Acrylamide Analytical Methods Working Group Backgrounder

The objectives of the Analytical Methods Working Group are to determine the current status of analytical methods for the determination of acrylamide in foods, the performance characteristics of those methods, needs for further methods development including the development of rapid methods, the availability of proficiency testing samples, and the collaborative testing of a reference method (multi-laboratory method validation). This Position Paper has been drafted to assist the Working Group with the discussions. This Position Paper is not being represented as a complete or thorough review of the literature with respect to the intended objectives of the Working Group. It serves only to promote and guide the discussions that will occur during the course of the meeting.

This Position Paper will provide some general background on acrylamide that is relevant to a discussion of analytical methods, general information on analytical methods, a summary of published methods, and a brief discussion of the status of proficiency samples and collaborative studies.

Introduction

As shown in Table 1, acrylamide is a very water-soluble organic molecule with low volatility. Thus, the analytical methods that have been developed have either been based upon derivatization of the molecule to increase its volatility or have employed liquid chromatography.

Acrylamide is a colorless to white odorless solid that melts at 84-85°C. On crystallization from benzene, leaf- or flake-like crystals are formed. Heating results in polymerization, which may be violent. Polymerization prevents the determination of a boiling point at ambient pressures, but at 3.34 kPa (25 mm Hg) acrylamide boils at 125°C. Polymerization also occurs with ultraviolet irradiation, and commercial solutions are stabilized with cuprous salts, *tert*-butylpyrocatechol or other antioxidants. The solid is stable if stored in a cool dry place.

Table 1

Molecular weight	71.06
Melting Point	84-86 ^{o}C
Solubility	216 g/100 g Water @ 30 °C. Other solvents: methanol (155), ethanol (86.2), acetone (63.1), ethyl acetate (12.6). It is sparingly soluble in benzene (0.35) and heptane (0.0068).
Boiling Point:	125 °C/ 25 mm
Stability	Thermally unstable

Analytical approaches for the measurement of acrylamide

There are a number of methods that have been developed for the analysis of acrylamide. Not all of these methods are suitable for use in foods.

Colorimetry

Acrylamide reacts with diazomethane in methanol-ether solution to form a pyrazoline derivative that reacts with 4-dimethyl-cinnamaldehyde to form a bright purple Schiff base complex. However, this reaction is not specific for acrylamide, and there can be interference by many other organic compounds.

Gas chromatography

Acrylamide is brominated to give its 2,3-dibromopropionamide derivative that is measured by gas chromatography using an electron capture detector (ECD). It can also be measured by a flame ionization detector (FID) but this is less sensitive.

Ultraviolet detection

Acrylamide or 2,3-dibromopropionamide is separated by means of high pressure liquid chromatography (HPLC) and measured by UV detection. This is a rapid and sensitive method.

High pressure liquid chromatography (HPLC)

Reverse phase HPLC can be used to determine the concentration of acrylamide or 2,3-dibromopropionamide.

Summary of Published Analytical Methods for the Determination of Acrylamide in Water and Foods

As mentioned in the previous section, current methods for the analysis of acrylamide in water and foods can be grouped into those that employ either separation by gas chromatography or by liquid chromatography.

GC Method

The published methods for acrylamide in foods that are based upon GC are very similar to the EPA method for acrylamide in water (1). Acrylamide in the extracted sample is derivatized with bromine (generated in situ with potassium bromide and hydrobromic acid) to dibromopropionamide. For water samples the brominated sample was extracted with ethyl acetate and an aliquot of the extract analyzed by GC with an electron capture detector. Where necessary, interferences from the extract can be removed by a Florisil cleanup column.

GC methods without derivatization of acrylamide are in development.

GC/MS Method

For food samples (2), the food is blended with water. The solids are removed by centrifugation and the supernatant is brominated. The brominated derivative is extracted into ethyl acetate. Further clean up of the sample can be done or the extracts can be analyzed directly by GC/MS. This method has been modified (6) by the addition of a water immiscible organic solvent as the food is blended with water to assist with the removal of lipids.

LC/MS/MS, LC/MS

Liquid chromatography based separation methods avoid the necessity of preparing a derivative. Thus, after the food samples are extracted with water, the extracts are cleaned up on various solid phase extraction columns. Two methods based upon LC/MS/MS method have been described (3,4,5). A method that integrates the extraction of the aqueous extract with ethyl acetate and excludes additional clean up has recently been described (6).

Table 2 summarizes the MS analytical parameters.

Table 2

	Ion Monitored	Quantified
LC/MS/MS	Acrylamide m/z: 27, 55, 72 (FDA) 54, 55 (Sweden) Internal Standard ^{13}C m/z: 29, 58, 75 (FDA)	Acrylamide m/z: 55 (FDA, Sweden) 72 / m/z: 58 (FDA, Sweden) 73
GC/MS	Acrylamide derivative m/z: 55, 56, 71, 72 m/z: 106, 108, 150, 152 (Sweden) Internal Standard m/z: 72 ^{13}C m/z: 110, 155 N,N-dimethyl acrylamide m/z: 78, 180 Dimethyl phthalate m/z: 163, 164	Acrylamide derivative m/z: 71 152 / Internal Standard m/z: 72 ^{13}C m/z: 155 N,N-dimethyl acrylamide m/z: 78, 180 Dimethyl phthalate m/z: 163

LC/UV Detection

Several laboratories have developed or are working on methods based upon liquid chromatography with UV detection, but thus far none of those methods have been published.

Table 3 presents a summary of the currently published methods.

Table 3

Method Reference	First Step/Extraction	Clean-up of Extract	Derivatization	Additional Clean-up	Separation/ Detection
EPA (water)			Brominate, extract w/EtOAC	Florisil cleanup, add IS (dimethylphthalate)	GC/ECD
Castle	50 g/ 200 ml water, acrylamide added to sample for quantification by standard addition	Centrifuge	Brominate, extract w/EtOAc, evap to dryness	Clean-up with BondElut (silica)	GC/MS
Tareke, et al	10 g/ 100 ml water	Filter, treat w/carbon column, add IS (N, N-dimethylacrylamide or $^{13}C_3$ Acrylamide)	Brominate, extract w/EtOAC-Hexane		GC/MS
FDA (Musser)	1 g/ 50 ml water, add $^{13}C_3$ Acrylamide as IS	Centrifuge, filter, OASIS SPE , followed by Varian/BondElut SPE			LC/MS/MS
Rosen and Hellenas	2-4 g/ 40 ml water, IS (deuterated acrylamide)	Centrifuge, filter, Isolute Multimode SPE			LC/MS/MS
Tareke, et al	10 g/ 100 ml water, add $^{13}C_3$ Acrylamide as IS	Centrifuge, filter, Isolute Multimode SPE, filter again			LC/MS/MS
Sanders, et al	6 g/ 40 ml water, add $^{13}C_3$ Acrylamide as IS, heat	Add 10 ml of EDC, Centrifuge, extract with EtOAc, Conc. extract			LC/MS

Method Performance Characteristics

Detection limits

Detection limits are matrix dependent. The estimated detection limits are shown below:

Method	Est. Detection Limit, ppb
GC/MS	10
LC/MS/MS	10
LC/MS w/EtOAc extraction	50

Precision and Accuracy

The precision of all of the published methods appears to be very good.

Method Reference	% CV
Sanders, et al.	4.7 (3.3 in spiked samples)
Rosen and Hellenas	6-21 (3-9 in spiked samples)
Tareke et al.	1.6-8.9

Accuracy

While most reported recoveries have been nearly quantitative, the results from other spiking experiments have not been as good. For example, according to a NFPA round robin project, the spike recovery in a food matrix was below 60 %.

Proficiency Testing

A very limited set of samples for proficiency testing was prepared in the United States by National Food Processors Association. Elsewhere, BgVV and FAPAS are sponsoring proficiency tests. Results from the FAPAS Round 1 with crisp bread were available in September.

Interlaboratory Method Validation

US FDA has indicated that a multi-laboratory method validation is being planned.

Questions to be considered by the Working Group

Draft of Questions for Analytical Methods Working Group

1. The calculation of the recoveries of acrylamide from a food matrix has generally been based upon a comparison to an internal standard. In such instances, the recoveries have been reported as being nearly quantitative. However, when recoveries are examined in comparison to added acrylamide, the recoveries are considerably less (approximately 60%).
    a. What effect does this have on the confidence in using the results for exposure assessment purposes?
    b. Does this indicate incomplete extraction of acrylamide from a food matrix, or does this indicate degradation of acrylamide?
    c. What studies should be done to answer question 1b, if the answer(s) are not already known?

2. What should be done to improve the level of confidence in analytical results from different laboratories?
    a. Should a reference method be established? Which method should be selected and under what umbrella (AOAC, CEN, AACC) should a collaborative test be conducted?
    b. Should reference materials and standards be formulated? What organization will maintain and distribute these materials?
    c. Is proficiency testing program for laboratories doing acrylamide testing necessary? If so, who should organize and conduct such a program?

3. What are the possibilities for the development of low cost, simple methods for the routine testing of foods for acrylamide? What are the priority areas for exploration (e.g., ELISA, biosensors)?

References

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