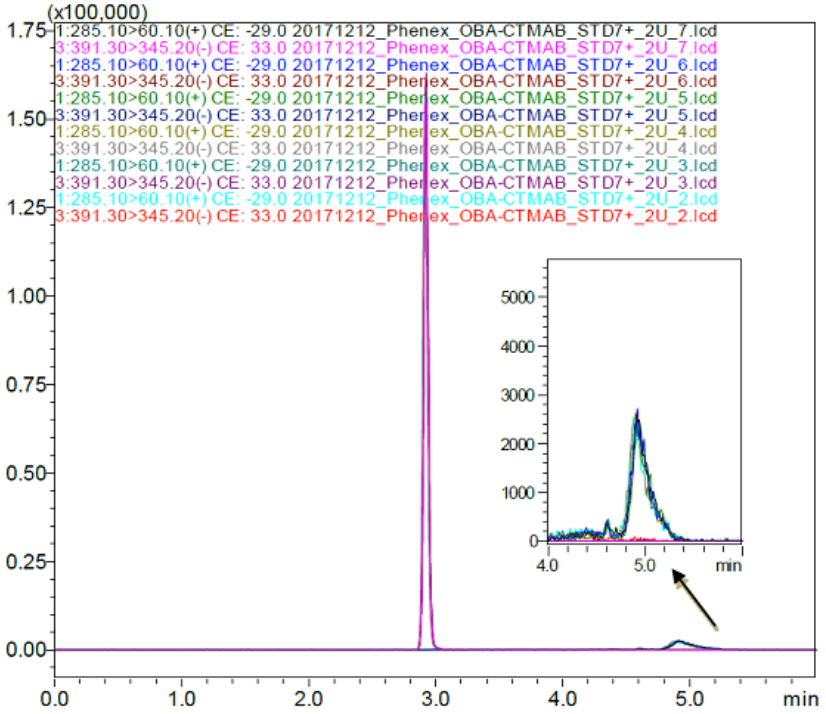

Figure 3. Chromatograms of DOC and CTAB, showing the overlay of six repeat injections

Measurement precision was evaluated by six repeat injections of the standard solution. Relative standard deviation (RSD%) of peak areas were found to be 1.24% and 0.91% for DOC and CTAB, respectively (Figure 3).

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Analysis of Real PPSV-23 Samples

The developed method was used to determine DOC and CTAB residues in each serotype of 23-PPSVs in their final formulations. Chromatograms for serotypes 19A and 23F were shown in Figure 4-5 to exemplify positive detection of both compounds. CTAB was present in most samples at concentrations ranging from 3.11 μg/L to as high as 13.1 mg/L (Table 3). Such high level of residual CTAB would require the manufacturer to assess the risks associated with intravenous injection of CTAB or to take measures to reduce the residue. The recovery rate of CTAB was evaluated for six representative serotypes (acidic, amphoteric and neutral), which resulted in favorable results ranging from 91.8% to 103%.

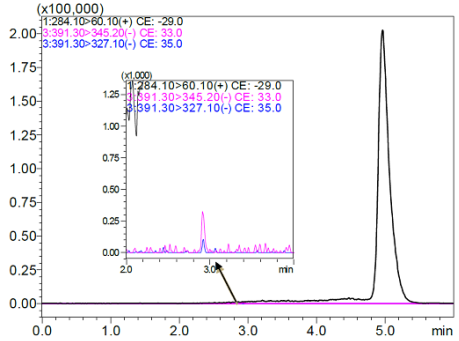

Figure 4. Detection of DOC and CTAB in PPSV serotype 23 Frepeat injections

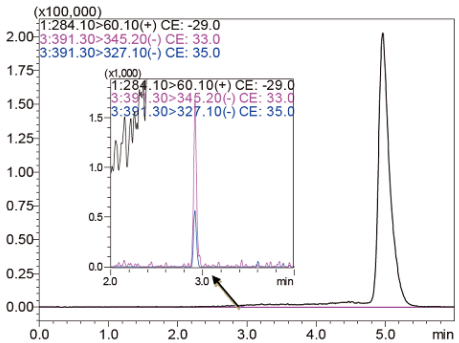

Figure 5. Detection of SDC and CTAB in PPSV serotype 19A

Testing for other Substances in Vaccines

Table 3. Content and recovery determinations of DOC and CTAB in 23-PSVs

Serotype	CTAB (μg/L)	DOC (μg/L)	CTAB recovery
1	120*	High background	103.0%
2	51.8	High background	/
3	13,110*	23.5	91.8%
4	-	-	95.6%
5	788*	-	92.7%
6B	6.18	-	96.3%
7F	16.2	-	/
8	3.76	-	/
9N	-	-	/
9V	-	19.0	/
10A	9.87	-	/
11A	63.9	-	/
12F	56.9	-	/
14	243*	-	/
15B	-	-	/
17F	53.6	-	/
18C	3.91	-	/
19A	431*	29.3	98.2%
19F	3.11	-	/
20	200*	-	/
22F	61.8	16.9	/
23F	32.9	13.4	/
33F	25.0	-	/

*: extrapolated beyond linear range
- : not detected
/ : not teseted

Testing for other Substances in Vaccines

3. Conclusion

In this paper, we presented a rapid LC-MS/MS detection method for simultaneous detection of DOC and CTAB in 23-PPSVs. The results demonstrated highly selective detection of the two components in real samples without interfering peaks and with high recovery rates. Compared prior art by UV/Vis spectrophotometry, the LC-MS/MS method required no derivatization of test compounds and has higher selectivity and resistance to interference.

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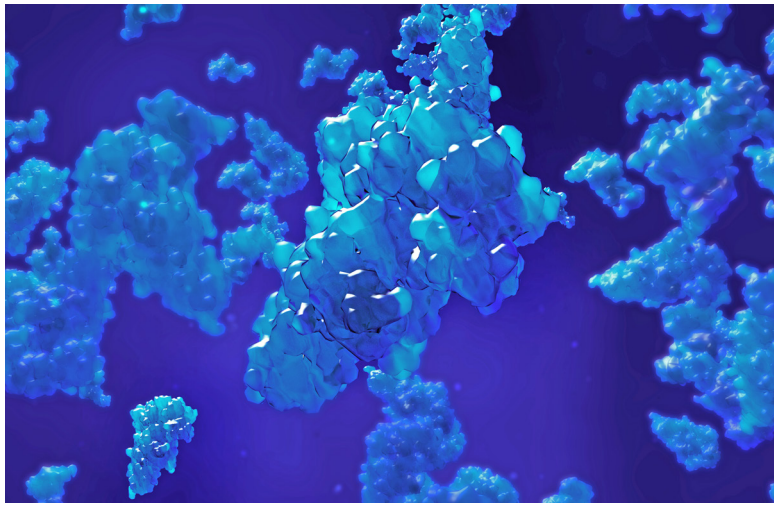
Testing for other Substances in Vaccines

5.2 Application of Aggregates Sizer in the Vaccine Aggregate Evaluation System

In this paper, a Shimadzu Aggregates Sizer was used to determine particle diameters and concentration distributions of vaccine formulation and to investigate the impacts of temperature and ambient pressure on vaccine aggregates. Our analyses showed that particle diameters and concentrations of aggregates were affected by both mechanical stress (stirring) and temperature fluctuation. Hence, the Aggregates Sizer could be used to monitor the vaccine production process and evaluate vaccine efficacy and safety.

Key words:

vaccine; aggregate; Aggregate Sizer



Testing for other Substances in Vaccines

1. Introduction

Aggregates are insoluble, microscopic particulates formed by non-specific and non-covalent multimerization of dissolved components such as proteins. Aggregate formation is typically monitored in pharmaceutical products for safety assurance as aggregates stimulate severe immunological side effects. Whereas the degree of aggregate formation is simply minimized in most pharmaceutical products, vaccines are characteristic in that their medicinal efficacy requires the immunogenic property of aggregates. Hence, more precise monitoring and control of aggregate particle sizes are required.

Particle diameter of vaccine aggregates are usually between 0.2 and 10 µm, and these are referred to as sub visible particles (SVP). Conventional methods of evaluating protein aggregates have not been able to cover the SVP size range in a single measurement, to measure in real time while stress or temperature is applied, to recover samples after measurement, and to perform quantitative measurements. All these were made possible with the Aggregates Sizer biopharmaceutical aggregation analysis system (Figure 1).

In this paper, changes in particle diameters and concentrations of vaccine aggregates were determined using a Shimadzu Aggregates Sizer, and the impacts of temperature and pressure on the vaccine aggregates were determined.

2. Experimental

A fresh vial of commercially available vaccine was sampled and diluted 100-fold with pure water. The batch reactor (Figure 2) was filled with the diluted sample, and particle diameters and concentrations were measured using Shimadzu Aggregates Sizer (SALD-7500nano) equipped with the batch reactor option, which enabled application of physical stirring stress and/or temperature stress and measurement in real time.

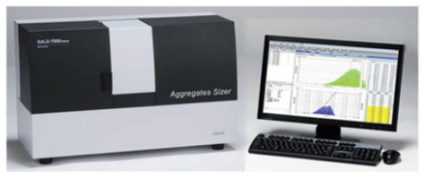


Figure 1. Aggregates Sizer™

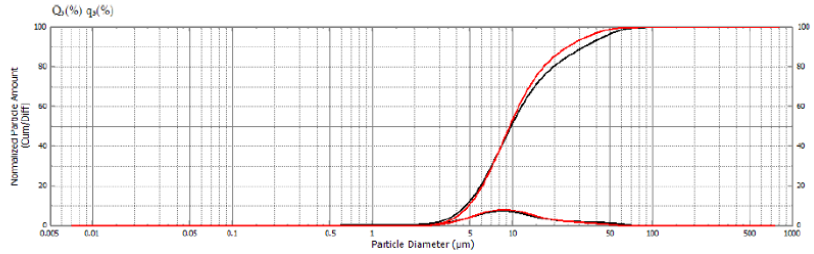
Figure 2. Batch Reactor

Testing for other Substances in Vaccines

3. Results

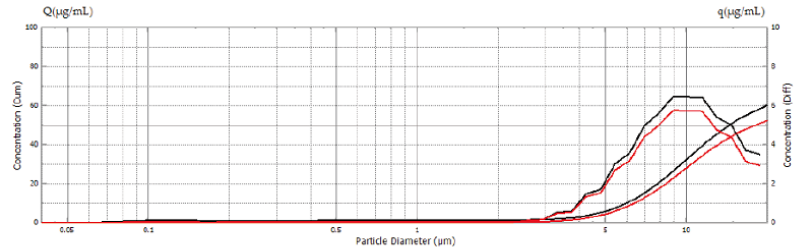
Impact of stirring stress on particle diameter distribution of vaccine

Measurement of aggregate was conducted before and after 1 hour of stirring in the batch reactor. The result shown in Figure 3 was that the 90-percentile diameter reduced from 32.0 to 24.4 μm after stirring, and the overall size distribution range was narrowed. This was a favorable outcome for a vaccine formulation, though the degree of change was not impactful in this experiment. However, determination of concentration revealed a notable loss across all diameter ranges after stirring for 1 hour (Figure 4). Taken together, these observations suggested that stirring does not impact the quality of the vaccine (if not improve) but may affect the recovery of the vaccine probably to container surfaces.



		File Name	Median Diameter	Modal Diameter	Mean Value	Standard Deviation	10.000%D	50.000%D	90.000%D
1	—	搅拌0h...	9.674	8.521	10.595	0.329	4.568	9.674	32.003
2	—	搅拌1h...	9.462	8.521	10.113	0.271	4.814	9.462	24.409

Figure 3. Particle diameter distributions of vaccine aggregates at different stirring times



		File Name	Cell Temp	0.040-0.100 μm	0.200-2.000 μm	0.100-10.000 μm	2.000-10.000 μm	10.000-20.000 μm
1	—	搅拌0h	25.5	0.315	0.496	32.299	31.160	27.767
2	—	搅拌1h	25.9	0.148	0.016	28.117	27.820	24.239

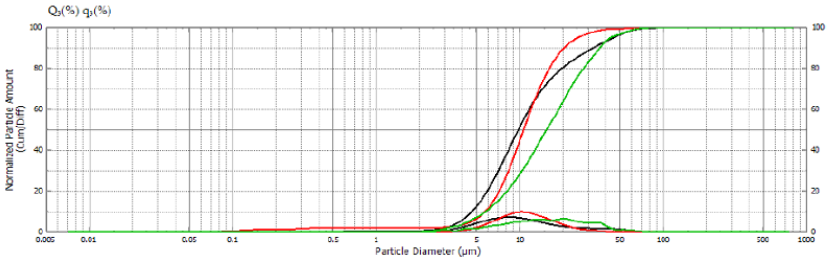
Figure 4. Concentration changes of vaccine aggregates with different mixing time

Testing for other Substances in Vaccines

Impact of temperature stress on particle diameter distribution of vaccine

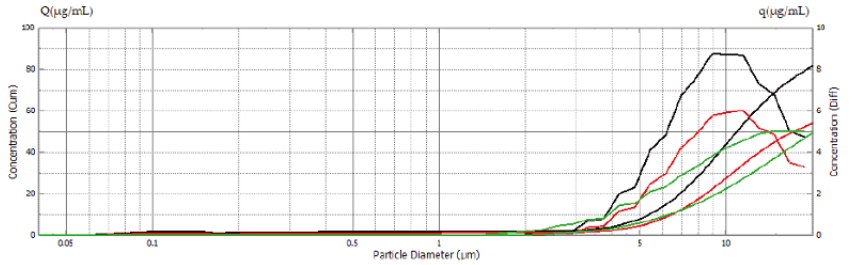
Temperature stress was applied in two ways. One sample was incubated at 35°C in the batch reactor and another sample was thawed from a frozen state and allowed to return to 25°C (freeze-thaw). The results were compared against the control, which was kept at 25°C.

As shown in Figure 5, elevated temperature resulted in increased modal diameter of the SVP from 8.5 to 11.0 μm while the 90-percentile decreased from 32.0 to 20.3 μm. Even though the SVPs improved in the uniformity, overall increase in particulate diameter was considered unfavorable for vaccine efficacy.
The freeze-thaw sample exhibited a greater impact on both modal and 90-percentile diameter of vaccine SVPs than simple temperature elevation. Notably, as shown in Figure 6, the freeze-thaw sample contained less of 0.1-10 μm particles (22.5 μg/mL) than 10-20 μm particles (27.1 μg/mL), indicating that the percentage of effective SVPs decreased considerably through the freeze-thaw process. These results suggest that freeze-thaw should be avoided during vaccine transportation.



	File Name	Median Diameter	Modal Diameter	Mean Value	Standard Deviation	10.000%D	50.000%D	90.000%D
1	T25℃	9.674	8.521	10.595	0.329	4.568	9.674	32.003
2	T35℃	10.548	11.024	9.911	0.348	5.641	10.548	20.297
3	T-20℃~25℃	15.152	20.101	14.674	0.307	5.696	15.152	35.894

Figure 5. Particle diameter distribution of vaccine aggregates at various temperatures

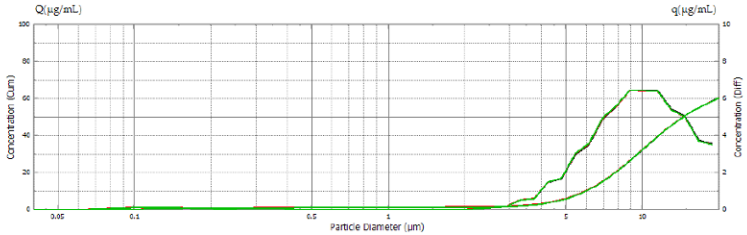


	File Name	Cell Temp	0.040-0.100 μm	0.200-2.000 μm	0.100-10.000 μm	2.000-10.000 μm	10.000-20.000 μm
1	T25℃	25.5	0.428	0.673	43.811	42.266	37.665
2	T35℃	34.9	0.270	0.428	27.501	26.528	26.441
3	T-20℃~25℃	25.3	0.000	0.302	22.526	22.224	27.164

Figure 6. Changes in concentrations of vaccine aggregates at various temperatures

Evaluation of instrument precision

Three repeat analysis was performed on a same sample without mixing to assess the precision of the instrument. As shown in Figure 7, the %RSD of the aggregate concentration in SVP was less than 1% over 3 measurements.



	File Name	0.040-0.100 μm	0.200-2.000 μm	0.100-10.000 μm	2.000-10.000 μm	10.000-20.000 μm
1	疫苗-1	0.342	0.586	32.181	30.902	28.065
2	疫苗-2	0.344	0.581	32.355	31.073	27.742
3	疫苗-4	0.315	0.496	32.299	31.160	27.767

Figure 7. Concentration results of vaccine aggregates (n=3)

4. Conclusion

The Aggregates Sizer allowed precise measurement of the size distribution and size-resolved concentration of vaccine SVPs and enabled quantitative and controlled assessment of quality change caused by stirring, temperature elevation and freeze-thawing. The results demonstrated that freeze-thawing exerted the greatest negative impact on the quality of vaccine formulation. These experiments exemplified the suitability of instrument for quality and safety assessment of vaccine products.

Summary and Outlook

The production of high-quality vaccines requires strict control from production management to strain fermentation and from active substance purification to harmful substance monitoring. This collection provides suggestions for future development of the vaccine industry in terms of data integrity, the optimization and regulation of vaccine production processes, vaccine valid substance detection, and the detection of other substances.

The data integrity section describes data security, audit trails, and data backup and recovery. The data security section describes the principles of user account management, permissions and password setting, and other issues of high concern. The audit trail section describes the frequent concerns of instrument, data and personnel, and setting audit trail management. The backup and recovery section provides graphical displays of multiple backup modes to meet the work requirements of different customers.

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Summary and Outlook

The vaccine production process optimization and regulation section, taking the cell culture supernatant composition for example, explains how to monitor the changes of 95 nutrients, including carbohydrates, amino acids, nucleosides, and vitamin compounds, in the cell culture supernatant during vaccine fermentation using LC-MS/MS, the results of which can provide a reference for detecting differences in quality between vaccines, thus optimizing the process of vaccine production.

The vaccine quality evaluation section, considering the two most immunized vaccines in the world (polysaccharide vaccine and viral protein vaccine) as examples, explains the role of modern analytical techniques in vaccine quality evaluation. LC-MS/MS technology is used to determine the content of saccharide units in the top 10 best-selling PPSVs. Compared with the European Pharmacopoeia methods, this approach has high throughput, fast speed, high accuracy, and a strong matrix interference resistance. MALDI-TOF is used for the testing of viral protein vaccine subunits and can be used for direct testing of the protein molecular weight without enzymatic hydrolysis or molecular weight restriction. Aluminum adjuvant is an important part of many vaccines, and its content is closely related to the effectiveness and adverse reactions of vaccines. This section thus provides ICP-OES and ICP-MS solutions for the determination of aluminum adjuvant content for the reference of vaccine workers using different experimental conditions.

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Summary and Outlook

The section regarding testing for other substances in vaccines also studies the polysaccharide vaccine and viral protein vaccine. LC-MS/MS provides solutions for the residue detection of the inactivator sodium deoxycholate and precipitant CTAB in the polysaccharide vaccine. This method accounts for the lack of detection of CTAB and has high selectivity and strong anti-interference capability compared with traditional UV spectrophotometry methods applied to sodium deoxycholate.

Larger vaccine aggregates can affect the effectiveness of drugs and result in unwanted side effects. In this section, the Aggregates Sizer is used to determine particle diameter and the concentration distribution of vaccine aggregates. This allows a discussion of the impact of the mixing process and temperature on the polymerization reaction of vaccine aggregates.

Shimadzu has as its primary concern the health of both humans and the planet, and our vaccine industry solutions are constantly being updated. Please contact us for more vaccine industry solutions or information on the development of new vaccine quality evaluation techniques.

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