Validation of identity by LC–MS

The experiments, statistics and acceptance criteria that establish, analyte by analyte, the identity call of SOP-03: what it refuses, what a mass cannot tell apart, and the mass accuracy behind its criterion.

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-02	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 ID, IDP, FULL; every analyte of the scope table, one report each	Web	cupel.ca
Supersedes	Nothing; first issue	Email	help@cupel.ca
Fingerprint	4984 9770 93dd 23ec		

1 Purpose

To establish, for one analyte at a time, that SOP-03's identity call accepts the named peptide and refuses what is not it: that no ion of a blank reads as the analyte, that the related peptides the laboratory can obtain are refused, which known variants the 10 ppm criterion separates by mass and which it cannot, and that the instrument's mass accuracy over replicate injections on several days leaves the criterion a margin.

2 Scope and status

The method is Identity by LC–MS (SOP-03). It is validated one analyte at a time: each analyte of the scope table gets its own data file and its own report, VR-02, against this protocol. Test codes ID, IDP, FULL.

Category: ICH Q2(R2) identification test, for which specificity is the one characteristic required; USP <1225> Category IV. The identity criterion is a number ([M+nH]n+ within 10 ppm of theoretical), so the mass accuracy behind it is measured too.

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 8
Mass accuracy and its distribution	not in the table	not in the table	section 9
Instrument suitability (resolving power)	not in the table	not in the table	section 7

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-03 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-03 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 Instrument suitability

SOP-03; results.MS_HIGH_RES_MIN_RP

A ppm criterion needs a resolved isotope cluster: it applies only on an instrument resolving the cluster at the charge states recorded.

Design

7.1 Before the first injection of each day, record the instrument class, the resolving power (FWHM) and the m/z it was measured at.

Instrument suitability, continued

Statistics

R = m/z / (peak width at half height, in m/z)

Acceptance criteria

Id	Criterion	Source
MS0	Resolving power (FWHM) at least 10 000	results.MS_HIGH_RES_MIN_RP (no ppm criterion on a unit-resolution instrument)

Recorded in the data file as instrument_class, resolving_power, resolving_power_at_mz.

8 Specificity

ICH Q2(R2) specificity; USP <1225> Category IV

The identity call is made on the analyte's ions only, refuses a related peptide, and states what it cannot distinguish.

Design

8.1 Acquire a blank (the purity diluent) under SOP-03 and extract every charge state of the analyte in the scan range (m/z 300–2 200) within the criterion over the analyte's retention window; record the largest intensity as percent of the analyte's base peak in a standard injection.

8.2 Acquire each related peptide the laboratory can obtain as a reference material and apply SOP-03's identity rule to it as if it were the analyte; record every charge state observed and its monoisotopic m/z.

8.3 Where a reference standard of the analyte is held, inject it under SOP-02 in the same sequence as the sample and record the retention time of its main peak and the retention time at which the sample's identity ions were taken, with the standard's lot and aliquot identifier. Mass alone is blind to every isobaric form of Annex B; the retention match is the evidence against them. With no standard held, the criterion is not applicable and the report says so.

8.4 For GHK-Cu the ions recorded for the copper complex (the adducts carrying copper) are listed in the data file and are not used for the identity call, which is made on the ions computed from the formula in the scope table; the report lists them as recorded.

8.5 The variants of each analyte are computed from lab/analytes_reference.json (the annex): the separation of each from the analyte at every charge state in the scan range, and whether one of its isotope peaks falls within the criterion of the analyte's monoisotopic peak, or the other way round.

Statistics

$m/z_{theo} = (M + z\,m_p) / z$, $m_p = 1.007276$ Da

error (ppm) = $(m/z_{obs} - m/z_{theo}) / m/z_{theo} \times 10^6$

variant separation (ppm) = $d_m / (M + z\,m_p) \times 10^6$, d_m the variant's mass difference

isotope overlap (ppm) = $(d_m - k \times 1.003354835) / (M + z\,m_p) \times 10^6$, k = ±1, ±2, ±3

Acceptance criteria

Id	Criterion	Source
SP1	Largest blank response at an analyte m/z, as percent of the analyte's base peak, at most 1.0%	laboratory default
SP2	Every related peptide run as a challenge is refused by the identity rule	SOP-03 identity rule
SP3	Retention time of the identity ions against the reference standard's main peak, where a standard is held, within ±0.20 min	SOP-03's main-peak link (results.MS_RT_LINK_MIN); CRITIQUE_17025 F18

Recorded in the data file as specificity.blank.rel_intensity_pct (one per charge state), .challenges (each: name, formula, observed), .retention (standard lot and aliquot, standard_rt_min, ions_rt_min; null when no standard is held) and .excluded_ions (the copper adducts of GHK-Cu, when any).

9 Mass accuracy and its distribution

SOP-03 criterion; ICH Q2(R2) precision

The error of every recorded charge state over replicate injections on several days, against the criterion the certificate applies.

Design

9.1 On each of three days, after the instrument's mass calibration, inject the reference standard six times.

9.2 Record for every charge state of each injection the monoisotopic m/z of the resolved isotope cluster.

Statistics

mean error (bias), s and RMS = sqrt(sum e_i^2 / n) over every observation

95% prediction interval = mean ± $t_{0.975,\,n-1}$ s sqrt(1 + 1/n)

Acceptance criteria

Id	Criterion	Source
MA1	Error of every observation (|error| rounded to 0.1 ppm) within ±10 ppm	the identity criterion (data.json method row)
MA2	95% prediction interval of one observation within ±10 ppm	laboratory default
MA3	Mean error (bias) within ±5.0 ppm	laboratory default (half the criterion)

Recorded in the data file as mass_accuracy.injections (each: day, label, observed with z and mz_obs).

10 Data and report

- The data file is lab/validation/ms-<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-02 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/ms-bremelanotide.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.

11 Annex A: analytes and their data files

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

12 Annex B: what the mass criterion separates, by analyte

Computed from lab/analytes_reference.json at the criterion of 10 ppm over the charge states in the scan range: the variants on file separated by mass, the isotope overlaps the criterion alone cannot exclude (the identity call reads the whole cluster), and the isobaric forms it cannot see at all.

Analyte	Separated	Isotope overlaps within the criterion	Isobaric
Semaglutide	6 of 6	Deamidation of Gln17 (4.7 ppm)	2
Tirzepatide	3 of 3	Deamidation of Gln19 or Gln24 (4.0 ppm)	3
Retatrutide	2 of 2	Deamidation of Gln3 or Gln19 (4.1 ppm)	3
BPC-157	1 of 1	none	4
TB-500 (Tbeta-4 17–23)	3 of 3	none	1
Ipamorelin	0 of 0	none	2
CJC-1295 (no DAC)	3 of 3	Deamidation of Gln8, Gln16 or Gln24 (5.7 ppm)	2
Tesamorelin	3 of 3	Deamidation of Asn8 or Asn35 (3.8 ppm)	3
Bremelanotide (PT-141)	3 of 3	none	1
Melanotan II	2 of 2	none	1
AOD-9604	4 of 4	Disulfide reduced to two thiols (4.9 ppm); 'HGH Fragment 176-191' as commonly sold: this sequence with both cysteines reduced (4.9 ppm)	1
Thymosin alpha-1 (thymalfasin)	2 of 2	Deamidation of Asn28 (6.2 ppm)	2
Epitalon (AEDG)	1 of 1	none	3
GHK (sold as GHK-Cu)	0 of 0	none	1

13 References and controlled sources

Standards and other documents

- ICH Q2(R2) (2023): identification tests; USP <1225> Category IV.
- lab/analytes_reference.json for the variants of each analyte (compiled, not measured; its own note says so).
- 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).

14 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.
- Isobaric forms are not separated by any mass criterion; the certificate's scope sentence says so for every identity result.
- No analyte has been validated under this protocol.

15 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.

16 Revision history

Revision	Change	Approval
1	First issue.	Not yet approved

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Current revisions

End of document