03 TdA-USP81 Capreomycin

Document-Id:	Document-1998 (Revision: 3)
Document type:	Turbidimetric assay (USP <81>, JP 4.02)/1
Last modified:	06.09.2021 11:02:49
Database:	O130_Permanent_PLA_305
Database GUID:	996faea8-9ec8-4fb7-8524-ea8686ffdf5d

Calculation information

Calculation performed:	06.09.2021 11:02:49, Benjamin Schneider (PLA 3.0.5 Build 816, NBARW01, 10014)
Report generated:	06.09.2021 11:12:42, Benjamin Schneider (PLA 3.0.5 Build 816, NBARW01, 10014)

Signatures

....
Responsibility   Review   Approval

Comments

Comment by Benjamin Schneider: (11.08.2021 16:43:04)

Example turbidimetric assay based on USP <81>: The standard consists of five concentration steps (S1 to S5) with S3 being used as reference. The relative potency of the unkown sample U3 is estimated based on the standard. The control sample CTL1 contains the test diluent but no antibiotic. Note that the interpolated concentration value of CTL1 is beyond the regression line and therefore does not allow any conclusion about the true estimate (e.g. nonlinear trend for high response values). In accordance with USP <81>, the test system includes tests on the combined standard deviation of the standard, the coefficient of determination (r^2), and the relative potency. For a direct comparison, the response values are taken from USP <81>. Moreover, the concentration is log10-transformed as recommended by USP <81>.

DOCUMENT-1998

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Documentation

Date	11.06.2021 15:44:17
Substance	Capreomycin, Polypeptide, Capreomycin is a cyclic polypeptide isolated from Streptomyces capreolus with antitubercular activity.

Inoculum
  Test organism

Species	Klebsiella pneumoniae
Incubation	20h, 36.5° C
Incubation medium	
Ingredient	Peptone, 6g
Ingredient	Pancreatic digest of casein, 4g
Ingredient	Yeast extract, 1g
Ingredient	Beef extract, 1.5g
Ingredient	Dextrose, 1g
Ingredient	Agar, 15g
Ingredient	Water, 1000ml
pH	6.6
Inoculum medium	
Ingredient	Peptone, 5g
Ingredient	Yeast extract, 1.5g
Ingredient	Beef extract, 1.5g
Ingredient	Sodium chloride, 3.5g
Ingredient	Dextrose, 1g
Ingredient	Dibasic potassium phosphate, 3.68g
Ingredient	Monobasic potassium phosphate, 1.32g
Ingredient	Water, 1000ml
pH	7
Inoculum concentration	0.05ml / 100ml
Inoculum volume per tube	9ml
Incubation	200min, 37°C

Assay overview

Assay elements	Summary
■ STD	Standard sample, 3 replicates, Sequence: 64, 80, 100, 125, 156
■ U3	Test sample, 3 replicates, Predilution: 1
■ CTL1	Control sample, 6 replicates, Predilution: 1

Property	Value
*Response data processing**	
Response adjustment	No adjustment
Response normalization	No normalization
Response transformation	No transformation
Replicate averaging	Averaging deactivated
Dose transformation	Logarithmic: Decimal logarithm (base 10)
Response unit	(not available)

* The response data processing steps are executed in the reported order.

Rack layout

	1	2	3	4
1	[1] 0.855	[2] 0.814	[3] 0.628	[1] 1.172
2	[4] 0.693	[5] 0.530	[1] 0.713	[1] 1.119

	1	2	3	4
1	[1] 0.842	[2] 0.827	[3] 0.695	[1] 1.180
2	[4] 0.685	[5] 0.578	[1] 0.796	[1] 1.085

	1	2	3	4
1	[1] 0.850	[2] 0.839	[3] 0.756	[1] 1.164
2	[4] 0.670	[5] 0.532	[1] 0.720	[1] 1.075

Assay suitability tests

	Passed	Failed (rejected)	Failed (warning)	Info
Assay suitability tests	2	0	0	0
Overall test result	**Passed**			

■ STD Test: Combined standard deviation of the standard Scope: Standard only; Severity level: Warning		Passed
Calculated value	0.03215	
Upper margin	0.10000	

■ STD Test: Coefficient of determination (R²), regular Scope: Standard only; Severity level: Warning		Passed
Calculated value	0.93037	
Lower margin	0.90000	

■ STD: Setup

Property	Value
Concentration unit	μg/ml
Number of replicates	3
Stepwise concentration sequence	64, 80, 100, 125, 156
Reference step	
Position	3
Concentration	100

■ STD: Result

Data plot
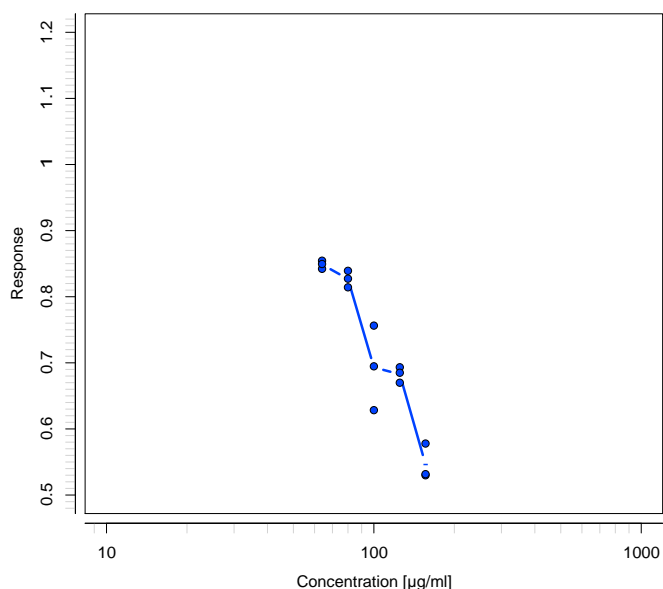
Regression plot
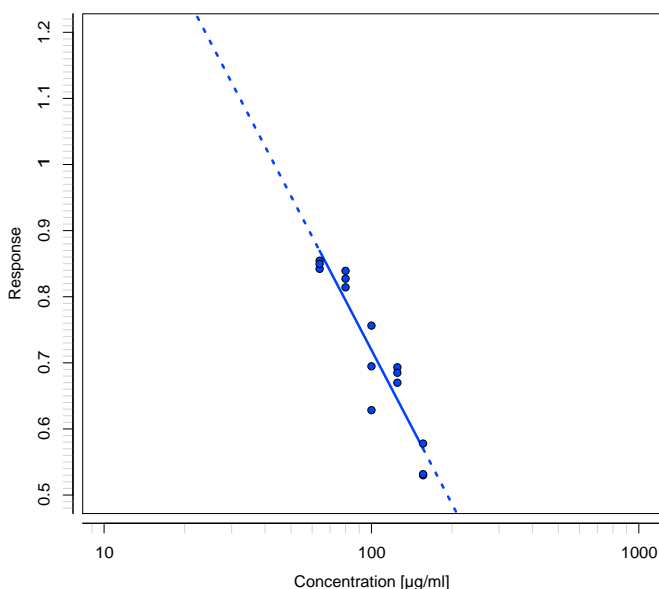
Response data

Dilution step	1	2	3	4	5
Working concentration [μg/ml]	64.00000	80.00000	100.00000	125.00000	156.00000
Response	0.85450	0.81420	0.62840	0.69330	0.52990
	0.84220	0.82730	0.69470	0.68500	0.57790
	0.84950	0.83920	0.75630	0.66990	0.53160
Mean	0.84873	0.82690	0.69313	0.68273	0.54647
SD	0.00619	0.01250	0.06396	0.01186	0.02724
CV	0.73%	1.51%	9.23%	1.74%	4.98%

Response data processing

Processing instructions

Adjustment → Normalization → Transformation → Averaging

Step	Details
Response adjustment	No adjustment
Response normalization	No normalization
Response transformation	No transformation
Replicate averaging	Averaging deactivated

Regression

Parameter	Estimate	Error
Intercept	2.26650	0.24491
Slope	-0.77351	0.12218

	Quality of regression
r^2	0.93037
r^2 adjusted	0.90715

Sample suitability tests

	Passed	Failed (rejected)	Failed (warning)	Info
Assay suitability tests	2	0	0	0
Sample suitability tests	0	0	0	0
Overall test result	**Passed**			

■ U3: Setup

Property	Value
Concentration unit	µg/ml
Number of replicates	3
Predilution	1

■ U3: Result

Interpolation plot
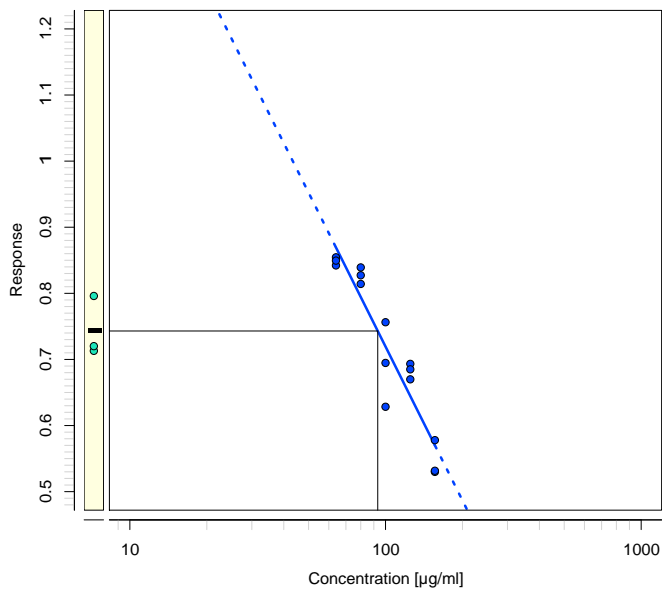
Response data

	Raw data
Response	0.71300
	0.79600
	0.72010
Mean	0.74303
SD	0.04601
CV	6.19%

Response data processing

Processing instructions

Adjustment → Normalization → Transformation → Averaging

Step	Details
Response adjustment	No adjustment
Response normalization	No normalization
Response transformation	No transformation
Replicate averaging	Averaging deactivated

Interpolation

Property	Value
Absolute potency [µg/ml]	93.23037
Potency of the working sample [µg/ml]	93.23037
Relative potency (percentage of reference concentration)	93.23%

Sample suitability tests

	Passed	Failed (rejected)	Failed (warning)	Info
Assay suitability tests	2	0	0	0
Sample suitability tests	1	0	0	0
Overall test result	**Passed**			

■ **U3** Test: Relative potency (percentage of reference concentration) Scope: Test samples only; Severity level: Warning		Passed
Calculated value	93.23037	
Margins	80.00000 to 125.00000	

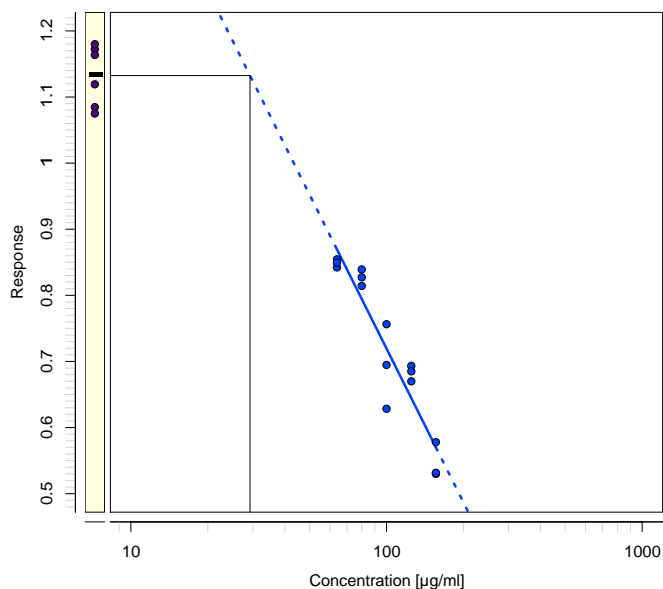| ■ **U3** Test: Relative potency (percentage of reference concentration) Scope: Test samples only; Severity level: Warning		Passed
Calculated value	93.23037	
Margins	80.00000 to 125.00000	

■ CTL1: Setup

Property	Value
Concentration unit	µg/ml
Number of replicates	6
Predilution	1

■ CTL1: Result

Interpolation plot

Response data

	Raw data
Response	1.17230
	1.11920
	1.17990
	1.08470
	1.16360
	1.07500
Mean	1.13245
SD	0.04597
CV	4.06%

Response data processing

Processing instructions

Adjustment → Normalization → Transformation → Averaging

Step	Details
Response adjustment	No adjustment
Response normalization	No normalization
Response transformation	No transformation
Replicate averaging	Averaging deactivated

Interpolation

Property	Value
Absolute potency [µg/ml]	29.24925
Potency of the working sample [µg/ml]	29.24925
Relative potency (percentage of reference concentration)	29.25%

Sample suitability tests

	Passed	Failed (rejected)	Failed (warning)	Info
Assay suitability tests	2	0	0	0
Sample suitability tests	0	0	0	0
Overall test result	**Passed**			