Purity by RP-HPLC–UV

Area percent of the main peak at one wavelength, with system suitability, one integration policy, ICH Q3A rounding and an expanded uncertainty on every certificate.

Not yet approved
No approval of this revision is on file. This revision states what the laboratory's results system enforces today; it takes effect when a named reviewer approves it.

Document		Laboratory	
Number	SOP-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 PUR, IDP, FULL		
Supersedes	Nothing; first issue	Web	cupel.ca
Fingerprint	38d9 b006 59f8 d113	Email	help@cupel.ca

1 Purpose

To determine the purity of a peptide as the area percent of its main peak by reversed-phase HPLC with UV detection at 214 nm, with the chromatogram, every peak at or above the reporting threshold, three injections and their spread, and whatever could not be resolved, all printed on the certificate.

2 Scope

Lyophilised peptides as sold, for the analytes of the scope table whose purity method is "RP-HPLC–UV 214 nm": Semaglutide, Tirzepatide, Retatrutide, BPC-157, TB-500 (Tbeta-4 17–23), Ipamorelin, CJC-1295 (no DAC), Tesamorelin, Bremelanotide (PT-141), Melanotan II, AOD-9604, Thymosin alpha-1 (thymalfasin), Epitalon (AEDG), GHK (sold as GHK-Cu). Test codes PUR and IDP; FULL includes it. Minimum sample, as the intake conditions state it: "one sealed vial as sold".

Validation: 0 of 14 analytes carry a signed validation report; a scope row reads validated only when one exists, and until then its status is "written".

3 Principle

The sample is reconstituted, injected three times on a C18 column under a water/acetonitrile gradient with 0.1% trifluoroacetic acid and detected by UV absorbance at 214 nm, where the peptide bond absorbs. Purity is the main peak's share of the integrated area, blank-matched peaks excluded; it is an area percent at one wavelength and not a mass percent.

What that figure cannot show, as the laboratory publishes it:

> RP-HPLC–UV purity is reported as area percent at a single wavelength. A co-eluting impurity with weak absorbance at 214 nm is under-counted; one with strong absorbance is over-counted. Where we suspect co-elution we say so in the impurities paragraph and name the retention time. Area percent also says nothing about how much of the powder is peptide. The counter-ion (trifluoroacetate or acetate), water and any bulking excipient do not absorb at 214 nm and are invisible to a purity figure; a 99% area powder is commonly 75–90% peptide by mass. Mass per vial, with water content, is the test that answers that question, and the

Principle, continued

> certificate prints the balance: peptide, water, counter-ion at its stoichiometric maximum, impurities, and what is left.
>
> The limits page, “Area percent is not mass percent”

4 Responsibilities

Role	Responsibility
Analyst	Prepares the sample and the mobile phases, runs the blank, the standard and the sample, integrates under the one policy below, enters the run and signs as analyst.
Reviewer	Reads the chromatograms, the suitability table and the warnings; decides whether a run with a missed criterion stands; signs the content as reviewed (SOP-07).

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

5 References and controlled sources

Standards and other documents

- USP <621> Chromatography (December 2022 harmonised text) and Ph. Eur. 2.2.46: system suitability (symmetry factor, plate number, resolution, repeatability) and the peak-width conventions.
- ICH Q3A(R2) Impurities in New Drug Substances: reporting, identification and qualification thresholds; rounding before comparison with a threshold (section 5).
- JCGM 100:2008 (GUM) 6.2 and Annex G; ISO/IEC 17025:2017 7.6 and 7.8.6.
- SOP-03 (the identity ions are taken from this run’s main peak), SOP-06 (the same chromatography quantifies against a standard), SOP-07 (review and issue), SOP-09 (the instrument).

Controlled sources, read at every build

- data.json method.rows (every chromatographic parameter below), scope.rows, limitations.
- lab/results.py: the suitability criteria, the normalisation, the rounding and the uncertainty; lab/CONTRACT.md section 1 (hplc).
- lab/entry_server.py: the peak-table paste and its rules.

6 Safety

- Read the current safety data sheet of every reagent before first use and keep it where the work is done. Wear a laboratory coat, safety glasses and chemical-resistant gloves for every step below.
- Treat every sample as a potent, biologically active substance of unconfirmed identity until its report says otherwise: open and weigh lyophilised powder so that it is not inhaled, and keep it off bare skin.
- Trifluoroacetic acid (in mobile phases A and B) is corrosive in its neat form and its vapour is harmful: dispense the neat acid with eye and hand protection, with ventilation that keeps the vapour from the face.
- Acetonitrile is highly flammable and toxic by inhalation, ingestion and skin contact: keep it from ignition sources and keep reservoirs capped.
- Neat trifluoroacetic acid is dispensed only in the fume hood, from a 1 mL ampoule where one is available, with chemical splash goggles and butyl or neoprene gloves; the 1 L mobile-phase preparation and every 4 L bottle transfer are done in the hood. Nitrile gloves are a splash barrier only for acetonitrile and methanol: change them at once when wetted.
- HPLC effluent goes to a closed waste container with a vented cap and the solvent lines through the cap, never an open funnel; its level is checked each morning; solvents and mobile phases live in the flammables cabinet.
- A stock of Semaglutide, tirzepatide and retatrutide diluted into mobile phase A can foam: cap before inverting. The needle used to vent a vial (SOP-06) and every broken vial go to the sharps container at the balance.

Safety, continued

- The one open-powder step the methods share, the reference standard's weighing (SOP-06), is done in the ventilated balance enclosure with double nitrile gloves.
- Collect solvent and reagent waste in closed, labelled containers for disposal as hazardous chemical waste; never pour it to drain.
- SAF-01, the laboratory safety document (the chemical hygiene plan, the waste plan and the exposure and spill response), is published beside this procedure and governs beyond the notes here: its hazard table, the protective equipment by task and the facts it marks as not yet supplied by the owner are the controlling text; the notes here are the bench reminders of it.

7 Equipment and materials

- A UHPLC with a binary gradient pump, a thermostatted column compartment and a UV or diode-array detector, under a chromatography data system that exports the integration report and the trace. 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.
- Column: C18, 2.1 × 100 mm, 1.7 µm. Its serial number or lot is recorded in the set and printed under Method parameters. Each column has a log sheet (SOP-09): serial, date installed, injections, the pressure at equilibration of every run, the storage solvent (acetonitrile and water 1 + 1 without acid); a column whose back-pressure at equilibration has risen by half, or whose standard symmetry factor leaves 0.8–1.8 twice, is retired.
- The instrument's own identifier, make and model and software are recorded in the set; the identifier is required to issue.
- A semi-micro balance (readability 0.01 mg) with a draft shield, an anti-static ionizer and a vial adapter, the balance SOP-06 and SOP-05 share; a calibrated thermometer reading to 0.5 °C for the medium's temperature; low-binding polypropylene autosampler vials for every solution at or below 1 mg/mL (SOP-09).

8 Reagents

Mobile phase A	Water, 0.1% trifluoroacetic acid. Recipe: to 1 000 mL of chromatography-grade water in a 1 L solvent bottle add 1.0 mL of chromatography-grade trifluoroacetic acid with a 1 mL positive-displacement pipette or from a 1 mL glass ampoule, in the fume hood; cap, invert ten times; sonicate or vacuum-degas 5 min.
Mobile phase B	Acetonitrile, 0.1% trifluoroacetic acid. Recipe: as A, with acetonitrile in place of water.
Labels and hold times	Each bottle is labelled with its name (MP-A, MP-B), the preparation date and time, the analyst, the expiry and the lots of water, acetonitrile and acid; a bottle is never topped up. Expiry: A 7 days, B 30 days, the laboratory's defaults until trended. The lots and bottle numbers are recorded on WS-04 (purity run).
Diluent and matrix	Mobile phase A, into which the stock is diluted; the sample's matrix is therefore its medium (LAL reagent water, or 10 mM Tris, pH 7.8, LAL grade for Semaglutide, tirzepatide and retatrutide) 1 + 1 in mobile phase A, and the blank is that matrix, not mobile phase A alone.
Water	Chromatography grade.
Acetonitrile	Chromatography grade.
Trifluoroacetic acid	Chromatography grade, at the concentration each mobile phase above states.

9 Reference standard

A reference standard of the analyte is injected for system suitability. Its name, lot and source are recorded in the set and printed as the suitability footnote, with its retention time against the sample's main peak; it assigns the main peak. Where mass per vial is also ordered (SOP-06) the same lot serves both, and one weighing serves both.

Reference standard, continued

- The suitability solution is the standard at the sample's own concentration and in the sample's own matrix: the SOP-06 calibration stock (2 mg/mL in the medium, WS-05 (the standard block)) diluted 1 + 1 in mobile phase A, exactly as the purity solution is made, so the standard and the sample are injected at the same load in the same solvent.
- Standard solutions are used within 24 h of preparation (the VP-05 solution-stability claim; until it is measured, 24 h is the limit), kept at 2–8 °C or in the autosampler at 5 °C, and labelled with name, lot, concentration, date, time, analyst and expiry.

10 Sample preparation

The preparation, as the methods page states it:

> Purity and identity: the stock 1 + 1 in mobile phase A, 1.0 mg/mL, 2 µL, so 2 µg on column; identity from the purity solution 1 + 9.
>
> The methods page, "Sample preparation" (data.json method.rows)

10.1 Stock: the whole vial dissolved by mass, to 2.0 mg/mL nominal in LAL reagent water (10 mM Tris of LAL grade for semaglutide, tirzepatide and retatrutide) (SOP-06, Sample preparation, which gives the weighings, the medium's mass and the clarity record; recorded on WS-03 (dissolution by mass)). PUR, IDP and ID with one vial: the same stock, from that vial.

10.2 Purity solution: the stock 1 + 1 in mobile phase A, 1 mg/mL nominal, in a low-binding polypropylene autosampler vial, capped and inverted five times at once; 2 µL injected. The Tris stock added to the pre-weighed mobile phase A with immediate mixing, never the reverse. The injection solvent is then about the acidity of mobile phase A, recorded as measured, not assumed.

10.3 The injected concentration is 1 mg/mL nominal for every fill, the concentration VP-01 validates; a run whose recorded concentration differs from it by more than 10% is a deviation (the model warns), handled under SOP-08.

10.4 Record the stock's source (preparation 1), the dilution, the vial type and the time of preparation (hplc.sample), the vials consumed (printed on page 1 beside the vials received and retained) and the clarity of the stock.

10.5 Hold times, until each VR-01 says otherwise: the stock 24 h at 2–8 °C from the time of dissolution on WS-03 (dissolution); the purity solution injected within 24 h of its preparation, autosampler at 5 °C (recorded); a sequence longer than 24 h is a deviation. The times of preparation and of the last injection are on WS-04 (purity run).

10.6 One preparation, three injections. The identity run (SOP-03) takes its ions from this preparation, diluted as SOP-03 says.

In a full panel the method as written consumes 5 vials (mass per vial 3; water content 2; HPLC from the mass per vial preparation; LC–MS from the HPLC preparation; endotoxin from the mass per vial preparation) (results.vials_required). This test takes no vial of its own there: its solution comes from the mass per vial preparation, 0 vials are recorded as consumed by it, and the certificate says "HPLC from the mass per vial preparation". Because the endotoxin aliquot (SOP-04) is drawn from that same solution, it is made in LAL reagent water and the aliquot is taken first, before the dilution in mobile phase A that this test injects.

11 Chromatographic conditions

Method parameters as written, read from data.json; the same rows are published on the methods page, and the defaults of every new result set are filled from them.

Column	C18, 2.1 × 100 mm, 1.7 µm
Mobile phase A	Water, 0.1% trifluoroacetic acid
Mobile phase B	Acetonitrile, 0.1% trifluoroacetic acid

Chromatographic conditions, continued

Gradient	5–60% B over 20 min; where co-elution is suspected, a 14-percentage-point window over 40 min centred on the composition at which the analyte eluted in the first run (for example 18–32% B for a 1 kDa peptide, 45–59% B for semaglutide)
Flow	0.30 mL/min
Column temperature	40 °C
Injection	2 µL
Sample preparation	Mass per vial: three vials, each vented, weighed and dissolved whole, by mass, in LAL reagent water (10 mM Tris of LAL grade for semaglutide, tirzepatide and retatrutide, the Tris stock added to mobile phase A with mixing) to 2.0 mg/mL nominal, the volume being the nominal fill in mg divided by 2.0 (a 5 mg fill in 2.50 mL), then the stock 1:10 in mobile phase A, 0.20 mg/mL; the masses and volumes are recorded and printed on every certificate. Purity and identity: the stock 1 + 1 in mobile phase A, 1.0 mg/mL, 2 µL, so 2 µg on column; identity from the purity solution 1 + 9. Endotoxin: an aliquot of that stock first, 1:40 in LAL reagent water, 0.05 mg/mL in the well. Water: two whole vials by oven Karl Fischer at the validated temperature
Detection	UV 214 nm for purity and content; 280 nm as a secondary channel, to flag aromatic-containing impurities (epitalon, GHK, BPC-157 and TB-500 have no chromophore there); reference wavelength off
Replicates	n = 3 injections of one preparation; their RSD is injection repeatability. Mass per vial uses three independent vials
Reporting threshold	0.05% area (ICH Q3A(R2)), meaningful once the analyte's measured limit of quantitation is at or below it; until then peaks are listed to 0.05% and no limit of quantitation is claimed

The cycle. The acquired window is the gradient (5–60% B over 20 min); the cycle is the gradient, a wash and a re-equilibration, and the run time recorded in the set must equal their sum or validate refuses. The certificate prints the cycle under Run time, never the acquired window alone.

Where co-elution is suspected, the second gradient the Gradient row describes is run; the result printed is the one the main peak is resolved on, and the open point says which gradient it was.

12 System suitability

Before the sample, a blank and the reference standard are injected and the following are recorded. Each row is printed on the certificate with its value and whether it was met.

Parameter	Criterion	Basis
Blank injection	No peak at or above the 0.05% reporting threshold at the analyte retention time	USP <621>; ICH Q3A(R2) 5 (reporting threshold)
Symmetry factor (standard, 5% height)	0.8–1.8	USP <621> symmetry factor, As = W0.05 / 2f (5% height); Ph. Eur. 2.2.46: 0.8–1.8 unless otherwise prescribed
Plate number (apparent, gradient), standard	Report	USP <621> plate number, N = 5.54 (tR / W0.5)^2 (half height); apparent in a gradient, reported, no criterion
Resolution, main peak / named pair (on the standard)	At least 1.5	USP <621> resolution, Rs = 1.18 (tR2 - tR1) / (W0.5,1 + W0.5,2) (half height); at least 1.5 for baseline separation
Area RSD of the standard injections	Not more than 2.0%, over 5 replicate standard injections (6 when the RSD limit is above 2.0%)	USP <621> system repeatability: 5 replicate injections when the RSD requirement is NMT 2.0%, 6 when it is more than 2.0%
Retention time of standard	Report (assigns the main peak)	retention time match of the reference standard (identity support, reported)
Resolution cross-check	Rs from the plate number and the peak spacing agrees with the stated Rs within 25%; a disagreement is a warning for the reviewer	USP <621>: Rs = 1.18 dt / (w1 + w2) at half height, w = tR sqrt(5.54 / N)

$A_s = W_{0.05} / 2f$ (symmetry factor at 5% height)

$N = 5.54\ (t_R / W_{0.5})^2$ (plate number at half height; apparent in a gradient)

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

Plate number and resolution from peak widths at half height; symmetry factor at 5% height (USP <621> / Ph. Eur. 2.2.46).

System suitability, continued

A missed suitability criterion does not block issue by itself: it is printed as not met and listed for the reviewer, who decides whether the run stands. The blank, the standard's identity and lot, and the number of standard injections are required on the record.

- The named pair for each analyte is the critical pair of its VR-01 (VP-01, robustness); until a report exists it is the main peak and the nearest impurity at or above 0.1% in the standard, named on WS-04 (purity run) with its relative retention time. Where the standard shows none, write "no pair at or above 0.1%" as the pair, leave the resolution blank, and set co-elution from the sample's own main-peak shape, recorded in the co-eluting pair.
- At the bench the suitability is read before the first sample injection (the sequence pauses after the last standard, or the standards run as their own sequence). If the blank shows a peak at or above 0.05% of the standard's main area at its retention time, the standard RSD exceeds 2.0%, or the symmetry factor is outside 0.8–1.8: stop. In order, a fresh blank and one more standard injection; the needle wash, the seal and the standard vial's level; re-equilibration for 15 min; a fresh standard solution. The samples are injected only after 5 consecutive standards pass, or with the reviewer's written agreement typed into the set's notes before the sample runs. Every injection stays in the data file and is named on the worksheet with its reason; nothing is deleted or renamed.

13 Procedure

13.1 Equilibrate the column at the starting composition, 0.30 mL/min and 40 °C for at least 15 min (about twenty column volumes) and until the pressure is within 10% of the column's last recorded value and the baseline at 214 nm drifts less than 1 mAU over 5 min; record the pressure and the time on WS-04 (purity run). A new column: 30 min, then the blank.

13.2 Record on the worksheet the data system's clock against the laboratory's UTC reference at the start of the sequence, and type the offset when importing; a clock more than 2 min from the reference is corrected before the run and the correction logged (SOP-09).

13.3 Inject the blank: the sample's matrix (the medium 1 + 1 in mobile phase A), prepared with the sample. Set the integration start from it: the first time after the void disturbance at which the baseline has returned to within 0.1 mAU of its level at 3 min, the same value for every injection of the set.

13.4 Inject the reference standard 5 times; record the suitability values above and the standard's retention time, and read them before the samples (System suitability).

13.5 Inject the sample exactly three times, acquiring every channel the Detection row above names. Sequence and sample names carry the accession (or the set id before logging) and the role: _BLK, _STD1 to _STD5, _S1 to _S3. For each injection record the label, the data file and the acquisition time (UTC); both are required to issue, per injection.

13.6 Integration uses the data system's processing method named for the analyte (name and version on the worksheet and in the set's notes); its parameters are not changed per injection. A manual integration event is made only on an impurity, never on the main peak, is recorded in the data system's audit trail and listed on the worksheet with its reason; a run with a manual event on the main peak is a deviation.

13.7 Integrate every injection from the one integration start time recorded for the set; a peak eluting before it is refused. Give each peak a role: main, impurity, excluded. Exactly one main peak per injection. A peak that also appears in the blank (void, solvent front, system) is excluded: listed with its share of the integrated area and left out of the normalisation.

13.8 Pasting the integration report from the data system is accepted (tab or comma separated, a header naming RT or Retention, Area, Height and optionally Name): the largest area becomes main unless a row is named main, and a peak below 1 min or named void or system or solvent or blank or inject or dead is excluded. Correct any row by hand.

13.9 Export each trace as t, mAU points and attach it to its injection. A trace of at least two points on every injection is required to issue; a draft without one previews a schematic that can never reach a certificate.

Procedure, continued

13.10 Record the baseline treatment, one of “as acquired” or “blank-subtracted”; the caption’s last sentence is printed from it (“Trace as acquired.” / “Trace blank-subtracted; raw trace on file.”).

13.11 Record co-elution as true, false, or blank to derive it from the resolution (below 1.5 means a component co-elutes and the purity is an upper bound). The open point, if any, is free text printed under the results table; it is never read as chromatography.

13.12 Where the online LC–MS run gave a mass for an impurity peak, record its retention time, observed m/z, charge and assignment (or “not assigned”); it is printed under the open point.

14 Calculations

Area normalisation (USP <621>): each peak’s area as a share of every integrated peak, blank-matched and system peaks (role “excluded”) left out. Peaks below the reporting threshold stay in the total and are not counted as impurities, so a purity is never raised by discarding small peaks; total impurities are 100 less the main peak, so the two add to 100.

Area percent = $100 \times A_{peak}$ / sum of A over every integrated peak not excluded

Purity = mean of the three main-peak area percents

RSD (%) = $100 \times s$ / mean, s the sample standard deviation (n – 1)

Total impurities (%) = 100 – main-peak area percent, per injection, then the mean

RRT = $t_R / t_{R,\,main}$

- Impurity peaks are grouped across the injections by retention time within 0.1 min and their areas averaged; the largest single impurity is reported with its RRT and RT.
- The reporting threshold is 0.05% of area (data.json). The comparison with the threshold is made on the share rounded to 2 decimals, as ICH Q3A(R2) section 5 compares a result rounded to the threshold’s precision.
- The threshold applied is the larger of the recorded threshold and the share the validated quantitation limit can see: the limit VR-01 establishes is an absolute concentration on the free-peptide basis (recorded against the run as hplc.sample’s ql_abs_ug_ml with its ql_basis; the model warns when the basis is not free peptide), divided by the main component found in the injected solution on the same basis (the content of SOP-06 over the stock volume, times the dilution), so a lot’s low content raises the threshold only as far as the detector’s limit requires and never by the counter-ion and water the powder basis would add. The certificate prints the recorded and the applied threshold with the reason (results.effective_reporting_threshold). For example, a limit of 0.4 µg/mL against 0.7 mg/mL of free peptide is 0.057% of the main peak, so the threshold applied is 0.06% (rounded to the threshold’s 2 decimals, as the certificate prints it). Without a recorded limit the recorded threshold stands, and the certificate says no limit of quantitation is established.
- ICH Q3A(R2) Attachment 1 thresholds for a maximum daily dose of 2 g or less, applied by analogy (Q3A(R2) excludes synthetic peptides from its scope; Ph. Eur. 2034 sets 0.1% reporting, 0.5% identification and 1.0% qualification for them); identification and qualification thresholds for information only: identification 0.1%, qualification 0.15%.
- Identification 0.10% and qualification 0.15% (Q3A), 0.5% and 1.0% (synthetic peptides): exceeded when the share as reported is greater than the threshold (0.0963% reports as 0.10% and does not exceed 0.10%); for information only, never a conclusion (ICH Q3A(R2) Attachments 1 and 2 (maximum daily dose 2 g or less, by analogy); Ph. Eur. 2034, peptides obtained by chemical synthesis; results.calculation_references hplc.identification_threshold).
- Rounding: ICH Q3A(R2): impurities below 1.0% to two decimals, at or above 1.0% to one decimal, rounded half up from the unrounded sum; purity one decimal on page 1 and in the headline, two decimals in tables labelled raw; RSD two decimals; RT and RRT two decimals. Rounding happens at presentation only, half up, never inside the model.
- When a component co-elutes with the main peak the purity is printed as an upper bound (at most the figure) and the plain-terms paragraph says so.

Calculations, continued

– A co-eluting component (stated, or Rs below 1.5) makes the purity an upper bound: it can show that a lower limit is failed and an upper limit met, never that a lower limit is met (conclusion Not evaluated) (logic of bounds; USP <621> Rs 1.5 for baseline separation; results.calculation_references hplc.coelution).

15 Measurement uncertainty and decision rule

Expanded uncertainty is reported with every quantitative result as U with k = 2. The standard error of the replicate mean is combined in quadrature with a method floor stated as an expanded value; for this method it is ±0.3% area, and the floor is a provisional pre-validation constant, the laboratory's prior estimate, until the method's validation has measured intermediate precision.

$u_{rep} = s / \sqrt{n}$

$u_{floor} = \text{floor} / k$

$u_c = \sqrt{u_{rep}^2 + u_{floor}^2}$

$U = k\, u_c,\ k = 2$

$nu_{eff} = u_c^4 / (u_{rep}^4 / (n - 1))$ (Welch–Satterthwaite, GUM G.4.1)

The model reports the effective degrees of freedom and the t factor they imply (GUM Annex G, Table G.2) and warns when nu_eff is below 10, where k = 2 gives less than 95% coverage. A single determination carries the floor alone.

Decision rule (ISO/IEC 17025:2017 7.8.6; ILAC-G8:09/2019): the conclusion is reached by simple acceptance (guard band w = 0) on the value rounded to its reported precision; the statement beside it says whether the conclusion still holds with the expanded uncertainty (k = 2) included, the guard band w = U. Where a limit is stated more coarsely than the result, comparing at the reported precision is stricter than USP General Notices 7.20 and Ph. Eur. General Notices 1.2, which round to the limit's own digits.

The result model carries this rule, with its sources, as results.calculation_references stats.decision_rule; the certificate's method page prints it.

Point comparisons with a limit are made on the value rounded to its reported precision: purity to 1 decimal.

The budget's terms (lab/results.py): the standard error of the three injections; the intermediate precision of the purity, which the floor stands in for while the method's floor status is "provisional"; and an integration term for the baseline and threshold choices (0.05% area, a design estimate until measured). No reference value enters an area-percent result, so the standard contributes no term. Once VR-01 measures intermediate precision its s_IP replaces the floor, and the report says whether the integration term is inside it (its precision runs integrated by one analyst) or added.

$U = 2 \times \sqrt{SE^2 + s_IP^2 + u_int^2}$; the certificate prints each term with its value and its source.

References: JCGM 100:2008 (GUM) 6.2 and Annex G; ISO/IEC 17025:2017 7.6 and 7.8.6.

16 Acceptance and reporting

What blocks issue (validate)

– Exactly three injections, each with exactly one main peak, positive areas, and retention times inside the acquired window and after the integration start.
– A data file, an acquisition time and an acquired trace of at least two points on every injection; the instrument identifier; the baseline treatment.
– A cycle that adds up: run time = gradient + wash + re-equilibration.
– A positive wavelength and run time; a reporting threshold of 0 or more.

Conclusion

– Against a specification supplied with the order: Conforms or Does not conform.

Acceptance and reporting, continued

- Against a purity on the sender's label ("label claim"): Meets claim or Below claim, the claim printed beside the result as customer-supplied and unverified.
- With neither: "None supplied; for information", and no conclusion is drawn, as the limits page says ("A verdict only against a specification you supply").
- The certificate prints the purity to one decimal on page 1 and in the headline, the injections and their mean to two decimals labelled raw, the RSD, the total impurities, the largest single impurity, the suitability table, the chromatogram of injection 1 with its peak table, the open point and the uncertainty sentence.

17 Deviations and open points

- A departure from this procedure, planned or found afterwards, is nonconforming work and is handled under SOP-08. One that bears on a result is stated on the certificate, in the open point or the notes of the section concerned, never left out.
- Co-elution, a missed suitability criterion, an impurity that could not be resolved: stated on the certificate in the open point and the co-elution row, never silently.
- The injected concentration is a fixed 1 mg/mL nominal (Sample preparation); each run records the concentration it used, the certificate prints it, and the model warns beyond 10% of it.
- The wash and re-equilibration of the cycle are not in the method parameters on file (only the gradient is); each run records them and the run time must add up.
- Limits of quantitation are not published until measured on the laboratory's own instrument in the matrix concerned; the scope table's LOQ column is empty until then.
- The backend's form does not yet take the stock, dilution, vial type and preparation time of hplc.sample, nor the mobile-phase lots and bottle numbers; they are on WS-04 (purity run) and in the set's notes until it does.

18 Records

- The result set lab/results/<set>.json, its audit trail and its emails; the register row; the certificate and record.json as issued (SOP-07).
- WS-04 (purity by RP-HPLC–UV), the original record of every value measured away from the backend (SOP-07, Records and retention): the mobile phases and their lots, the equilibration, the suitability read-out and any action, the sequence table, the processing method and manual events, the hold-time clock.
- Every peak, every trace point, the suitability values, and the data file and acquisition time of every injection, in the result set.
- The chromatography data system's raw data files, named on the certificate per injection; the column log (SOP-09).

19 Revision history

Revision	Change	Approval
1	First issue.	Not yet approved

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Current revisions

End of document