Validation of water content by coulometric Karl Fischer

The experiments, statistics and acceptance criteria that establish, analyte by analyte, the whole-vial water content of SOP-05: standard and spike recovery and precision against the provisional floor.

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-04	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 H2O, FULL; every analyte of the scope table, one report each	Web	cupel.ca
Supersedes	Nothing; first issue	Email	help@cupel.ca
Fingerprint	2e6f 20f2 878a 7aa1		

1 Purpose

To establish, for one analyte at a time, that SOP-05 releases the water of a whole sealed vial in the oven at the temperature it fixes, without decomposing the peptide, and recovers it quantitatively in the cell over the water amounts a vial holds; that the gross fill by three weighings of a vented vial carries the uncertainty the model assigns it; and how precise a whole-vial determination is within a day and between days, as s_r and s_run, the terms that replace the provisional ones of the certificate's budget (floor ±0.1% w/w) once this report is approved.

2 Scope and status

The method is Water content (SOP-05). It is validated one analyte at a time: each analyte of the scope table gets its own data file and its own report, VR-04, against this protocol. Test codes H2O, FULL.

Category: USP <921> Method Ic, evaporation technique; Ph. Eur. 2.5.32: the evaporation technique, water released from the whole sealed vial in an oven and carried to the coulometric cell by a dry gas. Accuracy and precision are shown as USP <1225> asks of a quantitative procedure; the range is the water amounts of the standard recovery. Specificity is not shown apart: the temperature study and the spike recovery are the tests that the oven releases the water, all of it and nothing but it.

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
Oven temperature, release and weighing	not in the table	not in the table	section 7
Accuracy: standard recovery	required	required	section 8
Accuracy: spike recovery in the matrix	required	required	section 9
Repeatability	required	required	section 10
Intermediate precision	required	required	section 10
Range	required	required	section 11

What the category requires, continued

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-05 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-05 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 Oven temperature, release and weighing

METHOD_DESIGN 3.2; METHOD_REVIEW M1, M6; USP <41>, <1251>

The oven temperature is the one that releases the vial's water completely without decomposing the peptide; the gross fill is a difference of three large masses and is trusted only when the vial was vented before the first and the weighing repeats inside the uncertainty the model assigns.

Design

7.1 Temperature ramp: one vial of at least 5 mg of material heated from ambient to at most 180 °C at the oven's ramp rate with the carrier flowing; record the water-release rate against temperature (the oven's record) and the temperature at which release is complete, below which no second rise begins. The ramp stops below the charring range; a second rise is decomposition, not water.

7.2 Isothermal runs: whole vials of one lot at T – 20, T and T + 20 °C (T the candidate temperature), three vials at each, the extraction ended on the relative-drift stop SOP-05 sets and the extraction time recorded; the carrier flow recorded. W(T) is the mean water in % w/w at each temperature.

7.3 The T – 20 side is reported for information: it is the sensitivity to an oven error, and with an oven tolerance of a few degrees against a change of at most 5% per 20 °C it is small; no criterion is set on it.

7.4 Release bias: when W(T + 20) exceeds W(T) by more than 2% the method under-releases by its own evidence, a bias replication does not shrink; the report states release_bias_rel_pct = (W(T + 20) – W(T)) / W(T) / sqrt(3) × 100, the Type B term SOP-05's budget takes (lab/results.py reads it from the set), and 0 when the difference is within 2%.

7.5 Residue check: the residue of a vial run at T is dissolved and run under SOP-02 and SOP-03 beside an unheated vial of the same lot; record the purity of both, the largest new peak, and whether the identity is confirmed.

7.6 Venting: for each vial format and lot, weigh one sealed vial as received and again after venting (the stopper lifted for a minute for content vials, a needle through the stopper for ten minutes for water vials); record the difference and the vendor's stoppering (air, nitrogen, partial vacuum) as far as it is known. The difference is the headspace term SOP-05 removes by venting; it is recorded, not judged.

7.7 Weighing study: for each vial format, ten repeats of the three weighings (the vented vial W0, the closure Wc, the bare glass W2) on each of two days on the balance SOP-05 names, with its readability and minimum weight recorded; G = W0 – Wc – W2 per repeat, its standard deviation per format and day against the u(G) the model assigns for the vent method (stopper lifted 0.04 mg; needle 0.09 mg).

7.8 The blank and drift convention: the instrument's drift compensation is on for blank and sample runs alike, from a drift measured with the carrier flowing immediately before each run; the blank entered is the drift-compensated mean of the day's empty-vial runs, and blank_drift_compensated is recorded true. The correction is made once (METHOD_REVIEW M4).

Statistics

release ratio (%) = W(T + d) / W(T) × 100; information: W(T – d) / W(T) × 100

release_bias_rel_pct = (W(T + d) – W(T)) / W(T) / sqrt(3) × 100 when the ratio exceeds 102%, else 0

$G = W_0 - W_c - W_2$; s(G) per format and day

$\text{purity change (\% area)} = \text{purity}_{\text{heated}} - \text{purity}_{\text{unheated}}$

Acceptance criteria

Id	Criterion	Source
TP1	Water at T + 20 °C as percent of the water at T (release complete at T) at most 105%	laboratory default (METHOD_REVIEW M6)
TP2	Ramp run on at least 5 mg to at most 180 °C	laboratory default (METHOD_REVIEW S5, S7)
RS1	Purity of the heated residue against the unheated vial within ±0.3% area	the purity floor (results.U_FLOOR_PURITY_PCT)
RS2	Largest new peak in the heated residue below 0.10% area	the identification threshold (results.Q3A_IDENTIFICATION_PCT)
RS3	Identity of the heated residue confirmed under SOP-03	SOP-03

Oven temperature, release and weighing, continued

VT1	A vented/unvented pair recorded for every vial format and lot, with the stoppering	laboratory default (METHOD_REVIEW M1)
WG1	Standard deviation of G over the repeats, every format and day, at or below the model's u(G) for the vent method	results.GROSS_FILL_U_MG_BY_VENT
WG2	Drift compensation on for blank and sample runs alike, the blank entered drift-compensated	METHOD_REVIEW M4

Recorded in the data file as oven (temperature_c, carrier_flow, extraction_s, blank_drift_compensated; ramp with max_c, mass_mg and complete_at_c; isothermal, one entry per offset with its vials; residue with the purities, the largest new peak and the identity; venting, one entry per format and lot; weighing, one entry per format and day with its repeats of w0, wc and w2).

8 Standard recovery

USP <921> Method Ic; Ph. Eur. 2.5.32; ICH Q2(R2) accuracy

The oven and the cell find the water they are given, over the amounts of water a vial holds.

Design

8.1 Weigh the certified solid water standard for the oven by difference into an oven vial at about 50, 100, 200, 400 µg of water, crimp, and run it through the programme at the oven temperature; three determinations at each amount.

8.2 Record the drift before each determination, the mass of standard and the water found, net of the drift-compensated blank.

Statistics

$W_{certified}$ (µg) = $m_{standard}$ (mg) × $w_{certified}$ (mg/g)

recovery (%) = W_{found} / $W_{certified}$ × 100

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

Acceptance criteria

Id	Criterion	Source
SR1	Recovery of every determination from 97.5 to 102.5%	laboratory criteria (results.KF_REC_LO, KF_REC_HI)
SR2	95% confidence interval of the mean recovery, both ends, from 97.5 to 102.5%	laboratory criteria
SR3	Drift before every determination at most 10 µg/min	laboratory criteria for the oven cell (results.KF_OVEN_DRIFT_MAX_UG_MIN)

Recorded in the data file as standard (name, lot, certified_mg_g); standard_recovery (each: level, determinations).

9 Spike recovery in the matrix

ICH Q2(R2) accuracy

Water added beside a whole vial is found again over the vial's own water: the matrix neither holds water back nor adds anything the cell titrates.

Design

9.1 Weigh the solid water standard into the oven vial beside a whole vial of the repeatability lot at about 50, 100, 150% of a vial's water, three vials at each level, crimp and run as SOP-05 prescribes.

9.2 The unspiked water is the repeatability vials' mean, per mg of sample, scaled by each spiked vial's mass.

Statistics

w_0 (µg/mg) = mean water content of the unspiked vials

$W_0 = w_0$ m; $W_{added} = m_{standard} \times w_{certified}$

recovery (%) = ($W_{found} - W_0$) / W_{added} × 100

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

Spike recovery in the matrix, continued

Acceptance criteria

Id	Criterion	Source
SK1	Mean recovery at every level from 95 to 105%	laboratory default
SK2	95% confidence interval of the mean recovery over all levels, both ends, from 95 to 105%	laboratory default

Recorded in the data file as spike_recovery (each: level; determinations with mass_mg, added_standard_mg and found_ug).

10 Precision, the blank and the quantitation limit

ICH Q2(R2) repeatability, intermediate precision; ISO 5725-2 and 5725-6

How far a whole-vial result moves between vials of one lot, within a day and between days, as the variance components s_r and s_run; the day's blanks, whose spread sets the cell's quantitation limit. "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: the vial-to-vial water of a real lot is inside s_r, and no criterion of the form 2 s_IP at most the floor is set (METHOD_REVIEW M3).

Design

10.1 Repeatability: six whole vials of one lot, one fill class, one analyst, one day; each vial's gross fill G from its three weighings (the vented vial, the closure, the bare glass), the weighings recorded beside the mass.

10.2 Intermediate precision: three further vials on each of two further days, by a second analyst where one is trained; the first day is the repeatability set. Report s_r, s_run and the repeatability limit r = 2.8 s_r, which SOP-05 applies to its duplicate.

10.3 Blanks: at least three empty-vial runs on every day of the study, drift-compensated, each day's mean and standard deviation recorded; the quantitation limit of the cell is 10 × s_blank (results.KF_LOQ_FACTOR), and the repeatability vials' water must sit at or above 1 × that limit (results.KF_QUANT_MIN_FACTOR) for the result to be reported with its uncertainty rather than as below the limit.

Statistics

$G\ (\text{mg}) = W_0 - W_c - W_2$; water (% w/w) = W (µg) / (G (mg) × 1000) × 100

RSD (%) = 100 s / mean

$r = 2.8\ s_r$; LOQ (µg) = $10\ s_{blank}$, the largest day's

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
RP1	Repeatability RSD at most 5%	laboratory default
IP1	Intermediate-precision RSD at most 7.5%	laboratory default
BL1	Standard deviation of each day's blanks (at least three a day) at most 3 µg	laboratory criterion (results.KF_BLANK_SD_MAX_UG)
LQ1	Water per vial of the repeatability vials, every vial, against 1 × LOQ:	results.KF_LOQ_FACTOR and KF_QUANT_MIN_FACTOR

Recorded in the data file as repeatability (each: mass_mg as G, w0_mg, wc_mg, w2_mg, vented, vent_method, water_ug, drift_ug_min); intermediate (each: run, then vials or from); blanks (each: day, values_ug).

11 Range

ICH Q2(R2) range

The water amounts over which recovery is shown cover the water a vial of this analyte holds.

Design

11.1 Derived; no further experiment.

Statistics

range = [lowest, highest certified amount of the standard recovery]

Acceptance criteria

Id	Criterion	Source
RG1	The mean water per vial of the repeatability vials lies within the range of the standard recovery	ICH Q2(R2)

Recorded in the data file as derived.

12 Data and report

- The data file is lab/validation/kf-<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-04 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/kf-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.

13 Annex A: analytes and their data files

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

14 References and controlled sources

Standards and other documents

- USP <921> Water determination, Method Ic; Ph. Eur. 2.5.32.
- ICH Q2(R2) (2023) and USP <1225> for accuracy and precision; ISO 5725-2 and USP <1210>.
- 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.

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).

15 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.
- No analyte has been validated under this protocol.

16 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.

17 Revision history

Revision	Change	Approval
1	First issue.	Not yet approved

The fingerprint in the control block is the SHA-256 of this document's text as composed, first 16 hex digits. A change in any controlled source changes it; a copy whose fingerprint differs from the approved one is not the approved text, and a change is approved as a new revision.

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Current revisions

End of document