# Water content by coulometric Karl Fischer

Water as mass percent of the sample as received, by coulometric titration of a methanol extract, whole vial by whole vial, with the drift, the blank and the water standard on the record.

**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 | |
|---|---|---|---|
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## 1 Purpose

To determine the water content of a lyophilised peptide as mass percent of the sample as received, by coulometric Karl Fischer titration of the water released from the whole vial in an oven and carried to the cell by a dry gas (USP <921> Method Ic, evaporation technique; Ph. Eur. 2.5.32), with the water per vial as the measurand and the mass percent derived from the vial's gross fill by weighing.

## 2 Scope

An add-on (code H2O, "Water content") to ID, PUR, IDP; the full panel includes it. Minimum sample, as the intake conditions state it: "two sealed vials as sold".

A whole-vial determination takes one vial per replicate, so it needs at least two vials, each weighed sealed, as its closure and as bare glass (SOP-06, the weighings). A weighed portion is the fallback for a vial format that fits no oven vial, and the certificate says so.

## 3 Responsibilities

| Role | Responsibility |
|---|---|
| Analyst | Conditions the cell, measures the drift, runs the water standard and the blanks, weighs and vents each vial, runs the determinations, records every value on the worksheet as it is read, and signs as analyst. |
| Reviewer | Checks the drift, the standard recoveries, the blanks, the weighings and the pair against the repeatability limit; signs the content as reviewed (SOP-07). |

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

## 4 References and controlled sources

**Standards and other documents**

- USP <921> Method Ic, evaporation technique; Ph. Eur. 2.5.32: the coulometric method with the water released by heating (the evaporation technique), and the requirement for a stable, recorded drift and a water-standard check. The chapters fix no numbers; the drift ceilings, the blank limits and the recovery window below are laboratory criteria and are cited as such on the certificate.
- USP <41> and <1251>: the balance, its minimum weight and the weighing of a difference of three large masses; ISO 5725-6 4.1: the repeatability limit r = 2.8 s_r for a duplicate.
- JCGM 100:2008 (GUM) 6.2 and Annex G; ISO/IEC 17025:2017 7.6 and 7.8.6.
- SOP-06 (the weighings, the venting and the gross fill, which close the mass balance), SOP-07 (review and issue), SOP-09 (the titrator, the oven, the balance and the vial-format table), VP-04 (the oven temperature per analyte and the weighing study).

**Controlled sources, read at every build**

- lab/results.py: the formula, the corrections, the criteria, the quantitation limit, the repeatability limit and the rounding; lab/CONTRACT.md section 1 (kf).
- data.json pricing and intake (minimum sample).

## 5 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.
- Methanol and the coulometric anolyte are flammable and toxic by inhalation, ingestion and skin absorption; the anolyte carries iodine and sulfur dioxide, which stain and burn, and releases sulfur dioxide if acid reaches the cell (an acetate or trifluoroacetate salt in the oven: ventilate the cell's exhaust): keep them from ignition sources, keep the cell closed, change the reagent with ventilation that keeps the vapour from the face, and read the safety data sheet of the reagent actually bought (a ketone-type reagent carries chloroform and its own waste stream; a diaphragm cell also has a catholyte).
- The oven block, the transfer needle and a vial pulled after a run are at the method temperature: tongs and a cooling rack, no re-crimping of a hot vial, the heated zone marked. The carrier gas is dry nitrogen from a cylinder (chained upright, regulator, cap on when moved; its size and the room's air changes decide whether an oxygen monitor is needed) or room air through the oven's drying train, changed on schedule.
- A needle left in a stopper to vent a vial is a sharps hazard and carries about 2 µg of peptide per µL of film: mark the rack, never recap, and discard into the sharps container at the balance.
- 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.

## 6 Equipment and materials

- A coulometric Karl Fischer titrator with an oven sampler, whose software records the drift, the blank, the end criterion and the extraction time of each determination; the open sample vial sits inside a crimped oven vial. 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 oven sampler's vial format is an owner fact the register does not yet carry: a 2 mL serum vial fits inside a 10 mL headspace vial, larger formats do not, and SOP-09's vial-format table, measured at the oven's operational qualification, says which formats go in whole, which are re-crimped with the oven's septum cap, and which can only be sampled as a weighed portion.
- The semi-micro balance (readability 0.01 mg) with its draft shield, anti-static ionizer and vial adapter, the day's check weight recorded, its minimum weight printed on it (SOP-09); the balance id and the check record go on WS-06 (water content). A gross fill of a few milligrams by difference does not meet USP <41>'s 0.10% rule on any semi-micro balance and is not claimed to: its uncertainty is carried in the budget instead.
- Oven vials and septa of the oven's format, a crimper and a decrimper that takes a shell off in one piece, venting needles, tongs and a cooling rack.
- The instrument identifier and the data file are recorded; the identifier is required to issue (there is no figure, so no acquisition time is).

## 7 Reagents

| | |
|---|---|
| **Anolyte** | A methanol-based coulometric reagent suited to the oven cell, its name and lot recorded, changed when the drift will not settle; a solvent extraction never takes a ketone-type reagent, and the model warns when one is named. |
| **Carrier gas** | Dry nitrogen or air through the oven's drying train, at the manufacturer's flow, recorded; the flow is a method parameter, and a change re-opens the temperature check of VP-04. |
| **Standard** | A certified solid water standard for the oven (certified value with its uncertainty on the certificate; weighed at 10–40 mg by difference into an oven vial), or the liquid standard at about 1 mg/g for a solvent cell; the certified and found micrograms are recorded and the recovery printed; a certificate that states no uncertainty is given 0.6% relative in the budget. |

## 8 Sample preparation

The preparation, as the methods page states it:

> Water: two whole vials by oven Karl Fischer at the validated temperature.
>
> The methods page, "Sample preparation" (data.json method.rows)

**8.1** A vial from refrigerated or frozen storage stands sealed in the balance room for at least 2 h before any weighing (condensation on a cold vial outweighs the fill's uncertainty); the time out of storage is on WS-06 (water content).

**8.2** Vent the vial before the first weighing: the sealed vial's headspace gas and pressure as the vendor stoppered it, 0.1–3 mg on a 2 mL vial, so the sealed vial holds room air at room pressure. For a water vial, where a minute's exposure would add water, vent through the stopper with a fine needle for at least 10 min in the hood, withdraw the needle and equilibrate 10 min; record the method on the worksheet (vented, vent method). The uncertainty of the gross fill follows the method: stopper lifted 0.04 mg; needle 0.09 mg; an unvented or unrecorded vial has no determined gross fill and the model warns.

**8.3** Three weighings on the semi-micro balance, each with its time and the analyst's initials: W0, the vented vial as received; Wc, the stopper and crimp shell within 2 min of decapping, dry; W2, the bare glass after the analysis, rinsed three times with water and once with methanol, dried 1 h at 105 °C and equilibrated open in the balance room for at least 2 h (not in a desiccator). G = W0 – Wc – W2. Powder visible on the stopper: rinse it into the vial with part of the medium, note it, and flag G. A decrimper that cuts the shell leaves G not determined.

**Sample preparation, continued**

**8.4** Whole vial in the oven: from pulling the stopper to crimping the oven vial at most 15 s (a lyophilised cake takes up water at about 0.1% a minute in room air; practise on an empty vial first); “within 15 s” is ticked on the worksheet. A format that fits the oven vial goes in whole; one that fits nothing is re-crimped with the oven’s septum cap when its finish allows, otherwise a weighed portion is taken with the exposure time recorded and the certificate says “weighed portion” (SOP-09’s format table; the decision is on the worksheet).

**8.5** At least two replicates, one whole vial each, numbered on WS-01 (receipt) (validate refuses fewer). A whole-vial mass more than 25% from the nominal fill is a warning to check the mass.

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 2 vials of its own there.

## 9 Oven temperature and programme

- The oven temperature is the one VP-04 establishes for the analyte from a temperature ramp on at least 5 mg of material (the release band returned to within 3 µg/min of the pre-sample drift, plus 10 °C, rounded to 5 °C; the ramp stops below 180 °C, where the salts’ acids and amines begin to reach the cell), proven complete and non-destructive by the isothermal runs 20 °C either side and the residue check; it is recorded per run (oven temperature) and printed.
- The extraction ends on a relative-drift stop, the rate within 2 µg/min of the baseline, rather than a fixed time, so the subtracted drift is as small as the release allows; at most 15 min. The extraction time is recorded (extraction time).
- Carrier gas and flow as validated; a change re-opens the temperature check.
- No analyte has a validated oven temperature yet (VP-04 has not been run): until it has, no result is reported under this procedure.

## 10 System suitability

| Check | Criterion | Basis |
|---|---|---|
| Drift before each run | Measured for 5 min with the carrier flowing through an empty oven vial at the oven temperature; at or below 10 µg/min; stable: changing by less than 2 µg/min over three consecutive readings a minute apart. Not reached within 30 min: septum and cell seals checked, cell dried, anolyte changed; the cell is not used until stable. | laboratory criteria |
| Water standard recovery | 97.5–102.5% at the start of the day and after the last sample, the two within 2% of each other (no cell poisoning by the salts’ acids) | laboratory criteria |
| Blank | An empty, cleaned and dried sample vial of the same format inside a crimped oven vial, through the same programme, at least three times a day (start, middle, end), drift-compensated as the samples are: mean at or below 25 µg, standard deviation at or below 3.0 µg; a higher blank means a wet septum or carrier | laboratory criteria |
| Drift after the last sample | At or below twice the drift before the first (acid or amine from a salt poisons the cell slowly) | laboratory criteria |
| End criterion | The titration end or extraction stop criterion is recorded and printed | USP <921> Method Ic; Ph. Eur. 2.5.32 |

A drift above the ceiling, a recovery outside the window or a blank beyond its limits means the day’s results are not reported: fresh anolyte, and the checks repeated. A missing drift, blank or standard check is listed as a missing accuracy check. The certificate says, in so many words, whether the drift and the blank were subtracted, and that the blank was drift-compensated.

## 11 Procedure

**11.1** Condition the cell; measure and record the drift as above, with the three readings.

**11.2** Titrate the water standard: the solid standard weighed by difference into an oven vial and crimped; record the masses, the certified and found micrograms and the recovery. A recovery outside the window: no sample is run; a fresh ampoule or weighing; a second failure takes the titrator out of service (SOP-09).

**11.3** Run the blanks: at least three empty vials through the programme with the instrument's drift compensation on, as it is for the samples; the blank entered is their drift-compensated mean, with its standard deviation and count, and the set records that it was drift-compensated. The drift is subtracted once, by the instrument, never again by hand.

**11.4** For each replicate: weigh W0 (vented) and Wc; load the open vial into the oven vial and crimp within 15 s; run; record the gross micrograms titrated, the drift the instrument subtracted and the extraction time; later W2. The water found is the drift-compensated figure less the blank, and the set records that each was subtracted.

**11.5** The pair: |w1 – w2| at or below the repeatability limit r = 2.8 s_r (ISO 5725-6 4.1, r = 2.8 s_r; the provisional r, the larger of 0.14% w/w (2.8 × half the ±0.1% w/w floor) and 10% of the mean, is the laboratory's estimate until VR-04 measures s_r). A pair beyond r is not averaged: a third vial is run when the retained vial allows and recorded as consumed, else the pair is reported with an open point and U from the pair.

**11.6** Titrate the standard again at the end of the day; record the drift after the last sample.

**11.7** Record the reagent, the end criterion, that the determination was whole-vial, the vials consumed, the balance id and the weighing statement.

## 12 Calculations

Water (% w/w) = water found (µg) / (sample mass (mg) × 1000) × 100

G (mg) = $W_0 - W_c - W_2$ (the sample mass: the vented vial, its closure, the bare glass)

water found (µg) = titrated water (drift-compensated by the instrument) – blank (the drift-compensated mean of the day's blanks)

mean, s (n – 1) and RSD (%) = 100 × s / mean over the replicates

for a duplicate (n = 2): s = $|w_1 - w_2|$ / sqrt(2), one degree of freedom

LOQ (µg) = 10 × $s_{blank}$; a water found at or above 1 × LOQ is reported with its U, below it as "< LOQ" with LOQ / G as the percent

water per vial (mg) = mean water found per whole vial (measured; the nominal fill is never used when whole vials were titrated)

With two determinations the replicate term of the uncertainty, s / sqrt(2), has one degree of freedom. The pair difference above which k = 2 no longer gives about 95% coverage (nu_eff below 10) is recomputed per result from the full budget and printed as a warning, not as an acceptance criterion; the acceptance is the repeatability limit r above. The floor-only case of that rule (0.068% w/w, lab/results.py) is the warning's lower bound.

The certificate prints the mean to two decimals, the SD to three decimals with its unit, the RSD to two decimals, the drift and blank sentences, the standard recovery to one decimal and the whole-vial footnote once.

## 13 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 ±0.1% w/w, and the floor is a provisional pre-validation constant, the laboratory's prior estimate, until the method's validation has measured intermediate precision.

**Measurement uncertainty and decision rule, continued**

$u_{rep} = s / \mathrm{sqrt}(n)$

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

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

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

$nu_{eff} = u_c^4 / (u_{rep}^4 / (n - 1))$ (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: water to 2 decimals.

The budget's terms (lab/results.py; the floor's status for this method is "provisional"): the pair's standard error (s_r / sqrt(2) from VR-04 once measured); the gross-fill weighing, u(G) / G, by the venting method (stopper lifted 0.04 mg; needle 0.09 mg); the blank, s_blank / W (2 µg when the day's blanks are not recorded); the drift subtraction (20% of the amount subtracted); the water standard's recovery and certificate (0.6% relative when the certificate states no uncertainty); the between-day term the floor stands in for; and, when the isothermal check of VR-04 passes with the T + 20 °C result more than 2% above the T result, a release-bias term from that difference. The water per vial carries the same budget without the weighing term, and its own U is printed.

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

## 14 Acceptance and reporting

- Against a supplied limit in % w/w: Conforms or Does not conform, with the decision-rule sentence; without one, "None supplied; for information".
- Reported as mass percent of the sample as received and as water per vial; the water per vial and the gross fills feed the mass balance and the fill-uniformity line of SOP-06 when both are run.
- What blocks issue: fewer than two replicates, a non-positive mass, a missing water figure, a negative blank or drift, a missing instrument identifier.

## 15 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.
- The oven sampler, its vial format and the carrier gas are owner facts not yet on the register (SOP-09); no oven temperature has been established for any analyte (VP-04), so no result is reported under this procedure yet.
- The repeatability limit is the laboratory's estimate until VR-04 measures s_r; the gross-fill, blank, drift and standard terms of the budget are design estimates until the records named beside them replace them.
- The backend's form does not yet take the oven fields, the three weighings, the venting method or the balance; they are on WS-06 (water content) and in the set's notes until it does.
- A weighed-portion determination gives no measured water per vial; the certificate then prints the figure at nominal fill and labels it so.

## 16 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-06 (water content): the titrator and oven settings, the anolyte, the drift readings, the blanks, the standard's masses and recoveries, the three weighings and the venting of each vial with their times, the 15-second check, the format decision, the pair against r (SOP-07, Records and retention).
- Every replicate with its mass, water and extraction time, the drift, the blank and the standard check, in the result set.
- The titrator's data file, named on the certificate.

## 17 Revision history

| Revision | Change | Approval |
|---|---|---|
| 1 | First issue. | Not yet approved |

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Current revisions

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