Quality by Design in the Development of Analytical Procedures

A discussion has been growing with a view to applying the Quality by Design (QbD) concept to the development of analytical procedures and promoting continuous improvement of such procedures. The European Federation of Pharmaceutical Industries and Associations (EFPIA) and Pharmaceutical Research and Manufacturers of America (PhRMA) proposed a new approach for design, development and use of analytical procedures, under the concept of analytical target profile (ATP).[1] The USP Validation and Verification Expert Panel has proposed the idea of an analytical procedure lifecycle[2] on the basis of the concept presented in the FDA Guidance for Industry regarding process validation.[3] The ICH plans to discuss the development and use of analytical procedures.[4]

By applying the QbD concept along with the newly proposed ATP, better understood and highly robust analytical procedures will be developed. Furthermore, a certain regulatory flexibility is expected to be offered. However, there is no guidance for this issue and there is just one for validation of analytical procedures at this moment.

In consideration of the background above, we are currently discussing the development and use of analytical procedures which incorporate this concept, as well as the descriptions in the Application Form, in Research on Quality Assurance Throughout the Lifecycle of Drug Products: Co-Research Project in "Research on Quality Assurance and Improvement Throughout the Lifecycle of Pharmaceuticals" supported by Health and Labour Sciences Research Grants for Research on Regulatory Science of Pharmaceuticals and Medical Devices.

The study group has investigated how the analytical procedure development process and its resultant knowledge could be shared with the regulatory authorities and has compiled the outcomes into a mock document as shown below. We call for comments on this mock document.

Since the current CTDs have no sections where such information is to be described, this mock document is not intended to incorporate the information into the current CTD sections specified in ICH M4. However, it is considered appropriate that most of these contents should be described as a part of CTD in the future. Some of the descriptions, such as risk assessment, are detailed and suitably explained for better understanding with a view to diffusing new ideas or approaches for analytical procedures development widely through this mock document. We hope that this mock document will help facilitate discussion in Japan about the application of the QbD concept and the lifecycle approach to the development of analytical procedures.

1) Implications and Opportunities for Applying QbD Principles to Analytical Measurements; Position paper from EFPIA ADS and PhRMA ATG. Pharm. Tech., 34 (2), 52, 2010

2) Lifecycle Management of Analytical Procedures: Method Development, Procedure Performance Qualification, and Procedure Performance Verification, Pharmacopeial Forum, vol 39 (5)

3) FDA Guidance for Industry, Process Validation: General Principles and Practices, January 2011

4) Yoshihiro Matsuda; Informal Quality Discussion Group, 30th ICH Immediate Briefing Session, Tokyo (July 2014)

1. Analytical Target Profile (ATP)

This analytical procedure is capable of quantifying related substances in XYZ drug product over the range of 0.1% (the reporting threshold specified in ICH Q3B) to 0.2% (specification criterion). The accuracy and precision of the procedure are maintained as reportable results fall within ± 0.02% of the true value with an 80% probability determined with a 95% confidence when 0.1% to 0.2% related substances are measured.

The concept of ATP shown above is explained in Appendix. The values provided above are for an illustrative purpose and not intended for recommendations.

To satisfy the ATP above, analytical procedures are required to conform to the "Performance criteria" shown below.

Specificity:	Not affected by the excipient components of the drug product, capable of determining the target impurities specifically with sufficient discrimination capability.
Sensitivity:	The quantitation limit (S/N ratio is not less than 10) is not more than 0.1%.
Range:	In the range between 0.1% and 0.2%, reportable results fall within ± 0.02% of the true value with an 80% probability determined with a 95% confidence. Figure 1 presents the relationship between acceptable accuracy and precision in this range.

Other requirements

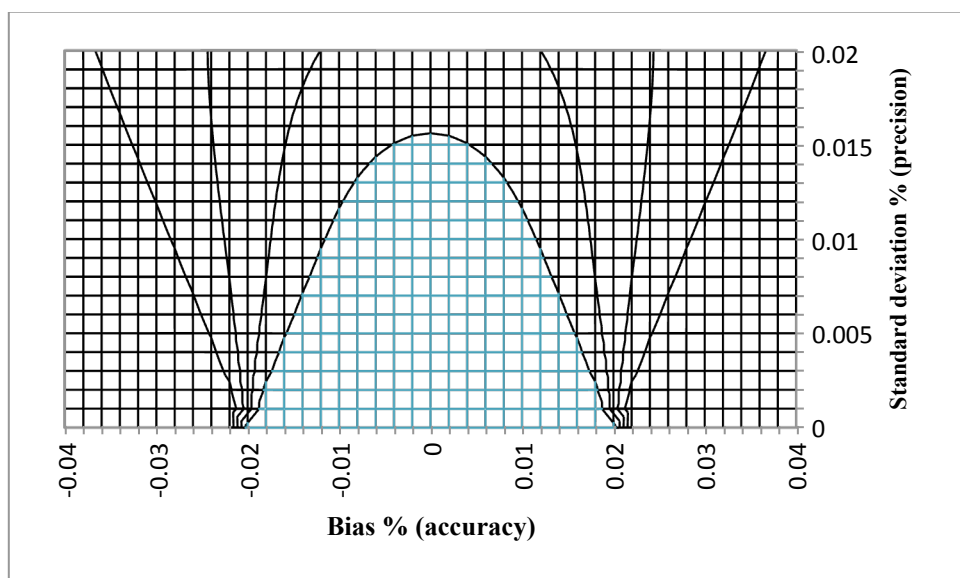|	
Linearity:	The analytical procedure shows linearity in the range between 0.05% and 1.0%. The correlation coefficient of the regression equation is not less than 0.99 and the regression line passes through the origin.

Figure 1   Performance criteria for accuracy and precision of the analytical procedure

The accuracy (bias) and precision (standard deviation) of the analytical procedure must be in the blue field.

Supplementary explanation

As for other requirements, targets set by the developer for developing an analytical procedure may be incorporated while they are not mandatory in terms of the performance criteria of the procedure. In this case, "Linearity" has been prescribed. Generally, analytical procedures do not always need to show linearity to satisfy the prescribed ATP, and linearity was included in "Other requirements" because the single-point calibration curve method is targeted for this analytical procedure, while quantitative determination by a multi-point calibration curve is feasible, too.

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2.   Analytical Procedure Development

2.1   Selection of analytical techniques

Candidate analytical techniques were selected based on the physicochemical properties of this compound (XYZ), the impurity profile related to the formulation process, information on the formulation and acquired knowledge and experience regarding analytical techniques, with primary evaluation in terms of analytical performance and usability.

2.1.1   Physicochemical properties of the drug substance

Figure X presents the chemical structure of XYZ. This is a non-volatile organic compound having a molecular weight of 300.00 and a melting point of about 180°C. It is sparingly soluble in water, the solubility in methanol and acetonitrile are 30 mg/mL and 100 mg/mL, respectively. The spectrum exhibits absorption in the ultraviolet region and a maximum at a detection wavelength of 254 nm. The pKa is 6.8 derived from dissociable group XXX.

Stability of XYZ in a solid state has been confirmed under heat and humidity stress conditions. XYZ shows no change in quality when stored at 25°C/60%RH for 36 months or at 40°C/75%RH for 6 months.

2.1.2 Formulation of the drug product

The formulation of this drug product is shown below. The drug product is a film-coated tablet formulated with lactose and calcium hydrogen phosphate hydrate as fillers, manufactured by wet-granulation method.

Function	Specification	Component	Amount per tablet (103 mg)
Active ingredient	Separate specification	XYZ	30 mg
Filler	JP	Calcium hydrogen phosphate hydrate	Appropriate amount
Filler	JP	Lactose	10 mg
Disintegrant	JP	Sodium starch glycolate	5 mg
Lubricant	JP	Magnesium stearate	2 mg
Coating agent	JP	Hypromellose	2.4 mg
Polishing agent	JP	Macrogol 6000	0.3 mg
Coloring agent	JP	Titanium oxide	0.3 mg
Coloring agent	JPE	Iron sesquioxide	Trace amount

2.1.3 Characterization of impurities (Target impurities)

Imps (impurities) 1, 3, 4 and 6 are derived from XYZ drug substance and controlled according to the drug substance specification "not more than 0.2%". Imp 1 (approx. 0.1%), Imp 3 (approx. 0.1%), Imp 4 (approx. 0.2%), and Imp 6 (approx. 0.1%) are consistently detected. These impurities do not increase during the manufacturing process of the drug products or during the storage period. Imp 2 (approx. 0.2%) and Imp 5 (approx. 0.1%) are constantly formed by the reaction of XYZ drug substance with lactose during the granulation process of drug product manufacturing, however, they do not increase during the storage period of the drug product.

2.1.4 Evaluation and determination of analytical techniques

Candidate analytical techniques were selected on the basis of knowledge and experience regarding analytical techniques, as well as information on the physical properties of XYZ, the formulation, and the impurity profile of XYZ, and were then evaluated in terms of analytical performance and usability. The characteristics evaluated were specificity, accuracy and precision for analytical performance and availability, operability, running cost and analytical time for usability. The results evaluated are shown in the table below. HPLC-UV was selected as the analytical procedure of this drug product because of its excellent analytical performance and usability.

Evaluation for analytical performance

	Specificity	Accuracy	Precision
HPLC-UV	M	H	H
HPLC-MS	H	H	M
UHPLC-UV	M	H	H
HPLC-MS	H	H	M
CE	M	H	M
TLC	L	M	L

H: High, M: Middle, L: Low

Evaluation for usability

	Availability	Operability	Cost	Time
HPLC-UV	A	A	A	B
HPLC-MS	C	B	B	B
UHPLC-UV	C	B	B	A
UHPLC-MS	C	B	C	A
CE	C	B	B	B
TLC	A	A	A	A

A: Excellent, B: Satisfactory, C: Unsatisfactory

2.2 Analytical procedure design

2.2.1 Initial screening study of analytical procedures

A screening study was carried out examining the organic solvent (acetonitrile) ratio in the mobile phase, buffer pH, and column temperature, the parameters known to have a significant impact on peak retention and separation in HPLC analysis. A detection wavelength of 220 nm was selected on the basis of the already determined UV spectrum of XYZ drug substance and the UV spectrum data for the target impurities. The analytical columns used were AAA, BBB, and CCC.

An experiment was made in a 2-level full factorial design with three factors to develop a multiple regression model for the number of peaks and the minimum resolution. AAA was selected as the analytical column because it produced the best peak shape. Figure 2 presents contour plots at column temperatures of 30°C, 35°C, and 40°C. The red area in each contour plot represents the region within which the number of peaks is less than 7 and all of the target impurities (Imps 1 to 6) are not separated from each other. On the other hand, each blue area indicates the region within which the resolution between the closest peaks is less than 1.5. It was predicted from the regression model that the number of peaks would be 7 or more and the resolution between the closest peaks would be 1.5 or more in cases where the acetonitrile ratio in the mobile phase would be about 40%, the buffer pH about 7 to 8, and the column temperature about 40°C (white area).
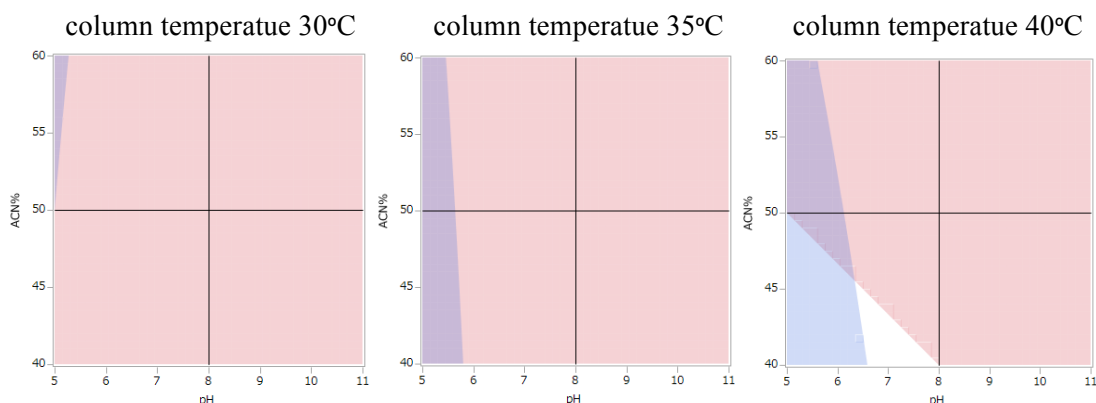
Figure 2   Contour plots at different column temperatures (column AAA)

Based on the above results, the following HPLC conditions were selected as the candidate conditions for optimization.

HPLC operating conditions
Detector: An ultraviolet absorption photometer (detection wavelength: 220 nm).
Column: AAA (4.6 mm ID × 150 mm, particle diameter 5 μm)
Mobile phase: A mixture of borate buffer solution, pH 8.0 and acetonitrile (60:40)
Flow rate: Adjust the flow rate so that the retention time of XYZ is about 15 minutes.
Column temperature: A constant temperature of about 40°C.

For sample solution preparation, 50% acetonitrile was selected as the extracting solvent, based on the study results for the solubility of XYZ, the stability in solution, and the effects on chromatograms. The procedures specified are as follows: Extract with the aid of ultrasonic waves for 10 minutes while shaking occasionally, and then centrifuge to prepare the sample solution (1 mg/mL).

Supplementary explanation

The initial screening study is intended to broadly design analytical conditions based on the knowledge obtained so far. In addition to conventional trial-and-error- or experience-based methods, screening study by experimental design or development study with commercial chromatography development software may be used to evaluate for column, buffer pH, salt concentration, type and ratio of organic solvent, gradient conditions, etc.

The findings on individual factors (parameters) acquired through the initial screening study will be useful in the primary risk assessment. Findings on other factors affecting analytical results, in addition to those under examination, may be obtained indirectly during the course of the initial screening study, which can also be made use of in the risk assessment. The evaluation for procedure parameters by experimental design is helpful for extracting more influential factors based on analytical results.

2.2.2   Primary risk assessment

The performance of an HPLC analytical procedure for impurities is greatly characterised by its specificity, namely, separation performance. Thus, factors considered to affect separation performance were extracted and compiled into a cause and effect diagram as presented in Figure 3. For each factor,

more detailed factors were extracted and classified according to respective characteristics as shown in Table 1. The assessment was carried out by utilizing the findings gained in the initial screening study and the general knowledge and the experience regarding the analytical technique.
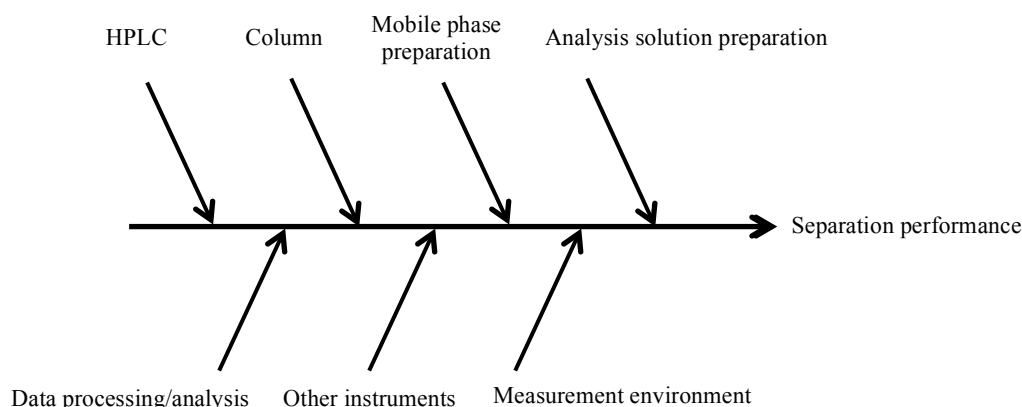

Figure 3   Factorial analysis for separation performance (cause and effect diagram)

Table 1   Factorial analysis for separation performance and classification results of factors

Factor			Mode	NCX	Set point	Comment
HPLC						
	Detector					
		Type of cell	Peak shape	C	High pressure resistant cell	Use the specified cell.
		Detection wavelength	Response	C	220 nm	Select in consideration of UV spectrum.
		Sampling frequency	Peak area, peak shape	C	10 Hz	Set the appropriate value based on the peak width at half time.
	Column oven					
		Column temperature	Peak retention (separation)	X		Study by DoE.
	Liquid feed pump					
		Flow rate	Peak retention time	C	1.0 mL/min	Set the flow rate suitable for column.
		Dead volume	Peak retention time	N		Under consideration.
	Injector					
		Needle cleaning/rinse solution	Memory (peak area)	C	Specified cleaning mode/50% MeCN	Set the appropriate conditions based on the results of the study.
		Sample injection volume	Peak area	C	5 μL	Set the appropriate conditions based on the results of the study.
		Cooler temperature	Solution stability	C	10ºC	Set at 10ºC considering both that degradation can be minimized at lower temperatures and that the specified lower limit temperature of Acquity UPLC sample cooler is "room temperature -18ºC."
Column						
	Column					
		Type of column	Peak retention, peak shape	C	Column AAA	Specify in the test methods.
		Inter-batch difference	Peak retention, peak shape	N		Under consideration.
		Column deterioration	Peak retention, peak shape	N		Under consideration.
		Size	Peak retention	C	4.6 mm × 150 mm	Specify in the test methods.
		Particle diameter	Peak shape	C	5 μm	Specify in the test methods.
Mobile phase preparation						
	Mobile phase composition					
		Type of buffer salt	Peak retention (separation)	C	Borate	Set conditions for buffer salt suitable for the intended pH based on the results of the study.
		Buffer concentration	Peak retention (separation)	C	10 mM	Set the appropriate conditions based on the results of the study.
		Buffer pH	Peak retention (separation)	X		Study by DoE.

Factor	Mode	NCX	Set point	Comment
Type of organic solvent	Peak retention (separation), column pressure	C	Acetonitrile	Select to reduce the column pressure.
Ratio of organic solvent	Peak retention (separation)	X		Study by DoE.
Mobile phase degassing	Liquid feeding, noise	C	5 minutes of ultrasonication	To prevent poor liquid feeding or spike peaks attributable to air bubbles.
Mobile phase preparation				
Reagent weighing operation	Accuracy of buffer concentration	N		Under consideration.
pH adjusting operation	Accuracy of pH	N		Under consideration.
Solution and solvent measurement				
Measuring operation	Accuracy of solution concentration	N		Under consideration.
Reagent/organic solvent				
Water	Baseline, system peak	C	Use of Milli Q water	To prevent interfering peaks due to water.
Grade	Baseline, interfering peak	N		Under consideration.
Manufacturer	Baseline, interfering peak	N		Under consideration.
Deterioration	Baseline, interfering peak	N		Under consideration.
Analysis solution preparation				
Sample solution composition				
Sample concentration	Peak area, SN ratio	C	1 mg/mL	To ensure adequate SN ratio.
Extracting solvent composition	Peak shape	X	Water/MeCN (1:1)	Study the relationship with mobile phase organic solvent composition.
Sample weighing				
Sample weighing operation	Accuracy of solution concentration	N		Under consideration.
Solution and solvent measurement				
Measuring operation	Accuracy of solution concentration	N		Under consideration.
Data processing/analysis				
Integration				
Integrating operation	Variability in peak area	N		Under consideration.
Other instruments				
Vial				
Material	Sample degradation, adsorption, interfering peak	C	Inert glass vial	To minimize adsorption or interfering peaks.
Measurement environment				
Indoor temperature and humidity	Variability in peak area	C	Specified temperature	Operate an air-conditioned measurement room.
Lighting	Change in peak area	C	Normal fluorescent lamp	Stable under indoor light. UV cut light is not necessary.

The above factors were classified into 3 categories: Factor C (Controllable) [i.e., factors whose effects on separation performance can be minimized by controlling properly], Factor N (Noise) [i.e., factors that are difficult to control and need measures to reduce effects on separation performance], or Factor X (eXperimental) [i.e., factors need to be experimentally verified for effects on separation performance]. For the factors placed under Factor N, measures to reduce effects on analytical results were studied by conducting a failure mode effects analysis (FMEA) after developing the analytical procedure (Section 2.2.4). For the factors placed under Factor X, their effects were verified by conducting an experimental evaluation as shown in the next section (2.2.3) and the optimal region was identified.

Supplementary explanation

The primary risk assessment identifies factors that may possibly affect the performance of an analytical procedure (specifically, separation performance in this mock document) on the basis of the findings obtained through the analytical procedure development study in the initial screening step and classifies the factors according to their characteristics. The factors provided here are not limited to the parameters directly evaluated in the initial screening study. Other factors may also be extracted by referring to the information on the compound accumulated so far, or the general knowledge and the experience regarding the analytical technique chosen. A large number of factors are identified at this stage, but can be addressed properly by classifying them into C, N, or X. Furthermore, the number of experimental verifications can also be reduced.

In the factorial analysis of this mock document, potential factors are exemplified in terms of both the design and use of the analytical procedure. In the factorial analysis during the development of analytical procedure, however, it may be appropriate to handle only factors that need to be studied in relation to design and to separately conduct the same analysis from an operational view after the analytical procedure has been optimized. In some cases, a factorial analysis is desirably carried out with variability of analytical results as the effect.

Factors such as the precision of equipment and apparatus (e.g., balance, pH meter, and glassware) are considered to fall into Factor N at the operational stage of an analytical procedure. In this mock document, such items were not extracted as factors, on the assumption that properly calibrated equipment and apparatus are used under a system where the reliability is ensured. In the case of the use of an analytical procedure, for example, in the technical transfer, it may be reasonable to regard instrument precision as a factor.

A detailed assessment was separately conducted regarding the impacts of factors on the variability in analytical results attributable to analysis solution preparation (since this report places emphasis on the development of HPLC conditions, the analysis solution preparation is not discussed in detail. Assessment of analysis solution preparation may be conducted independently or in parallel with the development of HPLC conditions).

2.2.3   Experimental evaluation

For the factors that were classified as Factor X in the primary risk assessment, their effects on separation performance were experimentally evaluated.

2.2.3.1   HPLC operating conditions

The results of the initial screening study and primary risk assessment revealed that the three factors categorized as Factor X, which are buffer pH of the mobile phase, column temperature, and acetonitrile ratio in the mobile phase, greatly affected the retention of the impurity peaks and their separation behavior of XYZ drug product.

Thus, the experiment with Box-Behnken design was performed to investigate the optimal levels for the buffer pH of mobile phase, column temperature, and acetonitrile ratio in the mobile phase. The experimental design was developed using as the startpoint the HPLC operating conditions set by the initial screening study. The experimental design and the obtained results are presented in Table 2, and the contour profiles of response surface are presented in Figure 4. The response surface of Figure 4 shows how the number of peaks and the minimum resolution vary depending on changes in buffer pH and acetonitrile ratio of the mobile phase, when column temperatures are between 36°C and 42°C. The red areas indicate that the number of peaks is less than 7 and the Imps 1 to 6 are not separated from

each other or from the other impurities. The minimum resolution was 1.5 or more at any point in the range of 36°C to 42°C for column temperature. The white areas denote that Imps 1 to 6 are separated and the resolutions between their peaks are 1.5 or more, thus representing a parameter region which satisfies the required separation performance. Based on these results, it was demonstrated that the required peak separation would be achieved under any conditions in the range of 38°C to 40°C for column temperature, 7.0 to 9.0 for buffer pH, and 40% to 50% for acetonitrile ratio of the mobile phase.

Table 2   Summary table for conditions and results of optimization experiments

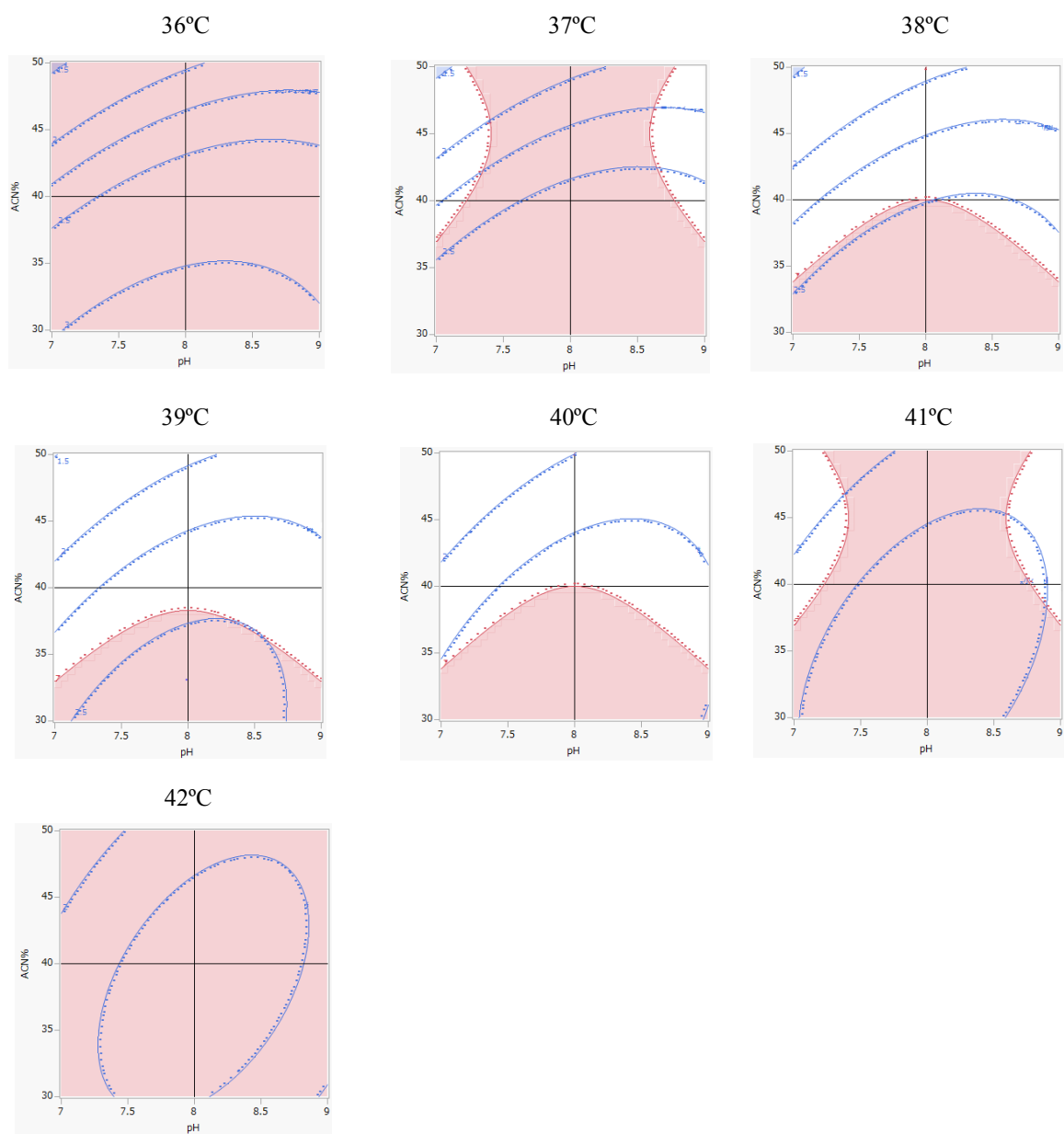| Experiment No.	pH	Column temperature	Acetonitrile %	Number of peaks	Minimum resolution
1	7	35	40	6	3.32
2	7	45	40	6	1.77
3	9	35	40	6	3.40
4	9	45	40	6	1.39
5	8	35	30	6	3.45
6	8	35	50	7	0.60
7	8	45	30	4	3.52
8	8	45	50	5	2.58
9	7	40	30	7	1.46
10	9	40	30	7	1.70
11	7	40	50	7	2.09
12	9	40	50	7	2.92
13	8	40	40	7	2.36
14	8	40	40	7	2.35
15	8	40	40	7	2.40

Figure 4   Changes in the number of peaks and the minimum resolution depending on variations in the buffer pH and acetonitrile ratio of the mobile phase at each column temperature

Based on the above results, the conditions in which the buffer pH was 8.0 and acetonitrile ratio was 45% for the mobile phase were selected as the optimal conditions showing high robustness as they were located near the center of the white area of the plot for the column temperature of 39ºC, and in whose white area it was indicated that a good separation performance was the largest among the plots. The selected HPLC conditions are shown below. The chromatogram obtained under these conditions is presented in Figure 5.

HPLC operating conditions
Detector: An ultraviolet absorption photometer (detection wavelength: 220 nm)
Column: AAA (4.6 mm ID × 150 mm, particle diameter: 5 μm)
Mobile phase: A mixture of borate buffer, pH 8.0, and acetonitrile (55:45)
Flow rate: A constant flow rate of 1.0 mL/min
Column temperature: A constant temperature of about 39ºC
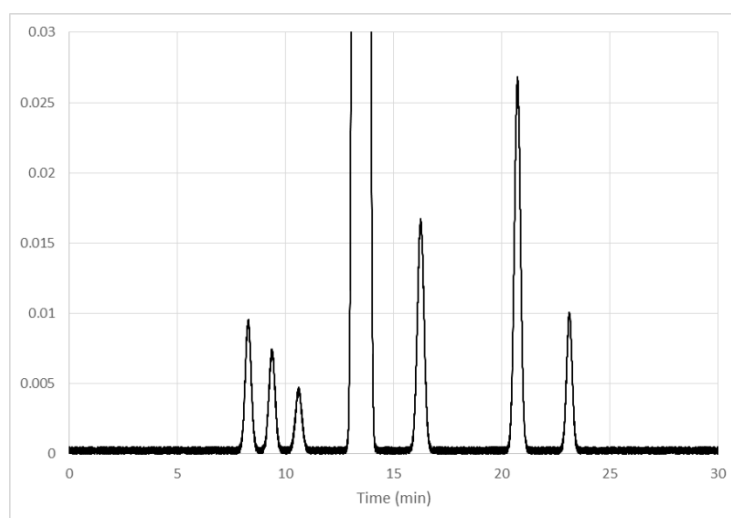Sample injection volume: 5 μL

Figure 5   Chromatogram obtained under the optimized conditions

2.2.3.2   Sample preparation

The primary risk assessment suggested that, of the factors categorized as Factor X, extracting solvent composition would affect the separation performance of the analytical procedure. Thus, considering the solubility of XYZ, 40%, 50%, and 60% acetonitrile were selected as the extracting solvents, and their effect on peak retention, shape, and separation were examined. The results confirmed that the sample solution of 1 mg/mL concentration could be prepared with a good peak separation maintained by selecting 50% acetonitrile.

Supplementary explanation

This report addresses only extracting solvent composition in assessment and experimental evaluation regarding sample preparation. In some cases, however, it may be necessary to discuss other factors in the actual development of analytical procedures.

2.2.4   Secondary risk assessment

For the factors categorized as Factor N in the primary risk assessment, an FMEA was conducted using the optimized analytical procedure. Prior to the determination of risk priority, Risk Priority Number was defined as follows:

Risk Priority Number (RPN) $= S \times O \times D$

Low (RPN 1-35)	Sufficiently acceptable risk level; generally, a further reduction of the risk is not required.
Medium (RPN 36-59)	Acceptable risk level; however, some measures to further reduce the risk are desirable.
High (RPN 60 or more)	Unacceptable risk level; some measures to reduce the risk are required.

Table 3 shows the results of the FMEA and those of the risk reductions achieved for the factors under Factor N with a medium or high risk priority. For the factors considered to bear a potential risk, a reduction of the risk was undertaken by setting system suitability or establishing SOPs.

Table 3   Results of the FMEA

Process/factor	Error mode	Before measures							Measures	After measures				
		Severity: Effect of error	Score	Occurrence: Cause of error	Score	Detectability: Detection method	Score	Risk priority: RPN	Rank		Severity Score	Occurrence Score	Detectability Score	Risk priority: RPN	Rank

HPLC

Liquid feed pump
| Dead volume | Peak retention | Peak retention | 5 | Wrong system configuration | 3 | | 4 | 60 | Medium | SST (retention time, theoretical plate number) | 5 | 3 | 1 | 15 | Low |

Column

Column
| Inter-batch difference | Peak retention, peak shape | Peak retention, peak shape | 5 | Variability in manufacture | 3 | | 4 | 60 | Medium | SST (resolution, theoretical plate number) | 5 | 3 | 1 | 15 | Low |
| Column deterioration | Peak retention, peak shape | Peak retention, peak shape | 5 | Life of material | 3 | | 4 | 60 | Medium | SST (resolution, theoretical plate number) | 5 | 3 | 1 | 15 | Low |

Mobile phase preparation

Buffer preparation
| Reagent weighing operation | Peak retention, peak shape | Peak retention, peak shape | 5 | Human operating error | 3 | | 4 | 60 | Medium | SST (resolution, theoretical plate number) | 5 | 2 | 1 | 10 | Low |
| pH adjusting operation | Peak retention, peak shape | Peak retention, peak shape | 3 | Human operating error | 3 | | 4 | 36 | Medium | SST (resolution, theoretical plate number) | 3 | 2 | 1 | 6 | Low |

Solution and solvent measurement
| Measuring operation | Peak retention, peak shape | Peak retention, peak shape | 3 | Human operating error | 3 | | 4 | 36 | Medium | SST (resolution, theoretical plate number) | 3 | 2 | 1 | 6 | Low |

Reagent/organic solvent
Grade	Interfering peak	Interfering peak	7	Variability in quality	3		4	84	High	Blank run	7	3	1	21	Low
Manufacturer	Interfering peak	Interfering peak	7	Wrong manufacturer	3		4	84	High	Designation of manufacturer	7	1	1	7	Low
Deterioration	Interfering peak	Interfering peak	7	Variability in quality	2	Use within the shelf-life	1	14	Low						

Analysis solution preparation

Sample weighing
| Sample weighing operation | Peak area | Peak area | 5 | Human operating error | 3 | Preparation record | 2 | 30 | Low | Training | 5 | 2 | 2 | 20 | Low |

Solution and solvent measurement
| Measuring operation | Accuracy of solution concentration | Peak area | 5 | Human operating error | 3 | Preparation record | 2 | 30 | Low | Training | 5 | 2 | 2 | 20 | Low |

Data processing/analysis

Data analysis
| Integrating operation | Variability in peak area | Peak area | 7 | Human error (proficiency) | 3 | Chromatogram | 4 | 84 | High | Control by SOP | 7 | 1 | 1 | 7 | Low |

Severity
8   High impact   Results critically affected and leading to an erroneous judgment
5   Moderate impact   Results considerably affected
3   Low impact   Results slightly affected, but limited
1   Very low impact   Results little affected

Occurrence
4   Frequent   Expected to occur periodically (10/100)
3   Occasional   Expected to occur with low frequency (5/100)
2   Rare   Expected to occur rarely (1/100)
1   Unlikely   Level with no or little occurrence

Detectability
4   Likely not detected   Not detectable because there is no method or system for detection in place
2   Regularly detected   Easily detectable
1   Always detected   There is a method or system for detection in place

3. Verification of Analytical Procedure Performance

The analytical procedure was verified for performance in accordance with the ICH Q2 Guideline on Validation of Analytical Procedures using the performance criteria laid down in Section 1 according to the ATP. The results confirmed that the established analytical procedure fulfilled the requirements based on the performance criteria, thus demonstrating the performance of this procedure.

A detailed description as to the results of the validation of the analytical procedure is omitted in this report.

3.1 Specificity

Using forced degradation samples or orthentic impurity samples, specificity of the analytical procedure was confirmed by conducting confirmatory verification of the separation performance elaborated during the development of the procedure.

3.2 Accuracy

Accuracy of the analytical procedure was evaluated in terms of the recovery of the impurity from the spiked samples. The spiked sample preparations were conducted at 3 concentrations in the range of 0.1% (reporting threshold) to 0.2% (specification value).

3.2 Precision (repeatability and intermediate precision)

Precision of the analytical procedure was evaluated in terms of the recovery of the impurity from the spiked samples. The spiked sample preparations were conducted at 3 concentrations in the range of 0.1% (reporting threshold) to 0.2% (specification value).

3.3 Linearity

Linearity was verified in the range of 0.1% (reporting threshold) to 0.2% (specification value). The results gained were analyzed for linear regression to evaluate for linearity.

3.4 Sensitivity

To verify the quantitation limit of this analytical procedure, sensitivity was evaluated by preparing a solution of drug substance XYZ at 0.5 µg/mL, equivalent to a 0.05% amount, and determining the signal to noise (S/N) ratio from the chromatogram obtained.

3.5 Range

The results for accuracy, precision, and linearity revealed that the values obtained from this analytical procedure fall within ± 0.02% of the true value between 0.05% and 0.25% with an 80% probability. Conformance to the ATP was thus verified.

3.6 Robustness

For the separation performance of this analytical procedure, the region within which the impurities of interest are separated has been identified, as shown in Section 2.2.3. Separation of the impurities of interest has been demonstrated over the range provided below in any combination of the parameters. Therefore, it has been verified that this range constitutes such a robust region that changes of the parameters do not affect measurement values. Based on the results for accuracy and precision, it has also been verified that the variations in measurement values from this analytical procedure have been reduced successfully to an acceptable level.

	Standard operating value	Lower limit	Upper limit
pH of buffer in the mobile phase	8.0	7.0	9.0
Column temperature (ºC)	39	38	40
Ratio of acetonitrile in the mobile phase (%)	45	40	50

3.7 Analysis solution stability

The sample solution and standard solution were verified for stability.

4. Control Strategy

For HPLC operating conditions, it has been experimentally demonstrated that the variability of parameters does not affect the separation performance of this analytical procedure within the verified region on the basis of the optimization study results shown in Section 2.2.3. Thus, the risks associated with Factor X had already been reduced during the development of this analytical procedure, indicating that the ranges presented in Section 3.6 can be a method operable design region (MODR), i.e., a robust region within which the analytical results are not affected.

Furthermore, the risk assessment of the established analytical procedure identified potential risks in the liquid feed pump, column, and solution preparing operation, as shown in Section 2.2.4. For controlling these risks, peak resolution, theoretical plate number, symmetry factor, and repeatability were included in the system suitability test, and a test for required detectability and test of a reference sample were incorporated as other parameters. This allows the reduction of the risks associated with these factors and it can also be confirmed during the routine tests that this analytical procedure maintains the expected performance.

System suitability

- System performance (specificity):
  When the analytical procedure is run with 10 µL of the solution for system suitability containing Imp 1 and Imp 4, the resolution between these peaks is not less than 1.5. The number of theoretical plates and the symmetry factor of the peak of Imp 1 are not less than 15,000 and not more than 1.2, respectively.

- System repeatability (precision):
  When the analysis repeated 6 times with 10 μL of the solution for system suitability containing Imp 1 and Imp 4, the relative standard deviation of the peak areas of Imp 1 and Imp 4 is not more than 10%.

- Test for required detectability (linearity and quantitation limit)
  The ratio of the peak area of XYZ from a 0.05% XYZ solution to that from a 1.0% XYZ solution is between 3% and 7%.

  Test of the reference sample
  When a solution of the reference sample (Lot xxx) which contains the impurities is prepared as directed under the preparation procedure for the sample solution, the quantities of Imp 1, Imp 3, Imp 4 and Imp 6 are about 0.1%, about 0.1%, about 0.2% and about 0.1%, respectively.

The solution for system suitability is a solution prepared from a sample containing Imp 1 and Imp 4.

Supplementary explanation

Periodic reviews of the accumulated system suitability data (resolution, repeatability, etc.) allow the verification of the performance of the analytical procedure in use. The approach that system suitability incorporates a measurement of a reference sample containing the impurities to be tested is also useful to confirm that the analytical procedure has the intended performance.

Appropriate reevaluation of the system suitability requirements by using the results of periodic review and information about OOS or OOT would lead to maintaining the analytical procedure performance set in the ATP. This can also be a basis for changing the analytical procedure as necessary.

Supplementary explanation

MODR can be established through evaluation of effects of factors by using DOEs (design of experiments). Application of the ATP concept may offer post-approval regulatory flexibilities with regard to analytical condition changes.

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5   Conclusion

An HPLC-UV analytical procedure was developed as the method for determining related substances in XYZ drug product. Evaluation of the developed analytical procedure for specificity, accuracy, precision, and range verified that the procedure satisfied the performance criteria set according to the ATP. In addition, evaluation for linearity showed that the linear regression equation is a straight line which passes through the origin. Based on the above results, it was demonstrated that this analytical procedure delivered the performance specified in the ATP. Furthermore, a control strategy was developed based on the findings acquired through the development activity to also ensure the intended performance of the analytical procedure during its use.

Commentary 1: Setting the ATP for Accuracy and Precision on the Basis of Probability

1   Introduction

Prior to developing an analytical procedure, an analytical quality by design (AQbD) approach sets up an analytical target profile (ATP) which represents the performance to be delivered by the procedure. In this report, the following ATP was applied, as an example, to related substances in the purity test.

> This analytical procedure was developed to determine related substances in XYZ drug product as percent with respect to the drug substance (XYZ) in the range from the reporting threshold specified in the ICH Q3B to 120% of the specification value. The procedure must achieve such a performance that reported values fall within ± 0.02% of the true value for the impurity level from 0.05% to 0.20% with an 80% probability with a 95% confidence.

The above ATP contains statements, such as "reported values fall within ± 0.02% of the true value for the impurity level from 0.05% to 0.20% with an 80% probability" and "reported values fall within ± 0.01% of the true value for the impurity level from 0.05% to 0.20% with a 95% probability" These descriptions correspond to accuracy and precision in the conventional analytical procedure validation.

2   Statistical model and the expression of analytical procedure performance

The relationship between analytical result (x) and the true value ($x*$) is assumed to follow the model given by the equation below.

$$\text{Equation 1} \quad x = x* + \delta + \varepsilon, \ \varepsilon \sim N(0, \sigma^2)$$

, where δ represents the deviation inherent in an analytical procedure, equivalent to the accuracy of the procedure. ε is an error. Error here does not mean merely repeatability (accidental error), but it may also be interpreted as reproducibility or intermediate precision according to the environment where the analytical procedure is placed throughout the lifecycle. In this model, ε is hypothesized as a random variable following a normal distribution with a mean of 0 and a standard deviation of σ. According to Equation 1, analytical result (x) can be said as a random variable following a normal distribution with a mean of $x*$ and a standard deviation of $x* + \delta$.

$$\text{Equation 2} \quad x \sim N(x* + \delta, \sigma^2)$$

By way of an example, Figure 1 presents the distribution of analytical results obtained when a sample with $x* = 100\%$ (the true value of content) is analyzed by an assay procedure with δ = 0% and σ = 3%. Figure 1 indicates how the analytical results are distributed. The figure can also provide an understanding of how wide the range of analytical results would spread with how much percentage (probability) in the long run.
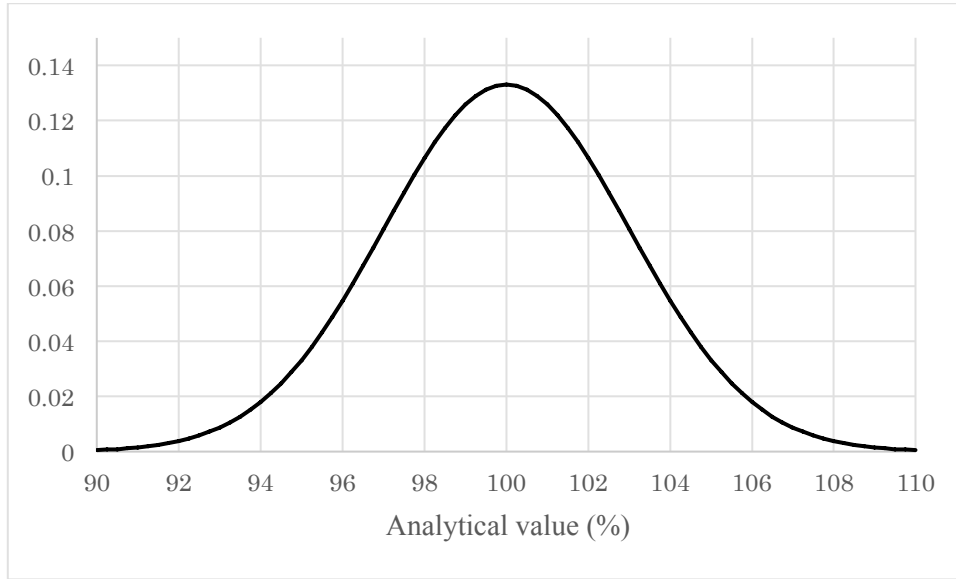
Figure 1 **Distribution of analytical results obtained when a sample with *x** = 100% is analyzed by an assay procedure with δ = 0% and σ = 3%**

Probability *P* is given as the following equation that analytical result *x* from an analytical procedure of such a performance as expressed by Equation 1 or Equation 2 is included in an interval defined by any lower limit *LL* and upper limit *UL*.

$$\text{Equation 3} \quad P = \int_{LL}^{UL} N(x * + \delta, \sigma^2)\, dx$$

Equation 3 provides for determination of the probability that an analytical result from an analytical procedure of a performance corresponding to any combination of *δ* and *σ* is included in any interval. For example, the probability is calculated to be about 90.4% that an analytical result from an assay procedure of such a performance as expressed by *δ* = 0% and *σ* = 3% is included in an interval equivalent to ± 5% of the true value. In the same manner, for an analytical procedure with *δ* = 2% and *σ* = 3% or *δ* = 0% and *σ* = 5%, the probability that an analytical result is included in an interval equivalent to ± 5% of the true value is about 83.2% and about 68.3%, respectively. Figure 2 displays the relationship between the distribution of analytical results, an interval equivalent to ± 5% of the true value, and the probability of inclusion in the interval when a sample with *x** = 100% is analyzed. The area surrounded by the *x* axis, a curve indicating the distribution of analytical values, and the 2 dotted lines equal the probability sought.
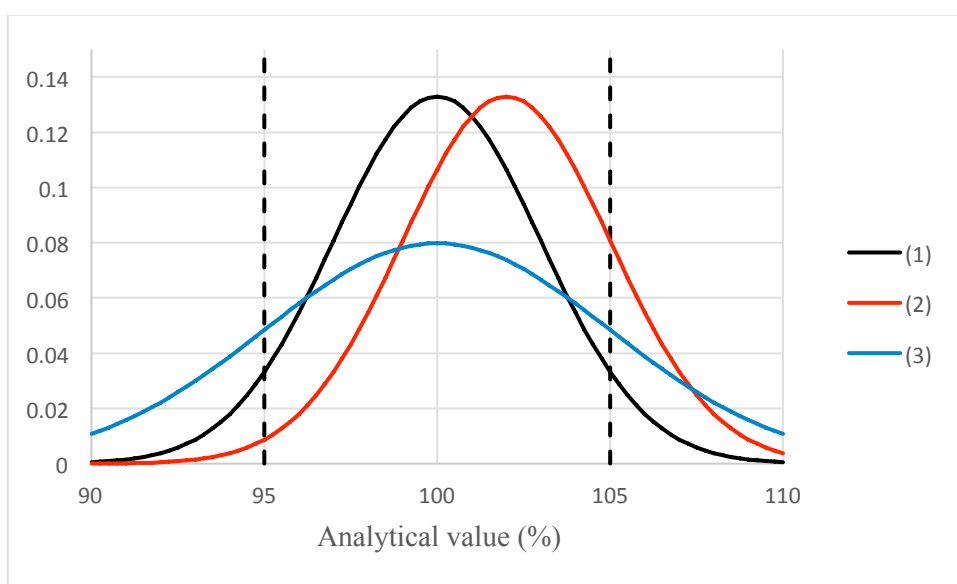
Figure 2   **Relationship between the distribution of analytical values with (1) δ = 0% and σ = 3%, (2) δ = 2% and σ = 3%, or (3) δ = 0% and σ = 5%, interval, and probability**

Figure 3 is created by similarly evaluating a probability that an analytical result is included in an interval equivalent to ± 5% of the true value for various combinations of δ and σ and plotting the results as a contour plot. Use of Figure 3 permits a visual representation of the performance of an analytical procedure. An analytical procedure with the true performance of δ = 2% and σ = 4%, for example, the performance of the procedure is plotted as a red symbol in Figure 3, showing that "the probability that an analytical result is included in an interval equivalent to ± 5% of the true value is between 70% and 75%."
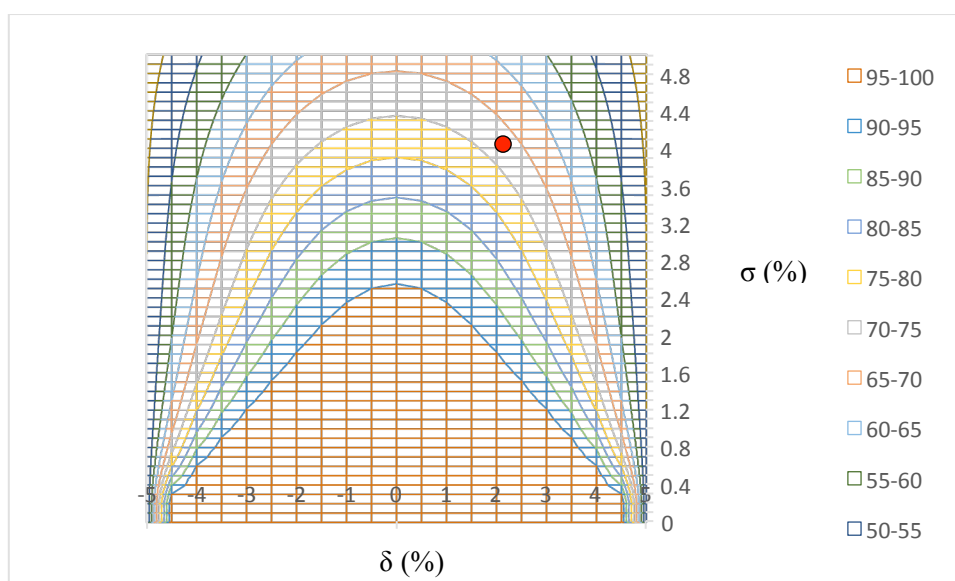

Figure 3   **Relationship between the accuracy/precision of an assay procedure and the performance of an analytical procedure**

3 Comparison with the acceptance criteria for the conventional analytical procedure

Accuracy and precision are separately prescribed in the acceptance criteria for the conventional analytical procedure validation. The criteria laid down are, for example, "the accuracy of the analytical procedure should be within ± 3.0% from the true value" and "the precision should not be more than 3.0% as standard deviation", respectively. By overlaying the conventional criteria on Figure 3, the criteria correspond to a region surrounded by a red solid line in Figure 4. Any analytical procedure with a combination of accuracy and precision included in this region would be judged to meet the above criteria, but the probability that analytical values falling within the ± 5% of the true value is shown to vary in a very wide range between 75% and 100%. In addition, although either blue symbol in Figure 4 corresponds to an analytical procedure for which its analytical values are included within ± 5% of true value with 95% probability, one is accepted and the other is rejected if judged according to the conventional criteria for analytical procedure validation.

As discussed above, analytical procedure performance can be described more reasonably by setting an ATP based on the statistical model proposed in the report.
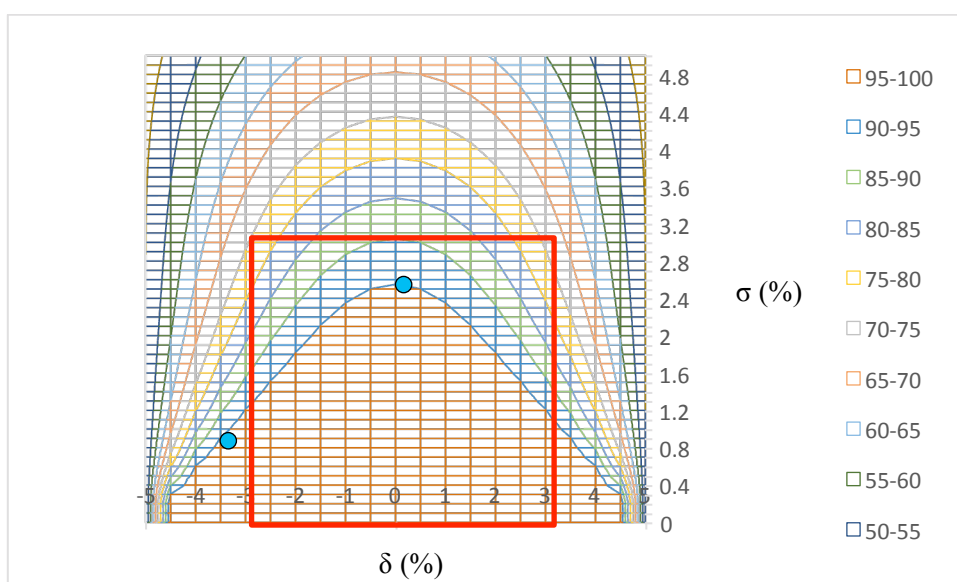

Figure 4   Comparison between the conventional criteria for analytical procedure validation and the statistics based ATP

4 Interval estimation for accuracy and precision and ATP

In the above discussions, the assumption is that the true accuracy and precision of an analytical procedure are already known. Generally, however, the true performance of an analytical procedure is unknown. Hence, performances of the analytical procedure are experimentally evaluated from limited experiments (e.g., analytical procedure validation).

Specifically, cases where the accuracy and precision of an analytical procedure are estimated from a small sample size will be explained below. Assume that values x_1, x_2, …, x_n are experimentally evaluated for accuracy, such as recovery values of an analytical procedure obtained from spike experiments. The true accuracy of the analytical procedure is the mean of the population distribution that generates the data of x_1, x_2, …, x_n, namely, population mean μ. However, μ cannot be

determined from a limited number of experiments. Therefore, μ must be presumed from a sample consisting of results from a limited number of experiments.

The mean (sample mean) of the data of $x_1, x_2, \ldots, x_n$ is given as the following equation.

$$\textbf{Equation 4} \quad \overline{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

$\overline{x}$ is an unbiased estimator of population mean μ and serves as an index for estimation of accuracy, but is only one value derived from a certain limited experiment. Even if such $\overline{x}$ as to meet the ATP is obtained in an experiment, a different $\overline{x}$ may result from another experiment. In other words, uncertainty in experiments is not taken into account when an analytical procedure is evaluated only in terms of the unbiased estimator.

Thus, an evaluation needs to be made in consideration of the confidence interval of $\overline{x}$, using an interval estimation.

Confidence interval is obtained by estimating a value for the parameter of interest based on experimental data in the form of an interval, and the probability that a confidence interval includes the true value of the parameter is referred to as the confidence level. For example, when the accuracy of an analytical procedure is experimentally evaluated, the confidence interval estimated with a confidence level of 95% (95% confidence interval) implies that 95% of the estimated interval contains the true accuracy of the procedure (the true value of accuracy).

If the degree of freedom for mean estimation is n, a *P*% confidence interval for population mean μ with an unknown standard deviation can be calculated by the following equation:

$$\textbf{Equation 5} \quad \overline{x} - t\left(n-1, \frac{100-P}{100}\right)\frac{s}{\sqrt{n}} \leq \mu \leq \overline{x} + t\left(n-1, \frac{100-P}{100}\right)\frac{s}{\sqrt{n}}$$

, where $t\left(n-1, \frac{100-P}{100}\right)$ denotes the positive one of the two-sided $100 - P$ percentile for a t-distribution with degree of freedom $n - 1$. Sample standard deviation *s* is an unbiased estimator of standard deviation, and is given as the following equation:

$$\textbf{Equation 6} \quad s = \sqrt{\frac{\sum_{i=1}^{n}(\mu - x_i)^2}{n-1}}$$

For the same reason as for accuracy, confidence interval needs to be evaluated for precision. If the degree of freedom for estimation of sample standard deviation *s* is $n - 1$, then the *P*% confidence interval for population standard deviation σ is:

$$\textbf{Equation 7} \quad \sqrt{(n-1)\frac{s^2}{\chi^2(n-1,(100-P)/200)}} \leq \sigma \leq \sqrt{(n-1)\frac{s^2}{\chi^2(n-1,1-(100-P)/200)}}$$

, where $\chi^2(n-1,(100-P)/200)$ indicates the $(100-P)/200$ percentile for a χ^2 distribution with degree of freedom $n-1$, whereas $\chi^2(n-1,1-(100-P)/200)$ represents the $1-(100-P)/200$ percentile for χ^2 distribution with degree of freedom $n-1$.

The results of one time of the analytical procedure validation provide a plane composed by a combination of confidence intervals for accuracy and precision according to Equation 5 and Equation 7. For example, assuming that the 95% confidence interval for accuracy is estimated to be -1.0% to +1.0% (as bias) and the 95% confidence interval for precision 0.50% to 1.0% (as standard deviation), the plane composed by these confidence intervals appears as a red shaded region in Figure 5. When analytical procedure validation is repeated to create planes defined by the combination of confidence intervals, it follows that about 90% ($0.95^2 = 0.9025$) of the created plains contain the true accuracy and precision of the analytical procedure. Therefore, if the entire plane created from one run of analytical procedure validation is included in the region designated based on an ATP, the procedure can reasonably be considered to meet the ATP.

Hence, it is necessary to estimate respective $100\times\sqrt{\dfrac{P}{100}}\%$ confidence intervals for both accuracy and precision to evaluate whether or not the performance satisfies the ATP. Following this procedure will ensure that the *P*% of the planes formed by the analytical procedure validation contain the true values of both accuracy and precision.
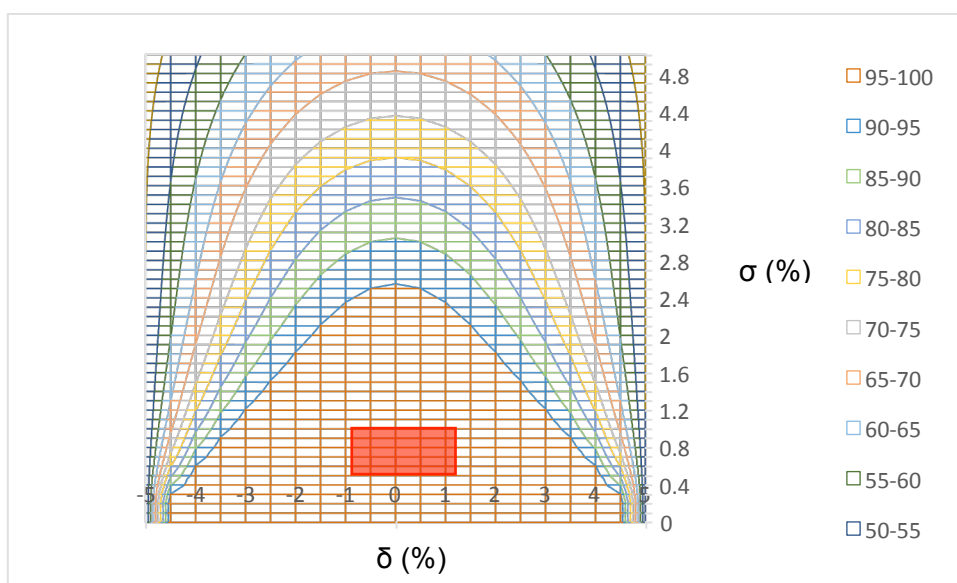

Figure 5   **Relationship between the accuracy/precision of an assay procedure and the performance of an analytical procedure**

Commentary 2: CNX Classification

A technique intended to acquire reproducible analytical results with small variations by classifying the factors listed in a cause and effect diagram into Factor C [i.e., factors that can be controlled properly according to respective characteristics], Factor N [i.e., factors that are difficult to control and affect analytical results], and Factor X [i.e., factors need to be experimentally verified for effects on analytical results] and by taking appropriate measures. Factors classified into N or X will be controlled properly as with Factor C by conducting appropriate action or verification. Or, their effects will be reduced to a low level to yield reproducible analytical results.

Factor C (controllable or constant)

Factors that are controlled properly, or those whose effects on analytical results can be minimized by controlling properly. Reproducible results with small variations can be obtained by taking appropriate measures (e.g., setting fixed values).

Factor N (not controllable or noise)

Factors that are difficult to control, or those for which measures to reduce their effects on analytical results are required because such effects are significant even if fixed values are to be set for controlling. Taking appropriate measures is expected to help produce analytical results with reduced variations and continued reproducibility. Such controlling measures may require much effort and cost.

Factor X (eXperimental)

Factors for which the impact on analytical results is unknown, or those needed to be further verified experimentally. Setting suitable fixed values or ranges based on verification results is expected to help produce analytical results with reduced variations and continued reproducibility. Verifying interaction with plural factors by experimental design, etc. is recommended for experimental investigation on these factors.