Validation of purity by RP-HPLC–UV

The experiments, statistics and acceptance criteria that establish, analyte by analyte, the purity method of SOP-02: specificity under forced degradation, the impurity response and its limits, precision, robustness 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-01	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 PUR, IDP, FULL; every analyte of the scope table, one report each	Web	cupel.ca
Supersedes	Nothing; first issue	Email	help@cupel.ca
Fingerprint	f197 d338 7b59 b2c0		

1 Purpose

To establish, for one analyte at a time, that SOP-02 measures the purity of that peptide and the share of each impurity it reports: that the main peak is free of interference and of its own degradation products, that the impurity response is linear and accurate from the quantitation limit to the top of the reporting range, what that limit is and whether it supports the 0.05% reporting threshold, how precise the impurity shares are within a run and between runs, how far the purity moves when the gradient and the temperature move, and how long a prepared solution may wait in the autosampler.

2 Scope and status

The method is Purity by RP-HPLC–UV (SOP-02). It is validated one analyte at a time: each analyte of the scope table gets its own data file and its own report, VR-01, against this protocol. Test codes PUR, IDP, FULL.

Category: ICH Q2(R2) quantitative test for impurities (purity is 100 minus the impurities of one area normalisation, so the impurity shares are what is validated); USP <1225> Category II, quantitative.

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
Response (linearity)	required	required	section 8
Response of the main peak at nominal	required	required	section 9
Range	required	required	section 15
Accuracy	required	required	section 10
Repeatability	required	required	section 12
Intermediate precision	required	required	section 12
Quantitation limit	required	required	section 11

What the category requires, continued

Detection limit	not required	not required	section 11
Robustness	not in the table	not in the table	section 13
Solution stability	not in the table	not in the table	section 14

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-02 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-02 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>; USP <621>

The main peak is measured alone: nothing in the blank sits under it, its degradation products are separated from it, and no co-eluting component hides in it.

Design

7.1 The nominal solution is the routine preparation of SOP-02: the vial dissolved whole by mass, to 2.0 mg/mL nominal in LAL reagent water (10 mM Tris of LAL grade for semaglutide, tirzepatide and retatrutide), then 1 + 1 in mobile phase A to 1.0 mg/mL nominal, 2 µL injected; for semaglutide, tirzepatide and retatrutide the stock is the Tris stock added to the pre-weighed mobile phase A with immediate mixing, never the reverse. The injection solvent is the medium 1 + 1 in mobile phase A, an acid mixture whose pH is recorded as measured (about 2.5), never assumed neutral. Record the nominal solution's concentration on the free-peptide basis, c_val = the nominal concentration × the reference standard's content fraction: the basis the quantitation limit below is stated on.

7.2 The blank is the sample's own matrix: the medium 1 + 1 in mobile phase A, prepared with the samples (for semaglutide, tirzepatide and retatrutide the medium is 10 mM Tris, pH 7.8, LAL grade, whose Tris elutes in the void, so the integration start is set from this blank and recorded). Inject it in every sequence and record the area at the main peak's retention time and the main-peak area of the nominal solution of the same sequence.

7.3 Stress portions of one sample preparation and keep an unstressed control at 2–8 °C, protected from light: acid, 0.1 M hydrochloric acid, 40 °C, 24 h, neutralised before injection; base, 0.01 M sodium hydroxide, 25 °C, 4 h, neutralised before injection; oxidation, 0.1% hydrogen peroxide, 25 °C, 2 h; heat, the solution at 60 °C, 24 h; light, ICH Q1B option 2 exposure: 1.2 million lux hours and 200 W h/m² near-ultraviolet. These are starting conditions: one that degrades the main peak by less than 5% or more than 20% is repeated milder or harsher, and the conditions as run are recorded.

7.4 Inject each stressed portion and the control under SOP-02, the diode-array detector recording spectra across the main peak, and acquire the same injections by LC–MS across the main peak (the identity solution of SOP-03, the mass spectrometer in line): the mass evidence is the specificity evidence, because related peptides share the main peak's spectrum at 214 nm; the diode-array peak purity is supporting.

7.5 For each, record the main-peak area, the total integrated area (blank-matched peaks excluded), the number of degradation peaks at or above the reporting threshold, the resolution of the main peak from the nearest of them, the peak-purity result of the main peak with its threshold, and from the LC–MS trace the largest co-eluting mass of a degradation product under the main peak, as percent area equivalent of the main peak (0 when none is seen).

7.6 Where a reference material of a related substance can be obtained (an epimer, an isoaspartate form, a truncation: the annex lists the isobaric forms of each analyte), inject it alone and spiked into the sample, and record its resolution from the main peak. Where none can be, the report says so and the isobaric forms stay an open point.

Statistics

blank interference (%) = $A_{blank} / A_{main,\ nominal} \times 100$

degradation (%) = $(1 - A_{main,\ stressed} / A_{main,\ control}) \times 100$

mass balance (%) = $A_{total,\ stressed} / A_{total,\ control} \times 100$

$R_s = 1.18\ (t_{R2} - t_{R1}) / (W_{0.5,1} + W_{0.5,2})$ (USP <621>)

Acceptance criteria

Id	Criterion	Source
SP1	Blank response at the main-peak retention time, as a share of the nominal main-peak area, at most 0.05%	the reporting threshold (data.json method row; ICH Q3A(R2) by analogy)

Specificity, continued

SP2	Resolution of the main peak from every degradation peak at or above the reporting threshold, in every stressed sample, at least 1.5	USP <621> (results.SS_RESOLUTION_MIN)
SP3	The main peak passes its peak-purity test in every stressed sample	laboratory default (the detector software's purity algorithm)
SP4	Loss of the main peak in at least one stress condition from 5 to 20%	laboratory default (the study shows the method sees degradation; ICH Q1A(R2) sets no number)
SP5	Mass balance of every stressed sample from 90 to 110%	laboratory default
SP6	Resolution of every related substance run as a reference material from the main peak at least 1.5	USP <621>
SP7	Largest co-eluting mass of a degradation product under the main peak by LC–MS, in every stressed sample, as percent area equivalent, below 0.10%	the identification threshold (results.Q3A_IDENTIFICATION_PCT); CRITIQUE_17025 F18

Recorded in the data file as specificity.blank (with the integration start it set and the medium), .c_val_mg_ml, .solvent_ph, .control, .stress (one entry per condition, with lcms_coeluting_pct) and .related (one per related substance).

8 Linearity of the impurity response

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

Impurities are quantified by the main peak's own response; the response must be proportional from the reporting threshold to the top of the range.

Design

8.1 From the reference standard (or, with none, a sample of the analyte, and the report says so) prepare a stock at the nominal concentration and dilute it to 0.05, 0.1, 0.5, 1, 2.5, 5% of nominal, each level by its own dilution.

8.2 Inject each level three times in one sequence, lowest first, after a blank.

8.3 Record every main-peak area with its level as percent of nominal.

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 (%) = $100\,a / (a + b\,x_{ref})$, x_{ref} = 1.0% of nominal (Ph. Eur. 2034 qualification threshold for synthetic peptides)

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	Correlation coefficient r at least 0.990	laboratory default (ICH Q2(R2) asks for r and sets no number)
LN2	Intercept, as a share of the response at 1.0% of nominal, within ±5.0%	laboratory default
LN3	Back-calculated value of every level above the lowest, as percent of its nominal value, from 90 to 110%	laboratory default
LN4	Back-calculated value of the lowest level, as percent of its nominal value, from 80 to 120%	laboratory default

Recorded in the data file as linearity.levels (each: x as percent of nominal, areas).

9 Linearity of the main peak at nominal

ICH Q2(R2) response; CRITIQUE_17025 F18

An area-percent purity needs the main peak inside the detector's linear range at the nominal load: a main peak that saturates reads every impurity share high.

Design

9.1 From the nominal solution prepare 50, 75, 100, 120% of nominal by dilution in the injection solvent and inject each level three times; record the main-peak area and height.

9.2 Fall-back when LN5 or LN6 fails (the detector is not linear at the nominal load): the injection volume is halved and the quantitation limit (the limits characteristic below) is re-established at that load. The limit is set by the amount on column, so halving the injection doubles its share of nominal; the documented outcome for that analyte is then a reporting threshold of 0.1% (Ph. Eur. 2034's reporting threshold for synthetic peptides), recorded in the data file and printed in the report, not a deviation.

Statistics

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

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

Acceptance criteria

Id	Criterion	Source
LN5	Back-calculated value of the 100% level, as percent of nominal, from 98 to 102%	laboratory default
LN6	Back-calculated value of the 120% level, as percent of nominal, from 97 to 103%	laboratory default

Recorded in the data file as main_linearity.levels (each: x as percent of nominal, areas) and .fallback (null, or the halved injection with the re-established limit).

10 Accuracy of the impurity share

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

A known share at impurity level is reported as that share. With no impurity standard, ICH Q2(R2) allows the drug substance's own response factor: the analyte is diluted to the level and measured as area percent of the nominal solution.

Design

10.1 Prepare the analyte at 0.1, 0.5, 1% of nominal, three independent dilutions at each level, and the nominal solution.

10.2 Inject each preparation once and the nominal solution six times. Where a related substance's reference material exists it may be spiked into the sample at the same levels instead, the unspiked sample injected as the background.

10.3 Record each area, any background area at the same retention time, and the nominal areas.

Statistics

found (%) = $(A - A_{background}) / A_{100,\,mean} \times 100$

recovery (%) = found / level × 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 90 to 110%	laboratory default
AC2	95% confidence interval of the mean recovery over all levels, both ends, from 90 to 110%	laboratory default

Recorded in the data file as accuracy.reference_areas and .levels (each: x, areas, background_areas).

11 Quantitation and detection limits

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

The smallest share the method quantifies, measured two ways and confirmed by precision at that level; the reporting threshold is meaningful only at or above it.

Design

11.1 Inject a solution at the reporting threshold (0.05% of nominal) six times. For each, measure the peak height H from the extrapolated baseline and the peak-to-peak noise h of a blank over at least five times the peak's width at half height, centred where the peak elutes (USP <621>; Ph. Eur. 2.2.46).

11.2 Prepare five levels near the expected limit (0.02, 0.03, 0.05, 0.07, 0.1% of nominal), inject each three times and record the areas.

11.3 Inject a solution at the candidate quantitation limit six times and record each area, H and h: the confirmed level is the established quantitation limit.

Statistics

S/N = 2H / h (USP <621>; Ph. Eur. 2.2.46)

$DL_{S/N} = 3\ c\ /\ (S/N)_{mean}$; $QL_{S/N} = 10\ c\ /\ (S/N)_{mean}$

DL = 3.3 $s_{y/x}$ / b; QL = 10 $s_{y/x}$ / b (ICH Q2(R2); $s_{y/x}$ and b of the low-range regression)

RSD (%) = 100 s / mean

Acceptance criteria

Id	Criterion	Source
QL1	QL by signal-to-noise, at most the confirmed level	ICH Q2(R2) (10:1)
QL2	QL by 10 s_y/x / b, at most the confirmed level	ICH Q2(R2)
QL3	Mean signal-to-noise at the confirmed level at least 10	ICH Q2(R2) (typical 10:1)
QL4	Area RSD of the injections at the confirmed level at most 10%	laboratory default
QL5	Confirmed quantitation limit, as percent of nominal, at most 0.040%	laboratory default (0.8 × the reporting threshold, data.json method row)

Recorded in the data file as limits.sn (x; injections, each with height and noise), .low_range.levels (each: x, areas) and .confirmation (x; injections, each with area, height and noise); the established limit is also stated as an absolute concentration, ql_abs_ug_ml = QL × c_val, with ql_basis free peptide, the fields the result model reads (lab/results.py hplc.sample).

12 Precision of the impurity shares

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

How far the reported shares move between preparations in one run, and between runs on different days, analysts and columns; the purity's intermediate precision is what the provisional uncertainty floor stands in for.

Design

12.1 Repeatability: six independent preparations of one lot, each run as SOP-02 runs a sample (the mean of its injections is the preparation's result), in one run.

12.2 Intermediate precision: three runs on different days, by two analysts where two are trained, on two columns of different lots, three preparations per run.

12.3 Record the purity (main-peak area percent) and every impurity at or above the quantitation limit, by its relative retention time.

12.4 Integration repeatability: one chromatogram of each precision run is re-integrated six times under SOP-02's integration policy, by two analysts (three each); record the purity of every re-integration. Its standard deviation is u_int, the integration term of the certificate's budget (results.HPLC_INTEGRATION_U_PCT, 0.05% area, a design estimate until this measures it). Each precision run is integrated once by its own analyst, so s_IP contains one integration of each chromatogram and not the spread between integrations: u_int is added to s_IP in the budget, not inside it, and the report says so.

Precision of the impurity shares, continued

Statistics

total impurities (%) = 100 – purity

RSD (%) = 100 s / mean, s with n – 1

u_{int} = s of the purity over the re-integrations of one chromatogram, the largest over the runs

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 of the total impurities at most 10%	laboratory default
PR2	Repeatability RSD of every impurity: at most 10% from 0.50%, 15% from 0.10% and 20% below	laboratory default
PR3	Intermediate-precision RSD of the total impurities at most 15%	laboratory default
PR4	Expanded intermediate precision of the purity, 2 s_IP, at most 0.3% area	the provisional floor it replaces (results.U_FLOOR_PURITY_PCT)
PR5	Standard deviation of the purity over the re-integrations of one chromatogram, every run, at most 0.05% area	the integration term of the budget (results.HPLC_INTEGRATION_U_PCT)

Recorded in the data file as precision.repeatability (each: main_pct, impurities by relative retention time), .intermediate (each: run, results) and .integration (each: run, analysts, purities of the re-integrations).

13 Robustness of the gradient, the temperature, the flow, the acid and the medium

ICH Q2(R2) and Q14 robustness

The result holds when the conditions move within what a laboratory can expect of them.

Design

13.1 Run the suitability standard and one sample preparation at the nominal conditions (40 °C; 5–60% B over 20 min; 0.30 mL/min) and under each variation, one factor at a time: column temperature 35 °C and 45 °C; gradient time 18 min and 22 min; initial composition 4% B and 6% B; flow 0.27 and 0.33 mL/min; trifluoroacetic acid in both mobile phases 0.08% and 0.12%; and for semaglutide, tirzepatide and retatrutide Tris in the medium 5 mM and 20 mM (the medium's buffer strength moves the stock's pH and the acid consumed on mixing; the injection solvent itself is the acid mixture and its pH is not a factor).

13.2 Record the resolution of the critical pair, the symmetry factor of the main peak and the purity under each condition.

Statistics

change = $\text{purity}_{condition} - \text{purity}_{nominal}$

Acceptance criteria

Id	Criterion	Source
RB1	System suitability in every condition: resolution at least 1.5 and symmetry factor 0.8–1.8	USP <621> (results.SS_*)
RB2	Purity in every varied condition, against the nominal result, within ±0.3% area	laboratory default (the provisional floor)

Recorded in the data file as robustness (each: condition, nominal, rs, symmetry, main_pct).

14 Solution stability

ICH Q2(R2) and Q14 robustness

How long a prepared sample may wait in the autosampler before its purity moves.

Design

14.1 Keep one sample preparation in the autosampler at its set temperature and inject it at 0, 12, 24, 48 h; record the purity and any peak at or above the reporting threshold not present at 0 h.

Statistics

change(t) = purity(t) – purity(0)

stable to t when every point up to t is within the limit and shows no new peak

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.points (each: hours, main_pct, new_peaks).

15 Range

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

The interval of impurity shares over which linearity, accuracy and precision have been shown: from the quantitation limit to the top linearity level.

Design

15.1 Derived from the characteristics above; no further experiment.

Statistics

range = [established QL, highest linearity level]

Acceptance criteria

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

Recorded in the data file as derived.

16 Data and report

- The data file is lab/validation/hplc-<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-01 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/hplc-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; the quantitation limit this protocol establishes is the Reported LOQ column.

17 Annex A: analytes and their data files

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

18 Annex B: isobaric forms each analyte's run must separate

From lab/analytes_reference.json: forms of the same formula, which mass cannot tell apart and only retention against a reference material can. Specificity runs any of them that can be obtained (SP6).

Analyte	Isobaric forms on file
Semaglutide	Epimers, identical formula: D-His1, D-Ser, D-Phe and others; Isoaspartate (beta-Asp) at Asp9
Tirzepatide	Regio-isomer acylated on Lys16 instead of Lys20; Epimers, identical formula: D-Tyr1 and others; Isoaspartate (beta-Asp) at Asp9 or Asp15
Retatrutide	Regio-isomer acylated on Lys16 instead of Lys17; Epimers, identical formula: including the alpha-methylleucine stereocentre; Isoaspartate (beta-Asp) at Asp9 or Asp15
BPC-157	Epimers, identical formula: any of the 15; Isoaspartate (beta-Asp) at Asp10 or Asp11; Prolyl cis/trans conformers of the Pro3-Pro4-Pro5 run; Sequence permutations (a Pro or Gly out of order)
TB-500 (Tbeta-4 17–23)	Sequence permutations (e.g. Ac-KLKTETQ)
Ipamorelin	Epimers, identical formula: L-2-Nal3, L-Phe4, D-His2; 1-Naphthylalanine positional isomer of the 2-Nal3 residue
CJC-1295 (no DAC)	L-Ala2 epimer (hGRF with Gln8, Ala15, Leu27); Epimers, identical formula: any other residue
Tesamorelin	(Z)-hex-3-enoyl isomer; Hex-2-enoyl positional isomer of the cap; Epimers, identical formula: any of the 44
Bremelanotide (PT-141)	Epimers, identical formula: L-Phe4, which is position 7 in alpha-MSH numbering; D-His3
Melanotan II	Epimers, identical formula: L-Phe4, D-His3
AOD-9604	Epimers, identical formula: any of the 16
Thymosin alpha-1 (thymalfasin)	Epimers, identical formula: any of the 28; Isoaspartate (beta-Asp) at Asp2, Asp6 or Asp15
Epitalon (AEDG)	Sequence permutations (ADEG, EADG ...); Isoaspartate (beta-Asp) at Asp3 (an Asp-Gly motif, the most prone); gamma-Glu-linked isomer (Ala-gamma-Glu-Asp-Gly)
GHK (sold as GHK-Cu)	Sequence permutations (HGK, KHG ...)

19 References and controlled sources

Standards and other documents

- ICH Q2(R2) Validation of analytical procedures (2023) and ICH Q14 Analytical procedure development (2023).
- USP <1225> Validation of compendial procedures; USP <621> Chromatography; Ph. Eur. 2.2.46.
- ICH Q1A(R2) and Q1B for the stress conditions; ICH Q3A(R2) and Ph. Eur. 2034 for the thresholds.
- ISO 5725-2 and USP <1210> for the variance components.

References and controlled sources, continued

– 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).

20 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.
– The reporting threshold is meaningful only at or above the quantitation limit this protocol establishes; until a report is approved, certificates say that no limit of quantitation is established.
– No analyte has been validated under this protocol.

21 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.

22 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