Designation: F2459 – 12

Standard Test Method for Extracting Residue from Metallic Medical Components and Quantifying via Gravimetric Analysis[1]

This standard is issued under the fixed designation F2459; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers the quantitative assessment of the amount of residue obtained from metallic medical components when extracted with aqueous or organic solvents.

1.2 This test method does not advocate an acceptable level of cleanliness. It identifies one technique to quantify extractable residue on metallic medical components. In addition, it is recognized that this test method may not be the only method to determine and quantify extractables.

1.3 Although these methods may give the investigator a means to compare the relative levels of component cleanliness, it is recognized that some forms of component residue may not be accounted for by these methods.

1.4 The applicability of these general gravimetric methods have been demonstrated by many literature reports; however, the specific suitability for applications to all-metal medical components will be validated by an Interlaboratory Study (ILS) conducted according to Practice E691.

1.5 This test method is not intended to evaluate the residue level in medical components that have been cleaned for reuse. This test method is also not intended to extract residue for use in biocompatibility testing.

NOTE 1—For extraction of samples intended for the biological evaluation of devices or materials, refer to ISO 10993–12.

1.6 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.7 *This standard may involve hazardous or environmentally-restricted materials, operations, and equipment. This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

G121 Practice for Preparation of Contaminated Test Coupons for the Evaluation of Cleaning Agents

G131 Practice for Cleaning of Materials and Components by Ultrasonic Techniques

G136 Practice for Determination of Soluble Residual Contaminants in Materials by Ultrasonic Extraction

2.2 *ISO Standard:*[3]

ISO 10993–12 Biological Evaluation—Sample Preparation and Reference Materials

3. Terminology

3.1 *Definitions:*

3.1.1 *ionic compounds/water soluble residue*—residue that is soluble in water, including surfactants and salts.

3.1.2 *non-soluble debris*—residue including metals, organic solids, inorganic solids, and ceramics.

3.1.3 *non-water soluble residue*—residue soluble in solvents other than water. Inclusive in this are oils, greases, hydrocarbons, and low molecular weight polymers. Typical solvents used to dissolve these residues include chlorinated or fluorinated solvents, or low molecular weight hydrocarbons.

3.1.4 *reflux system*—an apparatus containing an extraction vessel and a solvent return system. It is designed to allow boiling of the solvent in the extraction vessel and to return any vaporized solvent to the extraction vessel.

3.1.5 *reuse*—the repeated or multiple use of any medical component (whether labeled SUD or reusable) with reprocessing (cleaning, disinfection, or sterilization, or combination thereof) between patient uses.

[1] This test method is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.15 on Material Test Methods.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.

3.1.6 *single use component (SUD)*—a disposable component; intended to be used on one patient during a single procedure.

3.1.7 *surface area*—the projected surface area of a part. This area does not include the internal porosity of parts with cancellous, porous, or wire structure.

3.2 *Symbols:*

m_1 = weight of extraction vessel and component before extraction
m_2 = weight of extraction vessel, component, foil, and solvent after extraction
m_3 = mass of clean beaker and foil used to hold removed aliquot of extracted solution
m_4 = mass of beaker, foil, and aliquot of solution before drying
m_5 = mass of beaker, foil, and residue after evaporating solvent
m_6 = mass of new filter
m_7 = mass of filter following filtration and drying
m_a = mass of residue in removed aliquot
c_r = concentration of residue in solution
c_b = concentration of residue in blank solutions
m_r = mass of soluble residue in the overall extract, corrected for the blank runs
m_i = weight of insoluble debris
m_t = mass of soluble and insoluble residue
E = extraction efficiency

4. Summary of Test Method

4.1 This test method describes the extraction and quantitative analysis procedures used to detect and quantify extractable residue from metallic medical components. The residues are grouped into three categories: *(1)* water-soluble extractables; *(2)* non-water soluble extractables; and *(3)* non-soluble debris.

5. Significance and Use

5.1 This test method is suitable for determination of the extractable residue in metallic medical components. Extractable residue includes aqueous and non-aqueous residue, as well as non-soluble residue.

5.2 This test method recommends the use of a sonication technique to extract residue from the medical component. Other techniques, such as solvent reflux extraction, could be used but have been shown to be less efficient in some tests, as discussed in X1.2.

5.3 This test method is not applicable for evaluating the extractable residue for the reuse of a single-use component (SUD).

6. Apparatus

6.1 *Ultrasonic Bath,* for extraction. The bath must be large enough to hold an extraction beaker containing the medical component. This apparatus is used with the technique described in 11.5. Alternatively, an ultrasonic probe can be used with a bath.

6.2 *Solvent Reflux Extraction Assembly,* shown in Fig. 1. This assembly is composed of a vessel large enough to hold the medical component, and a water-cooled refluxing column. A heating manifold or hotplate stirrer capable of reaching the boiling point of the solvent is also included. This apparatus is used in the procedure described in 11.3. A Soxhlet extractor, as shown in Fig. 2, could be used as well using the procedure described in 11.3.

6.3 *Analytical Balance,* with 0.1 mg accuracy or better.

6.4 *Balance,* with accuracy of 10 mg of better and sufficient capacity to weigh the extraction beaker with the medical component and solvent combined.

6.5 *Glass Beaker and Extraction Vessel,* large enough to hold sufficient solvent to cover the medical component in the extraction vessel. Additionally, metal beakers could be used. Plastic beakers should not be used as low molecular weight residues could be extracted from the beakers.

6.6 *Desiccator.*

6.7 *Pipets,* for transferring liquid. Some solvents can leach extractable compounds from plastic pipets. Glass or metallic pipets are recommended for organic solvents.

6.8 *Aluminum Foil,* degreased in extraction solvent.

6.9 *Forceps, Tweezers, or Tongs,* cleaned with acetone or extraction solvent.

6.10 *Filtration Apparatus,* containing a removable 0.2 µm filter medium that is non-soluble in the extraction solvent.

7. Reagents and Materials

7.1 Each user needs to demonstrate solubility of all of their suspect sources of residue in the solvent(s) of choice. Several solvents may be required if more than one type of residue may be present on the component.

7.2 Spectroscopy or ACS-grade solvents should be used.

8. Hazards

8.1 Many organic solvents are toxic, flammable, or explosive and should be handled only with chemically protective laboratory gloves and used in a fume hood.

8.2 If sonication is used, the user should make sure that the solvent is not heated, directly or through sonication, to a temperature above the flash point of the solvent.

9. Sampling, Test Specimens, and Test Units

9.1 Metallic medical components should be taken in random groupings from different lots if available.

9.2 It is up to the user to determine the number of medical components that need to be used to establish known reproducibility.

9.3 It is up to the user to determine the number of test blanks that need to be used to establish known reproducibility.

9.4 Separate components should be tested for organic and aqueous extractions.

9.5 If a long medical component is cut, it is recommended that the original length and the cut lengths be recorded before the final cleaning operation for validation purposes. Individual

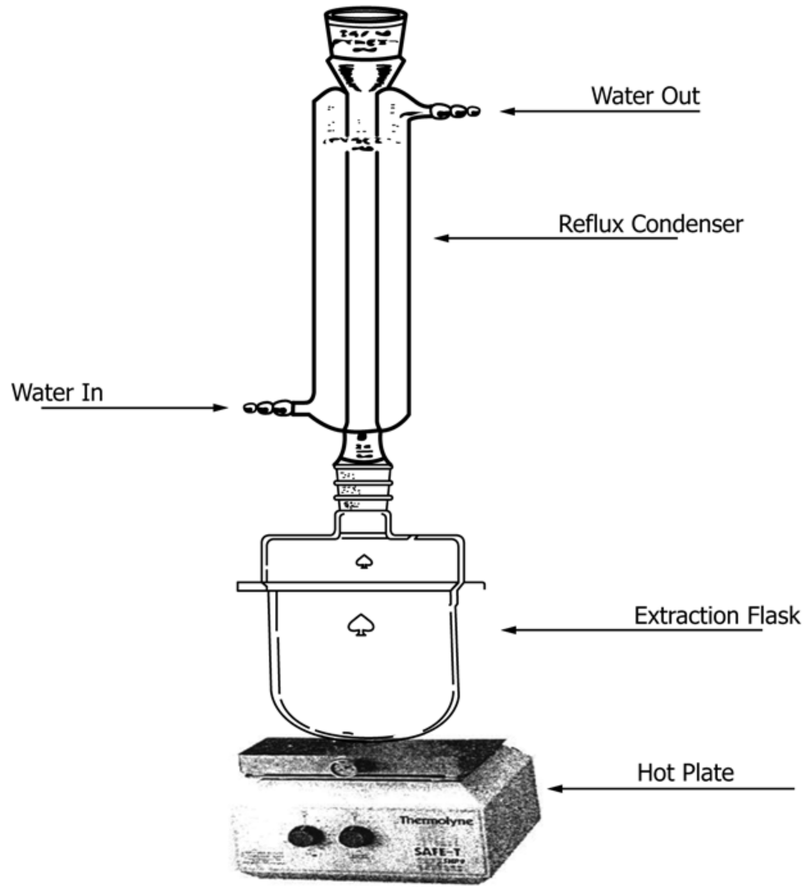

FIG. 1 Sample Solvent Reflux Extractor Assembly

cut lengths may be separately extracted and the results combined to provide a total residue value for the medical component. Cutting lubricants must be avoided in this procedure.

10. Limits of Detection and Recovery Efficiency

10.1 Standardized test coupons can be prepared according to Practice G121. Limits of detection for the two extraction techniques described in Section 11 can be assessed by placing known amounts of residues on the test coupons, and performing the extraction and analyses described in Section 11.

10.2 *Recovery Efficiency*—The recovery efficiency of the selected extraction technique can be determined by doping pre-cleaned medical components with known amounts of the target residue, then extracting and quantifying the target residue. When using this method, the extraction efficiency E is the ratio of the amount of recovered residue to the doped amount of residue. Recovery efficiency may also be determined by exhaustive extraction. The exhaustive extraction technique uses medical components which have not been cleaned and contain unknown amounts of the target residue(s). These components should be extracted using the selected extraction technique until no significant increase in the cumulative residue level is detected upon re-extraction, or until the incremental amount extracted is less than 10 % of what was detected in the first extraction. When using this approach, the extraction efficiency E is the ratio of the amount of recovered residue from the first extraction to the total amount of recovered residue from all extractions performed.

10.3 The user should adjust the extraction parameters in 11.3.11 or 11.5.8, or select the appropriate solvent, or both, in order to achieve an extraction efficiency of $E > 75$ %. This step should be performed if target residues are known a priori. In the case of mixed residues, extraction efficiency may not be able to be determined.

11. Procedure

11.1 If more than one specimen is to be extracted collectively, record the number of specimens.

11.2 If multiple specimens are to be extracted collectively, they must be of the same type and size.

11.3 *Reflux Extraction:*

11.3.1 Equipment may need to be cleaned with nitric acid or other appropriate means prior to solvent cleaning.

11.3.2 Clean the extraction equipment by rinsing at least three times with spectroscopy-grade hexane or another suitable solvent. The extraction solvent may be used.

11.3.3 Air dry all beakers and glassware at room temperature in a fume hood and store in a dessicator prior to use.

11.3.4 Assemble the extraction apparatus as shown in Fig. 1.

FIG. 2 Sample Soxhlet Extractor Assembly

11.3.5 Do not use any type of joint grease on the extraction assembly. It can dissolve in the solvent and contaminate the solution. Polytetrafluoroethylene (PTFE) sleeves or tape can be used to seal the joints if necessary.

11.3.6 Place the sample component in the extractor vessel and add a magnetic stirring bar or PTFE boiling stones to reduce the potential for boiling retardation in the system during reflux. The stir bar or boiling stones, or both, should be carefully cleaned in a suitable solvent prior to use.

11.3.7 Weigh the extractor vessel with the component on a balance and record the weight m_1.

11.3.8 Charge the flask with enough solvent to completely cover the component(s) and assemble the reflux system.

11.3.9 Start flow of cooling water through the condenser.

11.3.10 Adjust the hotplate stirrer or heating manifold to maintain the solvent at a brisk boil with moderate constant stirring.

11.3.11 Extract the component(s) for 4 h or for approximately 10 cycles if using a Soxhlet extractor. The extraction time or number of cycles can be adjusted by the user based on internal validation of their target residue.

11.3.12 After the extraction period is complete, turn off the hot plate and allow the system to cool. Carefully open the apparatus. If a Soxhlet extractor is used, heavy debris may stay in the top part of the extractor. This debris can be washed down into the collection vessel with fresh extraction solvent.

11.3.13 Weigh the extraction vessel, component, and solvent, and record the weight as m_2.

11.3.14 Weigh an aliquot beaker large enough to hold an aliquot of the extraction vessel along with a clean piece of foil and record the weight as m_3. The beaker should be weighed to a resolution of at least 0.1 mg.

11.3.15 Allow the insoluble debris to settle in the extraction vessel for 1 h. Withdraw an aliquot of the extracted solution that comprises at least 90 % of the total extracted solution and place in the aliquot beaker as described in 11.3.14, being careful not to withdraw any insoluble debris from the bottom of the extraction vessel. Weigh the solution with beaker and foil and record as m_4.

11.3.15.1 Allow the solvent to completely evaporate in a fume hood at room temperature. See X1.1.3 for more details.

11.3.15.2 Place the beaker, with residue, in a dessicator for a minimum of 2 h.

11.3.15.3 Weigh the beaker and foil again and record as m_5.

11.3.15.4 If the volume of the aliquot beaker is smaller than the aliquot, multiple aliquots can be removed from the extraction vessel, weighing each aliquot, evaporating the solvent, and collecting the next aliquot. The solution weight m_4 is the sum of the aliquot weights plus the foil weight. The final beaker weight m_5 should be recorded as described in 11.3.15.3.

11.4 *Blank Run:*

11.4.1 Conduct test blank(s) using the same amount of solvent and rinses, but no component, for the complete extraction and analysis procedure. Record all weights as above.

11.5 *Sonication Extraction:*

11.5.1 Background information on sonication extraction can be found in Practices G131 and G136.

11.5.2 Glassware may need to be cleaned with nitric acid or other appropriate means prior to solvent cleaning.

11.5.3 Clean the glassware by rinsing at least three times with spectroscopy-grade hexane or another suitable solvent. The extraction solvent may be used.

11.5.4 Air dry all beakers and glassware at room temperature in a fume hood and store in a dessicator prior to use.

11.5.5 Place the medical component in a beaker, cover with clean foil, and weigh. Record the weight as m_1.

11.5.6 Add enough solvent to completely cover the component.

11.5.7 Cover the beaker with the clean aluminum foil, then place in a sonicator bath. The aluminum foil should not contact the water in the sonicator bath.

11.5.8 Start the sonicator bath, and extract the component(s) for a time period and temperature determined by the user pending internal validation of their extraction efficiency on the target residues. The extraction temperature should be below the boiling point of the solvent. More details on sonication times can be found in X1.2.3.

11.5.9 After the extraction period is complete, remove the sonication beaker from the bath and blot dry. Weigh the beaker, foil, component, and solvent and to an accuracy of 10 mg. Record the weight as m_2.

11.5.10 Weigh an aliquot beaker with a clean piece of foil small enough to be weighed on the 0.1 mg resolution balance. Record the weight as m_3.

11.5.11 Allow the insoluble debris to settle in the extraction vessel for 1 h. Withdraw an aliquot of the extracted solution that comprises at least 90 % of the total extracted solution and place in the aliquot beaker in described in 11.5.10, being careful not to withdraw any insoluble debris from the bottom of the extraction vessel. Weigh the solution with beaker and foil and record as m_4.

11.5.11.1 Allow the solvent to completely evaporate in a fume hood at room temperature. See X1.1.3 for more details.

11.5.11.2 Place the beaker, with residue, in a dessicator for a minimum of 2 h.

11.5.11.3 Weigh the beaker with foil and residue and record as m_5.

11.5.11.4 If the volume of the aliquot beaker is smaller than the aliquot, multiple aliquots can be removed from the extraction vessel, weighing each aliquot, evaporating the solvent and collecting the next aliquot. The solution weight m_4 is the sum of the aliquot weights. The final beaker weight m_5 should be recorded as described in 11.5.11.3.

11.6 *Blank Run:*

11.6.1 Conduct test blank(s) using the same amount of solvent and rinses, but no component, for the complete extraction and analysis procedure. Record all weights as above.

11.7 *Insoluble Residue Analysis by Weighing:*

11.7.1 Insoluble debris remaining in the extraction vessel should be isolated by resuspending the residue in the extraction solvent remaining after taking the aliquot, then filtering the debris through a pre-weighed filter. Record the filter weight prior to filtering as m_6. The extraction vessel should be rinsed with additional fresh solvent which should be also be passed through the filter. The pore size of the filter should be reported.

11.7.2 Allow the filter to air dry until a constant mass is obtained. Record this mass as m_7.

11.7.3 Blank runs should be conducted on the filters, as discussed in 11.6.

12. Calculation or Interpretation of Results

12.1 If multiple specimens were used to collect one set of residues, then the total calculated residue should be divided by the number of samples.

12.2 *Total Soluble Residue:*

12.2.1 The total amount of soluble residue in the aliquot m_a is calculated as:

$$m_a = m_5 - m_3 \quad (1)$$

12.2.2 The concentration of residue in the solution c_r is calculated as:

$$c_r = \frac{m_5 - m_3}{m_4 - m_3} \quad (2)$$

12.2.3 Repeat this calculation for the blank runs, calculating the average concentration of residue in blank solutions as c_b.

12.2.4 The total mass of extractable residue m_r, corrected by the blank concentration c_b, is calculated as:

$$m_r = (m_2 - m_1)c_r - (m_2 - m_1)c_b \quad (3)$$

12.3 *Insoluble Residue:*

12.3.1 The insoluble debris m_i is calculated as:

$$m_i = m_7 - m_6 \quad (4)$$

12.4 *Total Residue:*

12.4.1 The total extracted debris m_t is calculated as:

$$m_t = m_r + m_i \quad (5)$$

13. Additional Analysis

13.1 The residues extracted above may be subjected to additional analysis to determine the chemical makeup of the residues. The residues can be re-dissolved in solvents of choice or stored for later analysis.

14. Report

14.1 All residue data should be reported in terms of mass/surface area if the surface area of the part can be accurately determined, [mg/cm^2], as well as total weight of extracted debris per component. The report should include the measured residue data, as well as the residue data corrected for the extraction efficiency.

14.2 The report should also detail the test conditions, including:

14.2.1 Extraction solvent used, including purity,

14.2.2 Number of components tested per extraction,

14.2.3 Time of extraction, and

14.2.4 Frequency, amplitude, and temperature of sonication, if used.

15. Precision and Bias

15.1 Because this testing protocol is dependent on the nature of the medical implant and the type of manufacturing residues that can come in contact with the implant, it was determined that a round robin study was not practical, in that it would be limited to a very specific set of conditions. As such, a precision and bias statement derived from this round robin would not have broad application.

16. Keywords

16.1 extractable residue; gravimetric analysis; metallic medical components; non-soluble extractables; non-soluble debris; water soluble extractables

APPENDIX

(Nonmandatory Information)

X1. RATIONALE AND NOTES ON EXTRACTION PROTOCOL

X1.1 Rationale

X1.1.1 The cleanliness of medical components, both permanent implants and single-use components, should be assessed in order to minimize potential adverse biological responses to surface contamination or extractable residue.

X1.1.2 Alternate beaker conditioning steps can be used. The same conditioning steps and times should be used for each step in order to ensure reproducible weight measurements.

X1.1.3 The extraction solution in 11.3.15.1 and 11.5.11.1 can be heated to decrease the evaporation time. The user should verify that the extracted residue is not volatilized or chemically altered by the heating procedure.

X1.1.4 During the evaporation step, the user should ensure that debris such as dust cannot enter the beakers, which would affect the weight measurement. Some users have placed a screen on the beaker or performed the evaporation step in a laminar flow hood.

X1.2 Notes on Extraction Protocol

X1.2.1 This test method describes the use of refluxing and sonication methods to extract soluble and insoluble debris from metallic components. The extraction method used will depend on the available equipment and the residues that are to be extracted. In an independent study,[4] researchers compared the extraction efficiency of an ultrasonic bath to a refluxing method. A buffing compound (Matchless V367) was applied to porous cobalt-chromium-molybdenum test coupons, heated to 83°C for 1 h, then extracted in hexane via an ultrasonic bath (6 h at 40°C) or a refluxing system (24 h). Gravimetric analysis of the extractable residue using the technique described in this standard showed that reflux extraction was successful in extracting 84 % of the soluble residue, while ultrasound extracted 92 %. For this particular residue, sonication proved to be more efficient than refluxing. Other residues may be extracted more efficiently with refluxing extraction. The buffing compound represents one of the more challenging manufacturing aids to remove from metallic components.

X1.2.2 *Solvent Choices*—It is the experience of several laboratories that carbon tetrachloride and hexane are good solvents for a variety of organic-based residues used in medical component manufacturing. Isopropyl alcohol has also been used with some success. However, regulatory agencies and safety concerns may inhibit the use of these solvents for extraction. The user should determine the appropriate solvent that is effective in extracting the residue of choice, while meeting the necessary regulatory and safety requirements. If the solvent is water, it is recommended that distilled water is used.

X1.2.3 *Sonication Times*—Typical sonication times used for oil-based residues on metallic implants are usually 3 min to 1 h at ambient temperature. In one study on a baked-on buffing compound, a sonication extraction time of 4 h at 40ºC was required to achieve the desired extraction efficiency. Because of the possibility of erosion of the metallic implant caused by excessive sonication conditions,[5] leading to an erroneously higher amount of insoluble debris generation than would be found from an as-manufactured device, the user should select sonication conditions with caution or refer to the manufacturer of the sonication equipment.

X1.2.4 *Aliquot Size*—Users may opt to remove 100 % of the extraction solution in 11.3.15 or 11.5.11 to determine the total combined mass of soluble and insoluble residue in one measurement.

X1.2.5 *Sensitivity Analysis*—The statistical confidence interval of mass change values can be calculated by propagating all known sources of error, including those introduced by intra-measurement and environmental conditions variation. Errors can be propagated as sample variance, s^2, depending on the type of operation being performed:

$$(A \pm a)+(B \pm b) = (C \pm c) \rightarrow a^2+b^2 = c^2 \qquad \text{(X1.1)}$$

$$(A \pm a)\cdot(B \pm b) = (C \pm c) \rightarrow \left(\frac{a}{A}\right)^2 + \left(\frac{b}{B}\right)^2 = \left(\frac{c}{C}\right)^2$$

where values {A,B,C} and associated errors {a,b,c} are used in calculations.

[4] Hooper, M. T., Moseley, J. P., and Bible, S. J., "Efficiency of Reflux Extraction versus Sonication for the Recovery of Buffing Compound from Porous Coated Implants," *Trans. 7th World Biomaterials Congress*, pp. 1246.

[5] Busnaina, A., et al, "Ultrasonic Cleaning of Surfaces: An Overview," *Particles on Surfaces*, ed. K. Mittal, Vol 3, Plenum Press, New York, NY, 1991, pp. 217–237.

Intra-measurement error arises from random variations in measured values, and is captured by the repeated measurements of all mass values. The mass value is calculated as the sample average, $\bar{x}$, and the intra-measurement error σ_{meas} is calculated as the 95 % confidence interval of the sample distribution error, $\sigma_{\bar{x}}$:

$$\sigma_{meas} = 1.96 \cdot \sigma_{\bar{x}} = \frac{1.96 \cdot \sigma}{\sqrt{n}} \quad (X1.2)$$

where:

σ = the sample standard deviation, and
n = the number of measurements in the sample.

X1.2.5.1 Variations arising from environmental conditions are implicitly included in the blank correction required by this test method because the variations in blank and sample masses caused by changing environmental conditions are assumed to be identical. Error in this correction arises from random differences between environmental effects on blank and sample masses. To determine this error, the masses of two identical glass aliquot beakers can be measured for several days under varying environmental conditions (temperature, humidity), and the difference in day-to-day mass changes between the beakers can be calculated for each day. These differences represent a sample of the range of variation between two identical samples under identically varying environmental conditions, and the measurement error σ_{env} can be calculated for this source of error.

X1.2.5.2 Accordingly, the error for each mass measurement σ_{tot}, and therefore the base error propagated through all calculations performed for this analysis, was propagated from the sum of its two sources:

$$\sigma_{tot}^2 = \left[\frac{1.96 \cdot \sigma}{\sqrt{n}} \right]^2 + \sigma_{env}^2 \quad (X1.3)$$

X1.2.5.3 This analysis represents one method of performing sensitivity analysis. It is up to the individual laboratory to establish a robust method.