National Pharmaceutical Control Bureau
MINISTRY OF HEALTH MALAYSIA

World Health Organization
WHO Collaborating Centre for Regulatory Control of Pharmaceuticals

PIC/S
Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme

QUALITY SYSTEM
SIRIM
Certified to ISO 9001:2000
Cert. No: AR 2293

STANDARDS MALAYSIA
ACCREDITED LABORATORY
MS ISO/IEC 17025:2005
[illegible]

Analytical Method Validation Common Problem 2

Centre for Quality Control
National Pharmaceutical Control Bureau
Lot 36, Jalan Universiti, 46200 Petaling Jaya, Selangor

DL: +6.03.78835400 | F: +6.03.79567075 |
WS : www.bpfk.gov.my |

OUTLINE

1) Linearity & Common Problems
2) Accuracy & Common Problems
3) Precision & Common Problems
   - Method precision
   - Intermediate precision

Type of analytical procedure characteristics	Identification	Testing For Impurities Quantitation Limit		Assay - dissolution (measurement only) - content/ potency
Accuracy	-	+	-	+
Precision				
Repeatability	-	+	-	+
Interm. Precision	-	+ (1)	-	+ (1)
Specificity (2)	+	+	+	+
Detection Limit	-	- (3)	+	-
Quantitation Limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+

- signifies that this characteristic is not normally evaluated

+ signifies that this characteristic is normally evaluated

(1) in cases where reproducibility has been performed, intermediate precision is not needed

(2) lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s)

(3) may be needed in some cases

Linearity and Range

Linearity

- The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample

Range

- … the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity

ICH Q2(R1) Guideline, 1996

Linearity and Range

- Assay of a drug substance or a finished (drug) product: normally from 80 to 120 percent of the test concentration;
- Dissolution testing: +/-20 % over the specified range;
- Determination of an impurity: from the reporting level of an impurity to 120% of the specification;

Linearity and Range - Assay

- Standard stock solution (S1)

25mg X —methanol→ 100mL

- Standard working solution

2mL S1 —methanol→ 50mL

- Calculate 100% conc.,

$$\frac{25}{100} \times \frac{2}{50} = 0.01 \text{ mg/mL}$$

- Requirement: 80 -120% of the *test concentration*
- 80% ??

  = 0.01 mg/mL x 80%

  = 0.008 mg/mL
- 120% ??

  = 0.01 mg/mL x 120%

  = 0.0012 mg/mL

Range: 0.008 - 0.0012 mg/ml

Linearity and Range – Impurity

- Requirement: LOQ to 120% of the *specification*
- For example, specification of Imp A = NMT 0.5%
- Working concentration = 1 mg/mL
- 100% = specification of Imp A, 0.5% (in this case)
- What is the concentration of 120% ??
  - = 0.5% x (120/100)% x 1mg/mL
  - = 0.6 mg/mL
- Range: LOQ - 0.6 mg/ml

Linearity and Range – Assay + Impurity

- Standard stock solution (S1)

25mg X —methanol→ 100mL

- Standard working solution

2mL S1 —methanol→ 50mL

- Calculate 100% conc.,

$$\frac{25}{100} \times \frac{2}{50} = 0.01 \text{ mg/mL}$$

- LOQ
- 120% ??

  = 0.01 mg/mL x 120%

  = 0.0012 mg/mL

Range: LOQ - 0.0012 mg/ml

Linearity and Range – Dissolution

- NLT 75% of the LC dissolved in 30 minutes.
- Standard stock solution (S1)

$$25\text{mg X} \xrightarrow{\text{methanol}} 100\text{mL}$$

- Standard working solution

$$2\text{mL S1} \xrightarrow{\text{methanol}} 50\text{mL}$$

- Calculate 100% conc.,

$$\frac{25}{100} \times \frac{2}{50} = 0.01 \text{ mg/mL}$$

- Requirement: +/-20 % over the *specified range*
- 75% - 20% = 55%
  - = 0.01 mg/mL x 55%
  - = 0.0055 mg/mL
- 75% + 20% = 95%
  - = 0.01 mg/mL x 95%
  - = 0.0095 mg/mL

Range: 0.0055 - 0.0095 mg/mL

Linearity

Data Required

- Testing Method
- Acceptance criteria
- Data for linear regression equation, Y-intercept, slope, r^2 and linearity graph.

Testing Method

- Minimum of 5 concentrations over a suitable range
- dilute stock solutions or separate weighings

Acceptance criteria

- visual – straight line graph.
- $r^2 > 0.995$
- Y - intercept at 100% working concentration $\leq$ 2%

NPCB
MOH

Example of Linearity Data and Curve

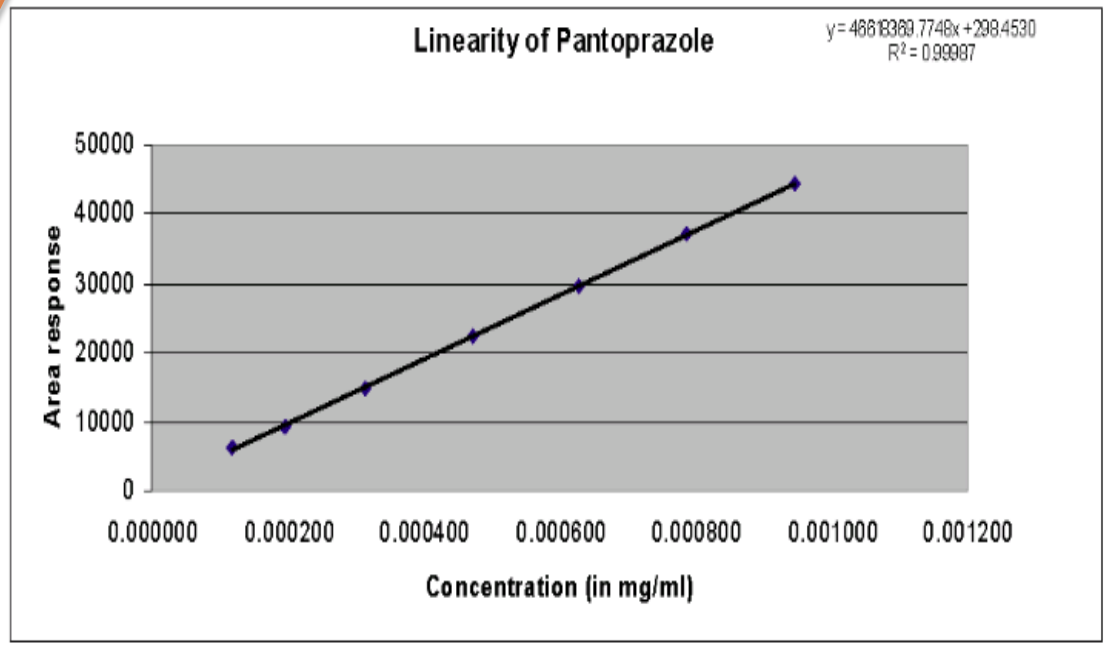
1) Common problem 1

- Testing method not given – preparation of each target concentration ??

2) Common problem 2

- Y – intercept at 100% not given

S. No.	Target Concentration (%)	Concentration (%, with respect to 0.423 mg /ml sample Concentration)	Concentration (mg/ml)	Area
1	(LOQ)	0.03	0.000118	6109
2	25	0.05	0.000197	9343
3	40	0.08	0.000315	14825
4	60	0.12	0.000473	22245
5	80	0.16	0.000630	29618
6	100	0.20	0.000788	37110
7	120	0.24	0.000945	44419
Correlation Coefficient [R]				0.99994
Regression Coefficient [R^2]				0.99987
Slope				46618370
Intercept				298.5



Conclusion: The Regression line of analysis shows linear relationship between concentration and response of Pantoprazole. The Correlation coefficient is 0.99994

NPCB
MOH

Example of Linearity Data and Curve

How to calculate y-intercept at 100%?

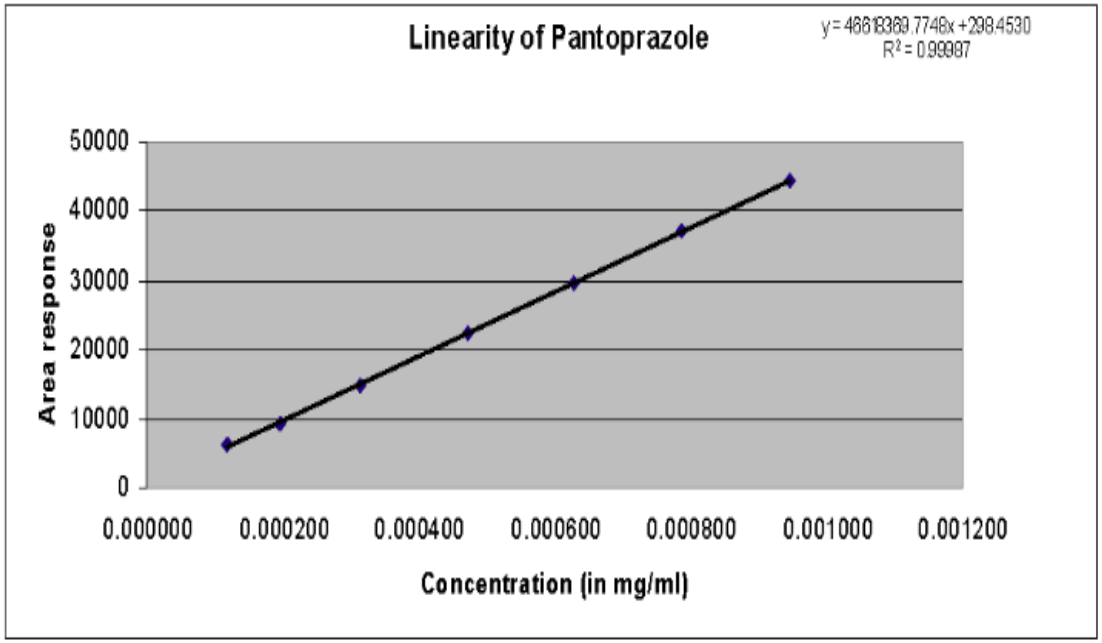
$$\frac{\text{Intercept}}{\text{Area at 100\% conc}} \times 100\%$$

In this case,

$$\frac{298.453}{37100} \times 100\%$$

= 0.8% (A.C. = NMT 2%)

S. No.	Target Concentration (%)	Concentration (%, with respect to 0.423 mg /ml sample Concentration)	Concentration (mg/ml)	Area
1	(LOQ)	0.03	0.000118	6109
2	25	0.05	0.000197	9343
3	40	0.08	0.000315	14825
4	60	0.12	0.000473	22245
5	80	0.16	0.000630	29618
6	100	0.20	0.000788	37110
7	120	0.24	0.000945	44419
Correlation Coefficient [R]				0.99994
Regression Coefficient [R^2]				0.99987
Slope				46618370
Intercept				298.5



Conclusion: The Regression line of analysis shows linear relationship between concentration and response of Pantoprazole. The Correlation coefficient is 0.99994

Common problem 3:
Do not provide data within the specified range

Linearity data for Cyanoguanidine
(an impurity of Metformin HCL)

Concentration (%)	Chromatogram Data name	Area	Average Area
0.016	188-300405-004-Rep1	96004	97305
	188-300405-004-Rep2	98703	
	188-300405-004-Rep3	97209	
0.018	188-300405-005-Rep1	109572	109064
	188-300405-005-Rep2	108090	
	188-300405-005-Rep3	109530	
0.020	188-300405-006-Rep1	118974	118670
	188-300405-006-Rep2	118251	
	188-300405-006-Rep3	118785	
0.022	188-300405-007-Rep1	130472	130314
	188-300405-007-Rep2	130026	
	188-300405-007-Rep3	130443	
0.024	188-300405-008-Rep1	145206	144599
	188-300405-008-Rep2	144217	
	188-300405-008-Rep3	144373	

Sr.No.	Concentration (%)	Chromatogram Data name	Area	Average Area
1.	(LOQ) 0.001	188-300405-003-Rep1	8144	8357
		188-300405-003-Rep2	8412	
		188-300405-003-Rep3	8514	
2.	0.016	188-300405-004-Rep1	96004	97305
		188-300405-004-Rep2	98703	
		188-300405-004-Rep3	97209	
3.	0.018	188-300405-005-Rep1	109572	109064
		188-300405-005-Rep2	108090	
		188-300405-005-Rep3	109530	
4.	0.020	188-300405-006-Rep1	118974	118670
		188-300405-006-Rep2	118251	
		188-300405-006-Rep3	118785	
5.	0.022	188-300405-007-Rep1	130472	130314
		188-300405-007-Rep2	130026	
		188-300405-007-Rep3	130443	
6.	0.024	188-300405-008-Rep1	145206	144599
		188-300405-008-Rep2	144217	
		188-300405-008-Rep3	144373	
Correlation coefficient (r)		: 0.9997		
Slope of regression line		: 5865637.7		
Y-intercept		: 2646.6		
Residual sum of squares		: 6319141		

Product with more than 1 strength ??

Common problem 4:
Do not provide sufficient data (1)

TABLE 4 : LINEARITY OF DETECTOR RESPONSE

Solution .No.	Concentration (µg/mL)	Peak Area
01	0.1988	13205
02	0.7954	46805
03	0.9942	73049
04	1.9884	125581

TABLE 4 : LINEARITY OF DETECTOR RESPONSE

Solution .No.	Concentration (µg/mL)	Peak Area
01	0.1988	13205
02	0.7954	46805
03	0.9942	73049
04	1.9884	125581
05	4.9711	343187
06	7.4567	509769
07	9.9422	677937
08	14.9133	1038641
09	17.3989	1189884
10	19.8844	1338150

Co-efficient of regression (m) = 68266.874804
Constant of Regression (b) = -570.445446
Co-efficient of Correlation = 0.999

Refer Register No. ADR0565/04, Page No: 31

Acceptance Criteria:

The Correlation coefficient should be not less than 0.997.

- Insufficient linearity data
- minimum of 5 concentrations over a suitable range

Common problem 4:
Do not provide sufficient data (2A)

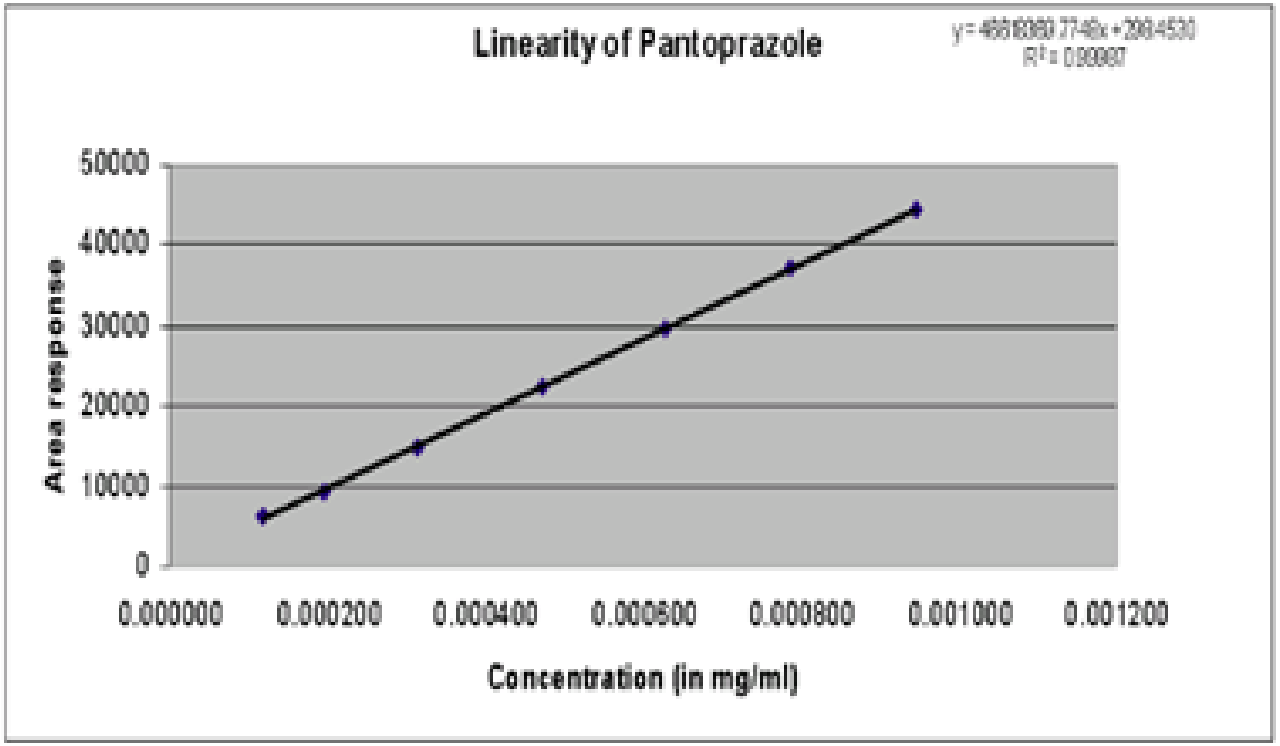
No. of Observations	Concentration of Impurity-1 in the solution µg / ml	Concentration of Impurity-1 in solution %
01	0.05 µg/ml	10%
02	0.1 µg/ml	20%
03	0.25 µg/ml	50%
04	0.375 µg/ml	75%
05	0.5 µg/ml	100%
06	0.625 µg/ml	125%
07	0.75 µg/ml	150%

No. of Observations	Concentration of Impurity-1 in the solution µg / ml	Area of peak corresponding to Impurity-1	Concentration of Impurity-1 in solution %
01	0.05 µg/ml	3690.25	10%
02	0.1 µg/ml	8015.6	20%
03	0.25 µg/ml	20648.3	50%
04	0.375 µg/ml	28976.79	75%
05	0.5 µg/ml	40429.3	100%
06	0.625 µg/ml	50843.5	125%
07	0.75 µg/ml	60202.92	150%

Model: y = Ax + B with 0, 0 A = 403.61 B = -138.76

- No peak area value for each point of linearity in tabulated form

Common problem 4:
Do not provide sufficient data (2B)



- No peak area value for each point of linearity in tabulated form

Type of analytical procedure characteristics	Identification	Testing For Impurities Quantitation Limit	Assay - dissolution (measurement only) - content/ potency
Accuracy	-	+ -	+
Precision			
Repeatability	-	+ -	+
Interm. Precision	-	+ (1) -	+ (1)
Specificity (2)	+	+ +	+
Detection Limit	-	- (3) +	-
Quantitation Limit	-	+ -	-
Linearity	-	+ -	+
Range	-	+ -	+

- signifies that this characteristic is not normally evaluated

+ signifies that this characteristic is normally evaluated

(1) in cases where reproducibility has been performed, intermediate precision is not needed

(2) lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s)

(3) may be needed in some cases

Accuracy

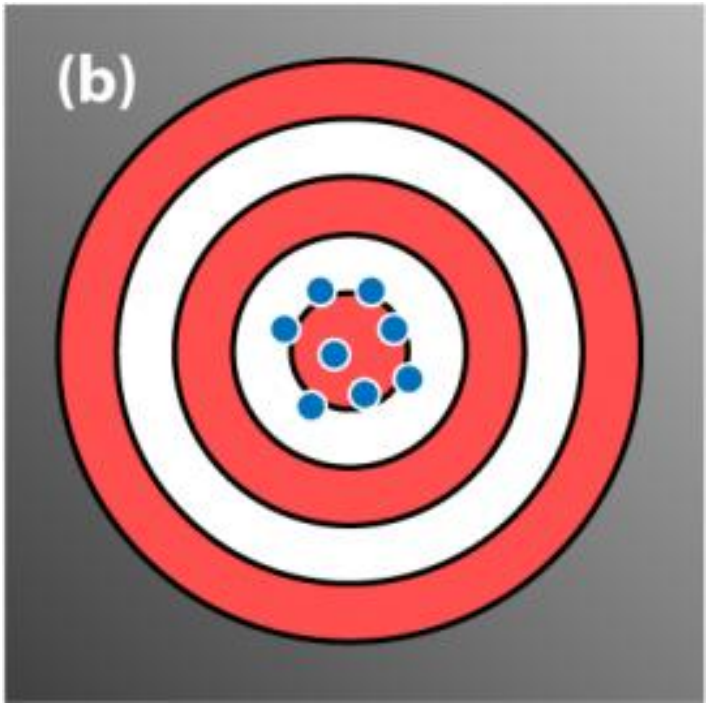
The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found

"Trueness"

Accuracy should be established across the specified range of the analytical procedure

ICH Q2(R1) Guideline, 1996

Accuracy



Accuracy

Data Required

- Testing Method
- Acceptance criteria
- Raw data in tabulated form
- % recovery or mean difference and confidence interval should be reported

Testing Method

- Minimum three (3) levels of concentration in triplicates covering the specified range

Acceptance criteria

- % recovery by the assay of known added amount of analyte in the sample (95 – 105%)

OR

- difference between the mean and the accepted true value (± 2%)

$$\%\ \text{Recovery} = \frac{\text{concentration found}}{\text{theoretical concentration}} \times 100\%$$

NPCB

Level	Amount Added (mg)	Area/Abs	Amount Recovered (mg)	% Recovery
10% Recovery	4.010	93.6184	3.968	99.0
50% Recovery	20.658	486.8524	20.635	99.9
100% Recovery	40.101	947.1021	40.143	100.1
200% Recovery	80.203	1951.2147	82.703	103.1
300% Recovery	120.305	932.4407	124.292	103.3

Level	Amount Added (mg)	Area/Abs	Amount Recovered (mg)	% Recovery
10% Recovery-1	4.010	93.6184	3.968	99.0
10% Recovery-2	4.010	94.5809	4.009	100.0
10% Recovery-3	4.010	94.6630	4.012	100.0
50% Recovery-1	20.658	486.8524	20.635	99.9
50% Recovery-2	20.658	494.5211	20.960	101.5
50% Recovery-3	20.658	492.6297	20.880	101.1
100% Recovery-1	40.101	947.1021	40.143	100.1
100% Recovery-2	40.101	943.2758	39.981	99.7
100% Recovery-3	40.101	948.5977	40.207	100.3
200% Recovery-1	80.203	1951.2147	82.703	103.1
200% Recovery-2	80.203	1945.7201	82.470	102.8
200% Recovery-3	80.203	1960.4708	83.095	103.6
300% Recovery-1	120.305	2932.4407	124.292	103.3
300% Recovery-2	120.305	2928.6372	124.131	103.2
300% Recovery-3	120.305	2914.8826	123.548	102.7
Average				101.4
CI 95% (±)				0.9
%RSD				1.6

Common Problem 1

- Insufficient data
- Minimum three (3) levels of concentration in triplicates covering the specified range

Common Problem 2

- No confidence interval

S. No	Recovery level	Amount added (mg)	Amount recovered (mg)	% Recovery	Mean % Recovery
1	50%	23.328	23.455	100.5	101.5
2	50%	23.366	23.827	102.0	
3	50%	23.356	23.790	101.9	
1	80%	37.341	38.012	101.8	100.7
2	80%	37.320	37.346	100.1	
3	80%	37.331	37.399	100.2	
1	100%	46.628	47.060	100.9	101.4
2	100%	46.624	47.284	101.4	
3	100%	46.625	47.465	101.8	
1	120%	55.940	56.834	101.6	101.3
2	120%	56.013	56.385	100.7	
3	120%	55.951	56.810	101.5	
1	150%	69.961	71.027	101.5	101.6
2	150%	69.972	70.861	101.3	
3	150%	69.997	71.416	102.0	
Average				101.3	
CI 95% (±)				√ 0.3	
% RSD				0.6	
% Average Recovery result for 15 replicates				101.0 - 101.6	
Acceptance criteria	The average recovery for each level should be between 98.0% to 102.0%.				

Type of analytical procedure characteristics	Identification	Testing For Impurities Quantitation Limit		Assay - dissolution (measurement only) - content/ potency
Accuracy	-	+	-	+
Precision				
Repeatability	-	+	-	+
Interm. Precision	-	+ (1)	-	+ (1)
Specificity (2)	+	+	+	+
Detection Limit	-	- (3)	+	-
Quantitation Limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+

- signifies that this characteristic is not normally evaluated

+ signifies that this characteristic is normally evaluated

(1) in cases where reproducibility has been performed, intermediate precision is not needed

(2) lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s)

(3) may be needed in some cases

Precision

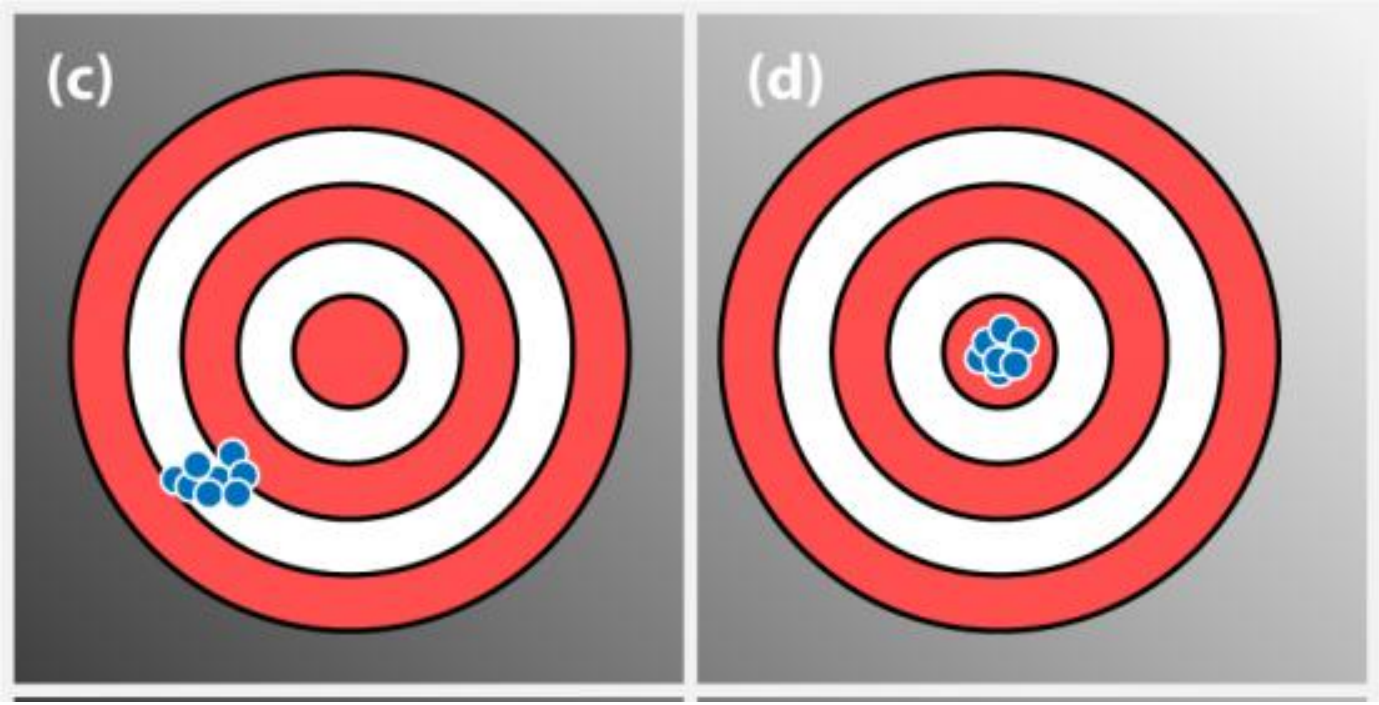
The precision of an analytical procedure expresses closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions

ICH Q2(R1) Guideline, 1996

Precision

Repeatability	• Same operating conditions • Over a short period of time • intra-assay precision
Intermediate precision (Ruggedness)	• Within laboratory variations: • *diff analyst • *diff days • *diff equipment
Reproducibility	• between laboratories

Precision



Repeatability

Data Required

- Testing Method
- Acceptance criteria
- Raw data in tabulated form
- standard deviation, relative standard deviation (coefficient of variation) and confidence interval should be reported for each type of precision investigated

Testing Method

- Minimum three (3) levels of concentration in triplicates covering the specified range

OR

- minimum six (6) replicates at 100% of the working concentration

Acceptance criteria

- RSD ≤ 2.0% (sample soln)

Method Precision !!!

NPCB

Sample	Sample Weight (g)	Equivalent $Al(OH)_3$ Weight in Sample (mg)	Sample Titre Volume (ml)	Volume EDTA VS needed (ml)	% Assayed
1	1.0859	30.68836	16.90	8.30	105.96
2	1.0916	30.84945	16.90	8.30	105.41
3	1.0861	30.69401	16.90	8.30	105.94

Sample	Sample Weight (g)	Equivalent $Al(OH)_3$ Weight in Sample (mg)	Sample Titre Volume (ml)	Volume EDTA VS needed (ml)	% Assayed	% RSD
1	1.0918	30.85510	16.20	8.30	106.00	
2	1.0910	30.83249	16.35	8.15	104.16	
3	1.0861	30.69401	16.30	8.20	105.27	
4	1.0912	30.83814	16.40	8.10	103.50	1.08
5	1.0873	30.72793	16.45	8.05	103.23	
6	1.0920	30.86075	16.40	8.10	103.42	
				Average	104.3	

Confidence interval ???

Common Problem 1

- Insufficient data
- minimum six (6) replicates at 100% of the working concentration

Common Problem 2

- No confidence interval

Replicate of Preparation	Ato
	Day 1 / Analyst 1
1	101.38
2	101.79
3	100.65
4	100.98
5	101.27
6	100.30
Average	**101.06**
CI 95% (±)	0.43
%RSD	0.53

Acceptance Criteria	**1.%RSD of six assay preparation result should not be more than 2.0%**

Intermediate Precision

Data Required

- Testing Method
- Acceptance criteria
- Raw data in tabulated form
- standard deviation, relative standard deviation (coefficient of variation) and confidence interval should be reported for each type of precision investigated

Testing Method

- Variation of analyst, date, equipment (at least 2 parameter

Acceptance criteria

- RSD $\leq$ 2.0%

Common Problem 1

- Intermediate precision not provided

Two Analyst in two days

Sample Replicate	Analyst A (Day 1)	Analyst B (Day 2)
1	106.00	105.96
2	104.16	105.41
3	105.27	105.94

Two Analyst in two days

Sample Replicate	Analyst A (Day 1)	Analyst B (Day 2)
1	106.00	105.96
2	104.16	105.41
3	105.27	105.94
4	103.50	106.41
5	103.23	106.90
6	103.42	106.75
Average	104.26	106.23
Average from two analyst in two days	105.2	
% RSD	1.27	

Calculation:

1) Average for two analyst in two days = $\frac{\text{Average of analyst A + Average of analyst B}}{2}$

2) Difference mean value of results = 106.2 % - 104.3 %

= 1.90%

Acceptance Criteria:

% RSD of combined results NMT 2.0%

Mean value of results within +/- 2.0%

Confidence interval ???

Common Problem 2

- Insufficient data

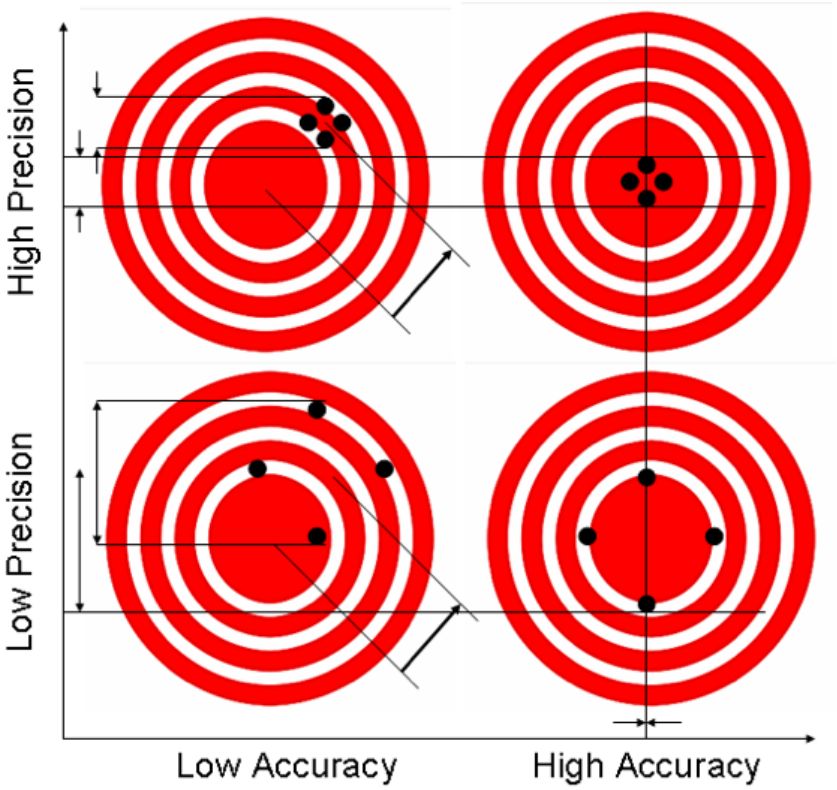
Common Problem 3

- Confidence interval not provided

NPCB

Replicate of Preparation	Atorvastatin (%)	
	Day 1 / Analyst 1	Day 2 / Analyst 2
1	101.38	101.88
2	101.79	101.76
3	100.65	102.27
4	100.98	105.26
5	101.27	103.16
6	100.30	102.43
Average	**101.06**	**102.79**
CI 95% (±)	√ 0.43	√ 1.04
%RSD	0.53	1.27
Grand Average	**101.93**	
CI 95% (±)	√ **0.74**	
% RSD	√ **1.29**	
Difference	**1.73**	
Acceptance Criteria	**1.%RSD of six assay preparation result should not be more than 2.0%** **2.The difference of the average result between both the sets should not be more than 2.0%**	

Accuracy vs Precision



Thank you