United States
Consumer Product Safety Commission

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Test Method: CPSC-CH-C1002-26

CPSC Staff Test Method for Determining Extractable Acrylamide Content from Water Beads Under 16 CFR § 1250.4(c)(2)

1.0 Introduction

This document explains and provides the test method that is used by U.S. Consumer Product Safety Commission (CPSC) Laboratory staff[1] to determine extractable acrylamide content from water beads contained in water bead toys and toys containing water beads under 16 CFR § 1250.4.

In 2025, the Commission published a rule[2] with requirements that apply to water bead toys and toys containing water beads. The requirements are specified in 16 CFR § 1250.4 and include a limit on the amount of extracted acrylamide in water beads from water bead toys and toys containing water beads.[3]

This document informs and provides clarity to interested parties of the test method utilized by CPSC testing laboratory staff for assessing potential human exposure to acrylamide in water beads under 16 CFR § 1250.4(c)(2). This test method is not required to be followed by other laboratories performing such analyses; however, other laboratories may consider using this procedure to meet third party conformity assessment requirements under 16 CFR § 1250.4(c)(2).

Guidance regarding the requirements for water bead toys and toys containing water beads can be found at: https://www.cpsc.gov/Business--Manufacturing/Business-Education/Business-Guidance/Water-Bead-Toys-Business-Guidance.

[1] This document was prepared by CPSC staff. It has not been reviewed or approved by, and may not necessarily reflect, the views of the Commission.
[2] The final rule notice is here: https://www.federalregister.gov/documents/2025/12/12/2025-22643/safety-standard-for-toys-requirements-for-water-beads; The requirements of the regulation can be found in the eCFR :: 16 CFR 1250.4 -- Requirements for water beads.
[3] See 16 CFR § 1250.4(c)(2) for the extractable acrylamide limit for water beads.

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2.0 Definitions

2.1 Water bead: a various shaped liquid absorbent polymer, composed of materials such as, but not limited to, polyacrylamide and polyacrylate, which expands when soaked in liquid.[4]

2.1.1 Small water beads are defined as <4 mm in all dimensions of the bead prior to hydration and large water beads are defined as ≥4 mm in any dimension of the bead prior to hydration.[5]

2.2 Laboratory method blank: a solution that is treated exactly as a sample including exposure to glassware, apparatus, and conditions used for a particular method, but with no added sample (*i.e.*, water beads). Laboratory method blank data are used to assess background contamination from the laboratory environment.

2.3 Stock standard: a neat or concentrated chemical (*i.e.*, acrylamide) purchased from a reputable commercial source at an acceptable level of purity, used to prepare calibration standards. It must be replaced before the expiration date.

2.4 Calibration standard: a solution containing a known level of analyte (*i.e.*, acrylamide). Calibration standards should be prepared, as needed, from the stock standard. Calibration standards should be replaced when experimental data demonstrates a decrease in quality.

2.5 Calibration blank: a solution containing no analyte that is used to verify blank response and freedom from carryover during instrumental analysis.

2.6 Calibration verification: a solution of known analyte concentration used to evaluate instrument response over time. Calibration verification standards are made, when possible, from a different stock standard than the calibration standards (*i.e.*, different lot number or manufacturer).

3.0 Equipment and Supplies

Equipment and supplies necessary for this test method, using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) instrument, are listed below.

3.1 Chemicals: must be a sufficient grade for the analytical instrumentation being used

3.1.1 Acrylamide monomer (C_3H_5NO) ≥98% purity, CAS No. 79-06-1

3.1.2 Deionized water (H_2O), CAS No. 7732-18-5

3.1.3 Hydrochloric acid (HCl), CAS No. 7647-01-0

3.1.4 Sodium hydroxide (NaOH), CAS No. 1310-73-2

3.1.5 Methanol (CH_4O) LCMS grade, CAS No. 67-56-1

3.1.6 Acetic acid ($C_2H_4O_2$) LCMS grade, CAS No. 64-19-7

[4] As defined in 16 CFR § 1250.4(b).
[5] 16 CFR § 1250.4(c)(2) defines small and large water beads.

3.2 Supplies
- 3.2.1 Volumetric glassware, a range of 10 mL of 100 mL volumetric flasks is recommended
- 3.2.2 Pipette tips
- 3.2.3 Glass beakers, a range of 100 mL to 1 L size beakers is recommended
- 3.2.4 Plastic sealing film
- 3.2.5 Plastic syringes
- 3.2.6 Syringe filters, such as 0.2 µm polyethersulfone membrane filters
- 3.2.7 LC-MS/MS vials
- 3.2.8 LC-MS/MS vial caps
- 3.2.9 Calipers or ruler, 1 mm precision or better

3.3 Equipment
- 3.3.1 Analytical pipettes
- 3.3.2 pH meter, pH paper including a pH of 7 with 0.5-unit readability (or more precise), or other equipment capable of measuring pH
- 3.3.3 Water bath capable of shaking and maintaining an elevated temperature, 0.1 °C or more precise
- 3.3.4 Analytical balance, 0.1 mg readability or more precise
- 3.3.5 Density meter
- 3.3.6 LC-MS/MS

4.0 Scope and Summary

The procedures required for acrylamide testing as described in 16 CFR § 1250.4(c)(2) must be followed when testing for compliance to 16 CFR § 1250.4(c)(2). The procedure used by CPSC, as described in this document, is consistent with the required procedures in 16 CFR § 1250.4(c)(2) and does not contradict anything therein. The test method described in this document is not a requirement for external laboratories but, rather, is provided here as guidance for testing laboratories as to how CPSC conducts acrylamide testing to 16 CFR § 1250.4(c)(2).

The procedure used by CPSC, and described below, consists of three major parts: sample preparation, extraction, and analysis. The general approach is to place the water bead(s) in a container, extract the acrylamide using pH-neutral deionized water over a 24-hour period, then analyze by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

5.0 Precautions

This test method requires the use of hazardous materials, including organic solvents and concentrated acids and bases. It is of paramount importance to properly handle all hazardous materials safely in a ventilated fume hood with adequate personal protective equipment such as gloves, lab glasses or goggles, and a lab coat.

6.0 Sample Preparation

Determine the number of water beads to be analyzed. A minimum of three trials is required. The number of water beads tested in each trial depends on the size of the water bead. For large water beads, defined as ≥4 mm in any dimension prior to hydration, test 1 bead per trial. For small water beads, defined as <4 mm in all dimensions prior to hydration, test 100 beads per trial. Use calipers or a ruler to determine whether the bead falls into the "large" or "small" category prior to testing.

Prepare enough pH-neutral deionized water to conduct three trials of each type of water bead being tested. Include at least one trial of a laboratory method blank. Prepare the extraction solution by adding an appropriate amount of acid or base (hydrochloric acid or sodium hydroxide) to the deionized water to obtain a pH of 7. Measure the pH of the extraction solution; a pH of 6.5-7.5 is acceptable. Do not re-measure or adjust pH again during the 24-hour extraction.

7.0 Extraction Method

Determine the appropriate container and volume of water to be used to perform the acrylamide extraction. Acrylamide extractions must be performed using both a volume of water that allows for full growth and coverage of the water bead(s) as well as a container that does not compress the bead(s) at any point during the 24-hour extraction period. Perform a trial run to determine an appropriate container and volume of water.[6] Multiple trial runs may be conducted simultaneously or iteratively.

7.1 Conduct a trial run by placing 100 small beads or 1 large bead in a container.
7.2 Add deionized water that has been pH neutralized.
7.3 Cover the container with plastic sealing film, place the container in a shaker bath at 37 °C, and shake it at a rate of 30 revolutions per minute (RPM). Leave the container and water bead(s) untouched in the shaker bath for 24 hours.
7.4 Examine the container and water beads at the end of the 24 hours.
- 7.4.1 If the water bead(s) has/have absorbed all the water, then more water is necessary for the extraction.
- 7.4.2 If the water bead(s) is/are no longer fully covered by water, then more water and/or a different container is necessary for the extraction.
- 7.4.3 If the water bead(s) has/have grown such that the sides of the container are compressing any bead, a different container is necessary for the extraction.
- 7.4.4 If there is a large quantity of water remaining, consider decreasing the amount of water used so that the concentration of extracted acrylamide is not unnecessarily diluted.

[6] 16 CFR § 1250.4(c)(2)(iv) discusses how optional tests may be performed to determine an appropriate volume of water and container.

7.5 Conduct further trial runs as necessary to determine optimal extraction conditions. Once an appropriate container and volume of water have been chosen, proceed to the final extractions.

7.6 For large water beads, perform three trials with one large bead per trial. For small water beads, perform three trials with 100 small beads per trial. Each trial is conducted in a separate container of deionized water that has been pH neutralized.[7] Include at least one trial of a laboratory method blank.

7.7 Place the water beads (one large water bead or 100 small water beads) as received in a container with deionized water that has been pH neutralized.[8] Prepare at least one container of a laboratory method blank, containing the extraction solution with no water beads.

7.8 Cover the containers with plastic sealing film, or an equivalent cap or covering, to prevent evaporation.[9]

7.9 Place the containers into a shaker bath heated to 37 °C and shake them at a rate of 30 revolutions per minute (RPM) for 24 hours. Leave the containers and beads untouched for the entirety of the 24-hour period.[10]

7.10 At the conclusion of 24 hours, remove the containers from the shaker bath.

7.11 Separate the water beads from the remaining volume of water (extraction solution), ensuring that the entire volume of remaining water is preserved.

7.12 Measure the volume of remaining water and record for each trial.[11] The volume may be determined by mass, using a graduated cylinder, or any other volumetric method.

7.12.1 If the volume is determined by mass, first determine the mass of remaining water for each trial using an analytical balance, and then remove an aliquot for instrumental analysis. Use a density meter to measure the exact density of the remaining water for each trial in order to calculate an accurate volume.

7.13 Filter an aliquot of the remaining water from each trial into an LC vial for instrumental analysis using a plastic syringe and syringe filter. Cap the vials.

8.0 Instrument Parameters

Acrylamide analysis is performed using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) instrument. For LC-MS/MS, instrument conditions may vary depending on the model and configuration of the instrument used. The following LC-MS/MS conditions (Table 1) can be used, though these specific conditions and instrumentation are not required.

[7] 16 CFR § 1250.4(c)(2)(iii) specifies the number of trials, number of beads per trial, and requires that each trial is conducted in a separate container of deionized water that has been pH neutralized.
[8] 16 CFR § 1250.4(c)(2)(i) describes this step.
[9] 16 CFR § 1250.4(c)(2)(v) requires that containers be covered to prevent evaporation.
[10] 16 CFR § 1250.4(c)(2)(ii) requires these extraction conditions.
[11] 16 CFR § 1250.4(c)(2)(vi) requires that the volume of remaining water be determined for each trial.

Table 1: LC-MS/MS Operating Conditions

LC Parameter	LC Condition			
Column	C_{18} 1.7 µm particle size, 2.1 mm x 50 mm			
Mobile Phase	Deionized water with 0.5% methanol and 0.1% acetic acid			
Flow Rate	0.2 mL/min constant			
Run Time	2.5 min			
Injection Volume	10 µL			
Seal Wash	Every 10 minutes, 50/50 Water/Methanol			
Strong Needle Wash	Methanol			
Weak Needle Wash	Deionized water with 0.5% methanol			
MS Parameter	**MS Condition**			
Capillary Voltage	3.80 kV			
Cone Voltage	5 V			
Desolvation Temperature	500 °C			
Desolvation Gas Flow	1000 L/hr			
Cone Gas Flow	10 L/hr			
Ionization Mode	ESI+			
MRM Method	**Time Segment**	**Mass Transition**	**Collision Energy**	**Dwell Time**
MRM Channel 1	0-2.5 min	71.75→54.75 m/z	3 V	0.332 s

9.0 Analysis

Analyze a filtered aliquot of the remaining water at the end of the 24-hour period, from each trial, to determine the concentration of extracted acrylamide present. Use an instrument that can quantitate acrylamide at levels equal to or less than the extracted acrylamide limit[12] specified in 16 CFR § 1250.4(c)(2). CPSC uses an LC-MS/MS instrument.

9.1 Prepare acrylamide calibration standards, including one calibration blank and at least one calibration verification, in volumetric glassware. The use of an internal standard and other types of quality control solutions is optional

9.2 Analyze the calibration standards, calibration verifications, and calibration blanks with a LC-MS/MS. Qualitatively analyze the results to ensure proper retention times and no contamination.

9.3 Integrate the peak areas from valley to valley for each standard.

9.4 Construct a calibration curve using the peak areas.

[12] 16 CFR § 1250.4(c)(2)(vi) requires this level of instrumentation.

9.5 Create a multi-point calibration curve with a 1/X weighted calibration model, generally with at least five points with a R^2 value >0.95. Separate low and high calibration curves may be required to quantify acrylamide in the method blank and samples.
9.6 Calibration standards should have calculated acrylamide concentrations within 20% of their expected values.
9.7 Calibration blanks should have calculated acrylamide concentrations that are less than 80% of the lowest calibration standard.
9.8 Analyze the laboratory method blank and all samples. The laboratory method blank and all samples should be preceded by at least one calibration blank and one calibration verification and followed by at least one calibration blank and one calibration verification. Other quality control solutions are optional.
9.9 Calibration blanks and laboratory method blanks should have calculated acrylamide concentrations that are less than 80% of the lowest calibration standard.
9.10 Calibration verifications should have calculated acrylamide concentrations within 20% of their expected values.

Laboratories are allowed to incorporate alternative and/or additional quality control checks and protocols to provide confidence in their results.

10.0 Calculations and Results

To determine the mass of extracted acrylamide (µg), multiply the measured concentration of acrylamide by the measured volume of remaining water for each trial, accounting for any necessary unit conversions.

Report acrylamide results in units of micrograms of extracted acrylamide. For small beads, this is micrograms of acrylamide extracted from 100 beads. For large beads, this is micrograms of acrylamide extracted from one large bead. Report the mass of extracted acrylamide for each trial. Water beads shall not have more than 325 µg acrylamide extractable from 100 small water beads or from one large water bead.[13] CPSC staff have no guidance or requirements regarding agreement among trials.

[13] 16 CFR § 1250.4(c)(2) specifies the acrylamide limit.