Validation of mass per vial by external standard

The experiments, statistics and acceptance criteria that establish, analyte by analyte, the mass per vial of SOP-06: specificity, linearity, accuracy with its confidence interval, precision by analysis of variance, range and solution stability.

Not yet approved
No approval of this revision is on file. This protocol states the validation the laboratory will perform; no run under it counts until a named reviewer approves it.

Document		Laboratory	
Number	VP-05	Name	Cupel Laboratories
Revision	1, first issue	Address	2599 Speakman Dr Mississauga, ON L5K 1B1
Status	Not yet approved		
Effective	Not yet in effect; takes effect when approved	Country	Canada
		Not on file	Not yet published: legal entity, postal address, telephone and country
Applies to	Test codes QNT, FULL; every analyte of the scope table, one report each	Web	cupel.ca
Supersedes	Nothing; first issue	Email	help@cupel.ca
Fingerprint	2d9b 26ea a0d0 3ab2		

1 Purpose

To establish, for one analyte at a time, that SOP-06 measures the mass of that peptide in a vial: that the whole vial dissolves and is recovered by the route the procedure prescribes, that the response is free of interference, linear and accurate over the range a vial's contents can fall in, how precise the result is within and between runs as s_r and s_run (the terms that replace the provisional ones of the certificate's budget, floor ±2% relative), and that solutions are stable for a sequence.

2 Scope and status

The method is Mass per vial (SOP-06). It is validated one analyte at a time: each analyte of the scope table gets its own data file and its own report, VR-05, against this protocol. Test codes QNT, FULL.

Category: ICH Q2(R2) assay (content of the named peptide); USP <1225> Category I.

Status: 0 of 14 analytes have a data file under this protocol and 0 are validated by it. No validation has been performed. This protocol takes effect when it is approved; no validation run counts before then.

3 What the category requires

Characteristic	ICH Q2(R2)	USP <1225>	This protocol
Specificity	required	required	section 7
Dissolution and recovery of the whole vial	not in the table	not in the table	section 8
Response (linearity)	required	required	section 9
Range	required	required	section 13
Accuracy	required	required	section 10
Repeatability	required	required	section 11
Intermediate precision	required	required	section 11
Quantitation and detection limits	not required	not required	not studied: not required
Solution stability	not in the table	not in the table	section 12

Required and not required as ICH Q2(R2) Table 1 and USP <1225> Table 2 set them for this category; a characteristic outside both tables is studied because the method's use needs it.

4 Responsibilities

Role	Responsibility
Analyst	Runs the experiments as written here, records every value in the data file as it is produced, and names the raw data files they came from.
Reviewer	Checks the data file against the raw data, composes the report, and signs it as reviewed; does not review their own runs.
Approver	Approves this protocol before any run, and the report after; a person named in the laboratory's people.

Named: Timothy Ian Benedicto, Medical Laboratory Technician; Roxanne Manlapaz, Laboratory Technician.

5 Prerequisites

5.1 SOP-06 is approved: its parameters are the ones validated, and a change to them after validation is a change to the method.

5.2 The instrument is installed, qualified and calibrated under SOP-09, and named in the data file. No instrument is listed yet: make, model and serial number are published on the methods page and on each certificate once the instrument is installed and its serial plate can be photographed, and never for an instrument the laboratory does not own.

5.3 Reference materials are on file with their certificates: the analyte's reference standard with its assigned content and the basis of it, and for each experiment the materials it names.

5.4 The people who run and review the work are trained on SOP-06 and on this protocol, and the training is recorded.

5.5 The data file is opened before the first run and filled as the work proceeds; a value is never entered from memory.

6 Decisions and the verdict

- Every criterion is evaluated on the value rounded to its reported precision, the decision rule the certificates apply: a limit of 102.0% is met by 102.04% (reported 102.0) and not by 102.05% (reported 102.1).
- Each criterion is decided Pass, Fail, Not evaluated (its data are missing or short of the design) or Not applicable (its condition is absent, with the reason stated); a statistic with no criterion is reported for information.
- A characteristic passes when none of its criteria fails or is missing. The method is validated for the analyte when every criterion of every characteristic passes, and the report is approved by a person named in the laboratory's people; the scope row then reads Validated, with the date.
- Criteria marked laboratory default are the laboratory's own and are not regulatory limits; a criterion from a cited text cites it. A criterion may be changed before data are taken, as an amendment with its reason, recorded in the data file and printed in the report beside the protocol's own; never after.
- A departure from the design is recorded as a deviation with its impact and printed in the report. A failed criterion is not repeated until a different answer comes: a repeat needs a cause found and recorded (SOP-08).

7 Specificity

ICH Q2(R2) specificity; USP <1225>

Nothing but the peptide contributes to the area the content is computed from.

Design

7.1 Inject the diluent blank; record the area at the analyte's retention time and the response of the 100% standard.

7.2 Record the peak-purity result of the main peak in the 100% standard and in a 100% sample solution.

Specificity, continued

7.3 The separation is SOP-02's: the forced-degradation evidence is VR-01's for the same analyte, cited by its data file and evaluated with this report.

Statistics

blank interference (%) = A_{blank} / A_{100} × 100

Acceptance criteria

Id	Criterion	Source
SP1	Blank response at the analyte's retention time, as a share of the 100% response, at most 0.05%	the reporting threshold (data.json method row)
SP2	The main peak passes its peak-purity test in the standard and the sample	laboratory default
SP3	VR-01 for the same analyte passes its specificity	VP-01

Recorded in the data file as specificity.blank_area, .response_100, .purity (one per solution) and .chromatographic (the VR-01 data file).

8 Dissolution and recovery of the whole vial

ICH Q2(R2) accuracy; METHOD_DESIGN 1.8, 2.3; METHOD_REVIEW S11, M2

The content is the mass the method recovers from the vial: the powder must dissolve completely in the routine medium, leave nothing on the glass, and lose nothing to the container between dissolution and injection. Each arm is run per analyte in its routine medium (10 mM Tris, pH 7.8, LAL grade for semaglutide, tirzepatide and retatrutide).

Design

8.1 Clarity: every preparation of this protocol is inspected against black and white before any aliquot is taken and recorded as clear and free of visible particles, or described, with the time to dissolve; a preparation that is not clear is a dissolution-completeness question under SOP-06, not a result.

8.2 Fourth-rinse residue: after the stock is withdrawn and the vial (and tube) rinsed three times as SOP-06 prescribes, a fourth rinse with the strong solvent (acetonitrile and water 1 + 1 with 0.1% trifluoroacetic acid) is injected under SOP-06 and the peptide found is expressed as percent of the content; six preparations.

8.3 Strong-solvent comparison: three vials dissolved by the routine route and three by the strong solvent, the content found by each; the ratio routine to strong, with the 95% confidence interval of its mean.

8.4 Prepared-vial recovery: at most 250 µL of a reference-standard solution in water (the salt only, no buffer) dispensed into empty vials of the routine format at 50, 100, 150% of the nominal fill, the mass dispensed taken by difference on the semi-micro balance, dried in a vacuum centrifuge or freeze-dried, stoppered, then dissolved and quantified by the whole SOP-06 route; three vials per level. Taken and found are both against the same standard, so its assigned content cancels: this arm is independent of the vendor's assignment, and it is the accuracy evidence the accuracy characteristic relies on.

8.5 Container comparison: the stock and each working dilution of it (the content solution and, for semaglutide, tirzepatide and retatrutide, the Tris stock diluted into mobile phase A, the Tris stock added to the pre-weighed mobile phase A with immediate mixing, never the reverse) held in the routine low-binding container and injected at 0, 6, 24 h, the response as percent of 0 h and the clarity recorded at each time; a loss located here is a container or mixing loss, which the prepared-vial arm detects but does not locate.

Statistics

residue (%) = $m_{rinse\,4}$ / $m_{content}$ × 100

ratio (%) = $m_{routine}$ / m_{strong} × 100; 95% CI of a mean = mean ± $t_{0.975,\,n-1}$ s / sqrt(n)

recovery (%) = m_{found} / m_{taken} × 100 (the standard's content cancels)

response(t) (%) = A(t) / A(0) × 100

Dissolution and recovery of the whole vial, continued

Acceptance criteria

Id	Criterion	Source
DR1	Clarity recorded for every preparation, each clear and free of visible particles before any aliquot	SOP-06
DR2	Mean fourth-rinse residue at most 0.2%	laboratory default
DR3	Fourth-rinse residue of every preparation at most 0.5%	laboratory default
DR4	Mean ratio of the routine to the strong-solvent content from 98 to 102%	laboratory default
DR5	95% confidence interval of that ratio, both ends, from 97 to 103%	laboratory default
DR6	Mean prepared-vial recovery at every level from 98 to 102%	laboratory default
DR7	95% confidence interval of the mean prepared-vial recovery over all levels, both ends, from 98 to 102%	laboratory default
DR8	Response of every held solution at every time, as percent of 0 h, from 98 to 102%	laboratory default

Recorded in the data file as dissolution.clarity (each: preparation, clear, time_min), .rinse (each: preparation, residue_pct), .strong_solvent (routine_mg and strong_mg, paired), .prepared (each level: x_pct; determinations with taken_mg and found_mg) and .container (each solution: name, clarity; points with hours and area).

9 Linearity

ICH Q2(R2) response; USP <1225> linearity

Area proportional to concentration over the range a vial can fall in, with the intercept small beside the 100% response.

Design

9.1 100% is the concentration a vial at its nominal fill gives under SOP-06: the stock at 2 mg/mL nominal (the whole vial dissolved by mass, the volume being the nominal fill divided by 2: a 5 mg fill in 2.50 mL) diluted 1:10 in mobile phase A, 0.20 mg/mL whatever the fill. From the reference standard prepare 25, 50, 75, 100, 150, 200% of that (0.30 mg/mL is the 150% level), concentrations corrected for the standard's assigned content.

9.2 Inject each level three times.

Statistics

$b = S_{xy} / S_{xx}$; $a = y_m - b\, x_m$; $S_{xx} = \text{sum}\,(x_i - x_m)^2$, $S_{xy} = \text{sum}\,(x_i - x_m)(y_i - y_m)$

$r = S_{xy} / \text{sqrt}(S_{xx}\, S_{yy})$; $s_{y/x} = \text{sqrt}(SS_{res} / (n - 2))$

$SE(b) = s_{y/x} / \text{sqrt}(S_{xx})$; $SE(a) = s_{y/x}\, \text{sqrt}(1/n + x_m^2 / S_{xx})$; 95% CI = estimate ± $t_{0.975,\, n-2}$ SE

relative residual (%) = $100\,(y_i - y_{fit}) / y_{fit}$

intercept (%) = $100\, a / (a + b\, x_{100})$

back-calculated level (%) = $100\,(y_{mean} - a) / (b\, x)$

lack of fit: $F = (SS_{lof} / (k - 2)) / (SS_{pe} / (n - k))$, $p = P(F_{k-2,\, n-k} > F)$ (information)

Acceptance criteria

Id	Criterion	Source
LN1	Coefficient of determination r^2 at least 0.999	laboratory criterion, the routine one (results.QNT_R2_MIN)
LN2	Intercept, as a share of the 100% response, within ±2.0%	laboratory default
LN3	Back-calculated value of every level above the lowest, as percent of its nominal value, from 98 to 102%	laboratory default
LN4	Back-calculated value of the lowest level, as percent of its nominal value, from 95 to 105%	laboratory default

Recorded in the data file as nominal_mg_ml; linearity.levels (each: x in mg/mL, areas).

10 Accuracy

ICH Q2(R2) accuracy; USP <1225> accuracy

A known mass is found again: recovery at three levels in triplicate, with the confidence interval of the mean.

Design

10.1 Prepare a second, independently assigned reference material at 50, 100, 150% of nominal, three weighings per level, and quantify each against the linearity calibration. “Independently assigned” is met by a second lot assigned by the laboratory by mass balance with its cross-checks (REFERENCE_STANDARDS route M), or by a quantitative NMR or isotope-dilution amino-acid analysis of the same lot at an accredited provider (routes Q and A); the data file names which, and the report prints it.

10.2 With one lot only, the experiment is independent weighings of that lot and is named for what it is: a precision of weighings, not an accuracy. Accuracy then rests on the lot’s assigned content, whose uncertainty the report prints beside the result, and on the prepared-vial recovery (dissolution and recovery, above), which is the accuracy evidence independent of the vendor’s assignment because the standard’s content cancels in it.

Statistics

found (mg/mL) = (A – a) / b

recovery (%) = found / taken × 100

95% CI of a mean = mean ± $t_{0.975,\,n-1}$ s / sqrt(n)

Acceptance criteria

Id	Criterion	Source
AC1	Mean recovery at every level from 98 to 102%	laboratory default
AC2	95% confidence interval of the mean recovery over all levels, both ends, from 98 to 102%	laboratory default

Recorded in the data file as accuracy.material and .levels (each: x_pct; determinations with taken_mg_ml and area).

11 Precision

ICH Q2(R2) repeatability, intermediate precision; USP <1225>

How far the result moves between whole-vial preparations of one material, within a run and between runs each with its own calibration, as s_r and s_run. “Validated” means this report’s s_r and s_run replace the provisional terms of the certificate’s budget (lab/results.py), not that a floor was met; no criterion of the form 2 RSD_IP at most the floor is set (METHOD_REVIEW M3).

Design

11.1 Repeatability: six independent whole-vial preparations by the SOP-06 route (the vented vial weighed, dissolved by mass, the content dilution by mass) from one homogeneous material (vials of one lot filled from the reference standard, or one lot of the analyte), quantified against the linearity calibration, so that the three weighings and the dilution are inside s_r.

11.2 Intermediate precision: two further runs on different days, each with its own five-level calibration, three preparations each, a second analyst where one is trained; the first run is the repeatability set. Report s_r and s_run.

Precision, continued

11.3 Budget on these data: the report tabulates the terms SOP-06's budget combines, with this report's s_r / sqrt(3) (the three preparations of a sample) and s_run in place of the provisional terms: the standard's content (u_RS, 3.0% relative when the certificate states none, results.QNT_STANDARD_U_REL_PCT_DEFAULT), the standard's weighing (0.5%, results.QNT_STANDARD_WEIGHING_U_REL_PCT), the preparation by mass (0.10%, results.QNT_PREPARATION_U_REL_PCT) and the calibration (s_y/x / (b x_100) from this report's regression, a planning figure); then U = 2 u_c for the fill classes 2, 5, 10, 50 mg. The certificate's own budget is lab/results.py's; this table shows what the validation changes in it.

Statistics

result (%) = found / taken × 100

RSD (%) = 100 s / mean

$u_c^2 = u_{RS}^2 + u_{weigh}^2 + u_{prep}^2 + u_{cal}^2 + s_r^2 / n + s_{run}^2$; $U = 2\ u_c$

one-way ANOVA, runs as groups: $MS_w = \text{sum}\ (y_{ij} - y_j)^2 / (N - k)$; $MS_b = \text{sum}\ n_j\ (y_j - y_m)^2 / (k - 1)$

$s_r^2 = MS_w$; $s_{run}^2 = \max(0, (MS_b - MS_w) / n_0)$; $n_0 = (N - \text{sum}\ n_j^2 / N) / (k - 1)$

$s_{IP} = \text{sqrt}(s_r^2 + s_{run}^2)$ (ISO 5725-2; USP <1210>)

Acceptance criteria

Id	Criterion	Source
PR1	Repeatability RSD at most 2.0%	laboratory default
PR2	Intermediate-precision RSD at most 3.0%	laboratory default

Recorded in the data file as precision.repeatability (each: taken_mg_ml, area, and the vial's w0_mg, wc_mg, w2_mg, vented, medium_mass_g) and .intermediate (each: run, then calibration and preparations, or from).

12 Solution stability

ICH Q2(R2) and Q14 robustness

How long the calibration standard and a sample solution may wait in the autosampler.

Design

12.1 Keep the 100% standard solution and one sample solution in the autosampler; inject each at 0, 12, 24, 48 h.

Statistics

response (%) = A(t) / A(0) × 100

stable to t when both solutions are within the limit at every point up to t

Acceptance criteria

Id	Criterion	Source
SS1	Established solution stability at least 24 h	laboratory default (the longest sequence)

Recorded in the data file as solution_stability.standard and .sample (each: hours, area).

13 Range

ICH Q2(R2) range; USP <1225> range

The concentrations over which linearity, accuracy and precision have been shown.

Design

13.1 Derived; no further experiment.

Range, continued

Statistics

range = [lowest, highest linearity level]

Acceptance criteria

Id	Criterion	Source
RG1	Linearity, accuracy and precision all pass, and every accuracy and precision level lies in the range	ICH Q2(R2)

Recorded in the data file as derived.

14 Data and report

- The data file is lab/validation/qnt-<analyte>.json, one per analyte (the annex lists every file name), with kind “plumbline-validation” and schema 1. Every value is entered as measured, with the raw data file it came from in the run’s record.
- The report VR-05 is composed from that file by python lab/validation.py report <file> <out.pdf>: every statistic computed from the file and printed with its formula and this data’s numbers, every criterion with its decision.
- lab/validation_examples/qnt-semaglutide.json shows the format; it is illustrative data, marked as such in the file and on every page of its report, and no scope row ever reads from it.
- The scope table reads each analyte’s file: Not yet validated without one, In validation with one, Validated when every criterion passes and the approval is signed.

15 Annex A: analytes and their data files

Analyte	Data file	Status
Semaglutide	lab/validation/qnt-semaglutide.json	Not yet validated
Tirzepatide	lab/validation/qnt-tirzepatide.json	Not yet validated
Retatrutide	lab/validation/qnt-retatrutide.json	Not yet validated
BPC-157	lab/validation/qnt-bpc-157.json	Not yet validated
TB-500 (Tbeta-4 17–23)	lab/validation/qnt-tb-500.json	Not yet validated
Ipamorelin	lab/validation/qnt-ipamorelin.json	Not yet validated
CJC-1295 (no DAC)	lab/validation/qnt-cjc-1295-no-dac.json	Not yet validated
Tesamorelin	lab/validation/qnt-tesamorelin.json	Not yet validated
Bremelanotide (PT-141)	lab/validation/qnt-bremelanotide.json	Not yet validated
Melanotan II	lab/validation/qnt-melanotan-ii.json	Not yet validated
AOD-9604	lab/validation/qnt-aod-9604.json	Not yet validated
Thymosin alpha-1 (thymalfasin)	lab/validation/qnt-thymosin-alpha-1.json	Not yet validated
Epitalon (AEDG)	lab/validation/qnt-epitalon.json	Not yet validated
GHK (sold as GHK-Cu)	lab/validation/qnt-ghk-cu.json	Not yet validated

16 References and controlled sources

Standards and other documents

- ICH Q2(R2) Validation of analytical procedures (2023); USP <1225> Category I.
- ISO 5725-2 and USP <1210> for the variance components.
- Cited for what they ask; this protocol claims no conformity with them, and a number they do not set is marked as the laboratory’s.

References and controlled sources, continued

Controlled sources, read at every build

- lab/validation.py: this protocol's characteristics, design and criteria, and the arithmetic of the report.
- data.json method rows (the routine conditions); lab/results.py (the routine criteria and the provisional uncertainty floors the validation tests).

17 Deviations and open points

- A departure from this protocol's design is recorded in the data file as a deviation with its impact and printed in the report; one found afterwards is nonconforming work, handled under SOP-08.
- No instrument is listed yet: make, model and serial number are published on the methods page and on each certificate once the instrument is installed and its serial plate can be photographed, and never for an instrument the laboratory does not own.
- Accuracy rests on the assigned content of the reference material; a second, independently assigned material is the design, and the report says which was used.
- No analyte has been validated under this protocol.

18 Records

- The data file and the raw data files it names; the report as composed and as approved; the approvals.
- Kept for the life of the method and never deleted while a report relies on the validation.

19 Revision history

Revision	Change	Approval
1	First issue.	Not yet approved

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Current revisions

End of document