7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring acrylamide, its metabolites, and other biomarkers of exposure and effect to acrylamide. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

7.1 BIOLOGICAL MATERIALS

Methods for the detection of acrylamide in biological materials are summarized in Table 7-1. Acrylamide can be detected in biological samples using gas chromatography (GC) with electron-capture detection. Acrylamide is converted to its 2,3-dibromopropionamide derivation in aqueous solution, plasma, or tissue homogenates by ionic bromination. The limits of detection for this method correspond to 9.5×10^{-12} g of acrylamide on the column or 8.4×10^{-9} g in the final biological extract of 0.5 mL. Acrylamide recovery by this method exceeds 80% at nanogram levels (HSDB 2009).

A liquid chromatography/tandem mass spectrometry (LC-MS/MS) assay was developed to examine unchanged acrylamide concentrations in bodily fluids, specifically urine, breast milk, and placental perfusion medium. A bioanalytical method was developed to analyze acrylamide in bodily fluids using LC-MS/MS following liquid/liquid extraction of acrylamide and evaporation under a stream of N_2 at 35 °C. A reversed-phase column eluted with an isocratic solvent system consisting of water, acetic acid, and an organic modifier was used for chromatographic separation. Method detection limits were 1 ng/mL for urine, 2 ng/mL for placenta perfusate, and 5 ng/mL for breast milk (Sorgel et al. 2002).

GC-MS methods have been used to determine the adducts of acrylamide and its metabolites (such as glycidamide) with hemoglobin in blood samples. These methods are sensitive enough to measure adducts at blood levels relevant to potential dietary acrylamide exposure, which allows for use of the adducts as

Table 7-1. Analytical Methods for Determining Acrylamide in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	Liquid/liquid extraction, evaporation under N_2 stream at 35 °C	LC-MS/MS	1 ng/mL		Sorgel et al. 2002
Breast milk	Liquid/liquid extraction, evaporation under N_2 stream at 35 °C	LC-MS/MS	5 ng/mL		Sorgel et al. 2002
Placental perfusion medium	Liquid/liquid extraction, evaporation under N_2 stream at 35 °C	LC-MS/MS	2 ng/mL		Sorgel et al. 2002
Biological samples	Ionic bromination of acrylamide in sample	GC with electron-capture device	Corresponds to $9.5x10^{-12}$ g acrylamide (column), $8.4x10^{-9}$ g (extract)		HSDB 2009

GC = gas chromatography; LC = liquid chromatography; MS = mass spectrometry

biomarkers of exposure (WHO 2002, 2003). Recent methods of detection for hemoglobin adducts of acrylamide and/or glycidamide utilize LC-MS/MS, High-Performance Liquid Chromatography (HPLC)/MS/MS, or GC-Negative Chemical Ionization (NCI)/MS/MS) (Fennell et al. 2003, Schettgen et al. 2010; Urban et al. 2006; Vesper et al. 2006, 2007, 2010; Von Stedingk et al. 2010; Wirfält et al. 2008).

Acrylamide and glycidamide were measured in serum and tissues of mice following dosing by intravenous, gavage, and dietary routes at 0.1 mg/kg acrylamide and by intravenous and gavage routes at equimolar amounts of glycidamide. LC-electrospray (ES)/MS/MS was utilized to measure acrylamide and glycidamide in serum and tissues with recoveries of 70% for acrylamide and 63% for glycidamide. The limit of detection was below 0.1 pmol/mg tissue for both acrylamide and glycidamide (Doerge et al. 2005b). Doerge et al. (2005a) detected DNA adducts derived from administration of acrylamide and glycidamide to mice and rats. A single intraperitoneal injection containing an aqueous solution of 100 µL of 50 mg/kg acrylamide or 61 mg/kg glycamide was given to the mice. Rats were treated with a single intraperitoneal injection of the same concentration using a volume of 100 µL per 100 g body weight. Six hours after dosing, the tissues were removed following gassing by carbon dioxide. Tissues were frozen and stored at -80 °C. DNA adducts were analyzed using a Quattro Ultima triple stage guadrupole mass spectrometer with an electrospray source, with an ion source temperature of 120 °C, desolvation gas temperature of 400 °C, and constant cone voltage of 35 V. DNA was isolated using a Blood and Cell Culture Maxi kit and adducts were quantified using LC-/MS/MS. The limit of quantification ranged from 1 to 1.5 adducts in 10^8 nucleotides and the limit of detection was 0.5 adducts in 10^8 nucleotides (Doerge et al. 2005a).

7.2 ENVIRONMENTAL SAMPLES

Methods for the detection of acrylamide in environmental samples are summarized in Table 7-2. Acrylamide detection in water samples can be achieved using direct injection and a reversed-phase high performance liquid chromatography (HPLC)-ultraviolet (UV) absorption procedure, which has a limit of detection of 5 µg/L (Cavalli et al. 2004). An HPLC method can be used to determine the amount of acrylamide monomer in natural and polluted aqueous environments. Acrylamide undergoes bromination and the resulting dibromopropionamide is assayed. The detection limit for the method was found to be 0.2 µg/L in river, sea, and estuarine waters as well as potable waters, sewage, and china clay works effluents (HSDB 2009).

Table 7-2. Analytical Methods for Determining Acrylamide in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Direct injection	Reversed-phase HPLC-UV absorption	5 µg/L		Cavalli et al. 2004
Natural and polluted water	Bromination of acrylamide in sample	HPLC	0.20 µg/L		HSDB 2009
Organic-free reagent water		HPLC	10 µg/L		HSDB 2009
Aqueous matrices		GC	0.032 µg/L		HSDB 2009
Drinking water	Direct injection of 500 µL	Ion-exclusion chromatographic separation/MS	0.20 ppb		Cavalli et al. 2004
Airborne dust, vapors		Differential pulse polarographic method			HSDB 2009
Food	Water extract food, brominates sample	GC-MS	5–10 µg/kg		WHO 2002
Food	Bromination of sample	GC-MS	5 µg/kg		Tareke et al. 2002
Food	Water extract food	LC-MS/MS	20–50 µg/kg		WHO 2002
Food		LC-MS/MS	10 µg/kg		Tareke et al. 2002
Food		LC-MS/MS	10 µg/kg	100–115%	Arisseto et al. 2007

GC = gas chromatography; HPLC = high performance liquid chromatography; LC = liquid chromatography; MS = mass spectrometry; SIM = selected ion monitoring; UV = ultraviolet

Solid phase extraction, extraction with activated carbon filter, and GC-MS analyses can also be used (Cavalli et al. 2004). A water sample is brominated to form 2,3-dibromopropionamide, which is then analyzed by GC-MS using methacrylamide as an internal standard (Ahn et al. 2002). Additional methods include polarography and electron capture GC (WHO 2003). EPA-OSW 8316 method determines acrylamide concentrations by HPLC in organic-free reagent water, with a limit of detection of 10 µg/L. The EPA-OSW 8032A method analyzes acrylamide in aqueous matrices using GC, with a detection limit of 0.032 µg/L (HSDB 2009).

Cavalli et al. (2004) utilized a method for detecting acrylamide in drinking water using a combination of ion-exclusion chromatographic separation and MS detection. Drinking water samples are injected directly into the microbore ICE-AS1 column and are then detected in the selected-ion monitoring mode by a single quadrupole system with electrospray ionization. The detection limit for the method was determined to be 0.20 ppb with an injection volume of 500 µL (Cavalli et al. 2004).

Airborne acrylamide vapors and dust can be determined in air by a differential pulse polarographic method (HSDB 2009). Two additional methods of detection in air were found (called Method 21 and OSHA PV2004), though only very limited details were provided (HSDB 2009).

Various methods are available to determine the acrylamide content of food. The most common methods utilize either GC-MS or LC-MS/MS (Ahn et al. 2002; Arisseto et al. 2007). Water is often used as the extraction solvent. Solid-phase extraction (SPE) is typically use to prepare samples. SPE phases that have been used for this method include graphitized carbon black, mixed mode anion and cation exchange, and polymeric materials (Arisseto et al. 2007)

The GC-MS method of determining acrylamide content in food is well established. A water extract of the food is brominated to form 2,3-dibromopropionamide, a derivative of acrylamide with enhanced GC properties. The derivative is then analyzed by GC-MS using methacrylamide as an internal standard (Ahn et al. 2002; WHO 2002). Detection limits for the GC-MS method are typically in the 5–10 µg/kg range (WHO 2002). Tareke et al. (2002) analyzed food samples for acrylamide using an improved GC-MS method involving bromination. A detection limit of 5 µg/kg was achieved.

The LC-MS/MS method was developed over concerns that artifacts were forming during the bromination procedure used with the GC-MS method. LC-MS/MS allows for direct acrylamide analysis without the need for a derivative (WHO 2002). In this method, a water extract of food is tested by LC-MS/MS using

deuterated acrylamide as an internal standard (Ahn et al. 2002). Acrylamide is then identified by its retention time and the relative ion intensities. WHO (2002) reports that the detection limits of this method are typically 20–50 μg/kg, although lower detection limits have since been obtained for this method.

Tareke et al. (2002) also utilized the LC-MS/MS method to determine underivitized acrylamide concentrations. This method had a detection limit of 10 μg/kg. Sorgel et al. (2002) used a modified LC-MS/MS method, similar to that developed for analysis of acrylamide in bodily fluids, to determine acrylamide content in food. Brazilian foods were analyzed by LC-MS/MS to determine acrylamide content (Arisseto et al. 2007). Detection limits of 10 μg/kg were achieved, along with a limit of quantification of 20 μg/kg and mean recoveries ranging from 100 to 115%.

7.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of acrylamide is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of acrylamide.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect.

Exposure. Exposure to acrylamide can be determined directly by LC-MS/MS for biological samples, including urine, breast milk, and placental perfusion medium with detection limits of 1, 5, and 2 ng/mL, respectively (Sorgel et al. 2002). GC-MS methods are also used to detect the adducts of acrylamide and its metabolites in blood and tissue samples (Fennell et al. 2003, HSDB 2009; Schettgen et al. 2010; Urban

et al. 2006; Vesper et al. 2006, 2007, 2010; Von Stedingk et al. 2010; WHO 2002, 2003; Wirfält et al. 2008). Selected methods are described in Table 7-1. Additional methods for the detection of acrylamide and its metabolites in biological samples would be helpful.

Effect. Information on biomarkers of effect for acrylamide would be useful.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. There are several variations on methods for identifying acrylamide in water, air, and food. The methods are summarized in Table 7-2. The methods for water, primarily involving the use of HPLC and GC are fairly sensitive (e.g., ~0.03-10 µg/L for water (Cavalli et al. 2004; HSDB 2009). In air, a differential pulse polarographic method was identified, although limited details were provided (HSDB 2009). Additional information concerning the detection of acrylamide in air, including detection limits, would be useful. Various methods are available to determine acrylamide content of food using primarily GC-MS or LC-MS/MS (Ahn et al. 2002; Arisseto et al. 2007). GC-MS involved bromination, while LC-MS/MS can determine acrylamide content directly. Detection limits for GC-MS methods typically range from 5 to 10 µg/kg, while limits of detection for the LC-MS/MS method are typically in the range of 10–50 µg/kg (Tareke et al. 2002; WHO 2002). More sensitive methods for direct determination of acrylamide in food via the LC-MS/MS method would use useful.

7.3.2 Ongoing Studies

The Federal Research in Progress (FEDRIP 2009) database contains a study sponsored by the U.S. Department of Agriculture in which new methods are being developed for detecting and controlling acrylamide formation in deep-fat fried foods.

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