Measurement and Uncertainty of Residual Inorganic Content by Micro-ROI Analysis for Certification of Reference Materials

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Introduction

Certified reference materials for pharmaceutical, forensic/toxicology, clinical/diagnostic and environmental applications require thorough characterization. Understanding the traceability and uncertainty of the reference materials is critical for laboratories seeking to ensure compliance with ISO/IEC 17025.

The purity of a reference material cannot be reliably stated in terms of chromatographic purity alone. Residual impurities such as water, solvent, and inorganic impurities must be considered when using reference materials for quantitative applications.

Residual inorganic content is typically determined through residue on ignition techniques such as sulfated ash. USP <281> Residue on Ignition (ROI) requires large sample sizes of several grams. Reference materials, particularly impurities, metabolites, and stable labeled materials may not be available in large quantities.

Residue on Ignition using micro-analytical techniques (micro-ROI) have been developed for measurement of residual inorganic content using small samples sizes. Uncertainty of this technique was evaluated along with its impact on the overall uncertainty of the reference material.

Method

The micro-ROI method is based on USP <281> Residue on Ignition (ROI). Samples are accurately weighed, sulfated and ignited repeatedly at 600°C until a constant weight is achieved.

Residual inorganic content (ash) is calculated using the following equation:

$$\%ROI = \frac{(m_3 - m_1)}{(m_2 - m_1)} \times 100$$

where
m1: mass of empty crucible
m2: mass of crucible + sample
m3: mass of crucible + sample residue after ignition

This equation is also the measurement equation for uncertainty calculations.

Mass measurement procedures and sample handling techniques have a large impact on the overall uncertainty of the method.

Sample Handling

- Samples are handled in a manner to minimize contamination and ensure mass measurement accuracy.
- Crucibles are handled with forceps to eliminate heat transfer during weighing.
- Ionizers are used to eliminate the influence of static charge.
- Special precautions are employed for hygroscopic materials.
- Qualified 6 place analytical balance is used.

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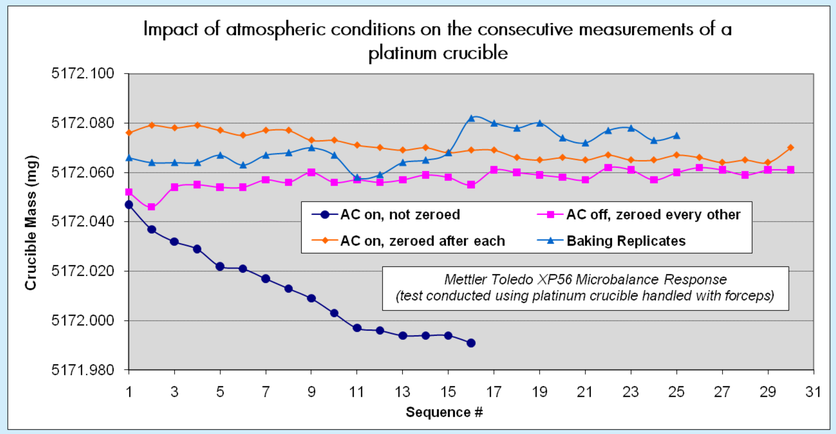
Mass Measurement

When smaller sample sizes are used, on the order of 10-100 mg, the uncertainty in the balance measurements becomes the primary contributor to the uncertainty of the result.



- Strong influence of temperature (air conditioner [AC] on/off) on the repeatability of the measurements if the balance is not zeroed between each measurement.
- Zeroing the balance before each measurement yields more consistent values, with the range dropping from 0.056 mg without zeroing to 0.016 mg with zeroing between measurements.
- When the crucible is ignited and cooled between measurements, the variance in measurements increases to 0.024 mg.

Specific mass measurement procedures were developed incorporating sample handling and balance parameters to minimize the uncertainty of the method.

Repeatability studies were performed employing these controls.

Method Precision

Repeatability

- Selected samples were analyzed in duplicate to demonstrate method repeatability.
- Sample size 10-25 mg.
- Controls representative of process impurities were included.
- Consistent results were obtained across a residue range of 0.055% to 100%.

Compound Name	% Ash	Absolute Difference	Percent Difference
Diethylpropion HCl	2.462	0.047	0.95%
	2.509		
Ethyl ß-D-glucuronide	0.124	0.002	0.81%
	0.122		
1% silica Control Sample	0.563	0.013	1.14%
	0.576		
9% silica Control Sample	7.580	0.050	0.33%
	7.530		
Morphine-D6	0.336	0.000	0.00%
	0.336		
Norbuprenorphine-3ß-D-glucuronide Lot A	2.381	0.000	0.00%
	2.381		
Glass wool Control Sample	102.743	0.783	0.38%
	103.526		
6-Acetylnormorphine	0.196	0.016	3.92%
	0.212		
10-Hydroxyoxymorphone	0.373	0.032	4.48%
	0.341		
Norbuprenorphine-3ß-D-glucuronide Lot B	0.057	0.002	1.79%
	0.055		

Intermediate Precision

- A control sample was selected for multiple analyses to demonstrate the intermediate precision of the method.

Sample Size (mg)	10.514	10.264	17.493	15.330	17.697	16.690	16.071	17.939
% Ash	1.146	1.116	1.246	1.239	1.006	1.321	1.033	1.120
					Average		1.153%	
					RSD		9.43%	

Analysis was repeated by three different analysts over four days.

Uncertainty

Uncertainty in the micro-ROI measurement was estimated following the guidelines provided in the EURACHEM/CITAC guide "Quantifying Uncertainty in Analytical Measurement" and evaluated over a range of input values.

- Input values to the measurement equation are mass measurements made on a qualified micro-balance installed on a marble balance table.
- The primary contributor to uncertainty of the micro-ROI method was the repeatability of crucible mass measurement before and after ignition. This was quantified using the standard deviation of repeated mass measurements.
- For the measurements made prior to ignition (s_{p1}), repeated measurements were made of a single platinum crucible. The standard deviation of these measurements was 0.016 mg.
- For measurements made after ignition (s_{p2}), the crucible was baked between each repeated measurement. The standard deviation of repeated measurements made post-ignition was 0.024 mg.
- Uncertainty was calculated using sequential perturbation in a spreadsheet format as described by Kragten[1].

Uncertainty of the micro-ROI value was determined for a range of sample mass and theoretical inorganic content and is presented in the table below. As expected, the uncertainty of the measurement has an inverse correlation with the sample mass and residual inorganic content.

micro-ROI Relative Uncertainty (%)		% Ash			
		0.2	*0.5*	*1*	*5*
Sample Mass (mg)	*15*	96.1	38.4	19.2	3.8
	25	57.7	23.0	11.5	2.3
	50	28.8	11.5	5.8	1.1

The micro-ROI uncertainty was propagated into the neat reference material purity calculation which factors in measurements of chromatographic purity, residual solvent, residual water and residual inorganic content. These values are shown below. The impact of micro-ROI sample size on purity uncertainty is limited.

Purity Factor Relative Uncertainty (%)		% Ash			
		0.2	*0.5*	*1*	*5*
Sample Mass (mg)	*15*	0.25	0.25	0.25	0.26
	25	0.19	0.19	0.19	0.20
	50	0.16	0.16	0.16	0.16

For the purposes of reference standard preparation an acceptable uncertainty can be obtained with sample masses as low as 15 mg.

1. J. Kragten. Calculating Standard Deviations and Confidence Intervals with a Universally Applicable Spreadsheet Technique. *The Analyst* 119: 2161-2165 (1994)

Comparison to USP <281>

Control samples were prepared that contained a known amount of Sodium Chloride in an organic matrix and tested by Cerilliant and a third-party laboratory.

% ROI theoretical*	3.53	1.22	0.63	0.30
Cerilliant micro-ROI (sample size 10 – 25 mg)	3.46	1.26	0.57	0.35
% recovery Cerilliant	-1.04%	1.41%	-5.39%	7.83%
Third-party USP <281> (sample size 1 g)	3.71	1.3	0.7	0.42
% recovery Third-party	2.49%	3.17%	5.26%	16.67%

Micro-ROI results are an average of 4 analyses by 2 analysts on different days.

Results indicate the Cerilliant method is comparable to the USP ROI method.

*%ROI theoretical is based on conversion of all inorganic content to the sulfated equivalent.

CONCLUSIONS

- This study demonstrates the feasibility of smaller sample sizes for residual inorganic content determination. Sample size is critical where materials have high cost and are available in limited quantities but must be fully certified for quantitative applications.
- The micro-ROI method is comparable to USP <281>.
- At low sample masses, the largest contributor to micro-ROI uncertainty is repeatability of the mass measurement.
- For the purposes of reference standard preparation an acceptable uncertainty can be obtained with sample masses as low as 15 mg.
- Micro-ROI uncertainty has minimal impact on overall uncertainty of the reference material purity factor.