



Environment Agency

The determination of acrylamide in waters using
chromatography with mass spectrometric detection (2009)

Methods for the Examination of Waters and Associated Materials

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This booklet contains guidance on the determination of acrylamide in waters.

Method A has been validated in only one laboratory and consequently details are included for information purposes only as an example of the type of procedures that are available to analysts. Information on routine multi-laboratory use of both methods would be welcomed to assess their full capabilities.

Whilst this booklet may report details of the materials actually used, this does not constitute an endorsement of these products but serves only as illustrative examples. Equivalent products are available and it should be understood that the performance characteristics of the method might differ when other materials are used. It is left to users to evaluate methods in their own laboratories.

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About this series

Introduction

This booklet is part of a series intended to provide authoritative guidance on recommended methods of sampling and analysis for determining the quality of drinking water, ground water, river water and sea water, waste water and effluents as well as sewage sludges, sediments, soil (including contaminated land) and biota. In addition, short reviews of the most important analytical techniques of interest to the water and sewage industries are included.

Performance of methods

Ideally, all methods should be fully evaluated with results from performance tests. These methods should be capable of establishing, within specified or pre-determined and acceptable limits of deviation and detection, whether or not any sample contains concentrations of parameters above those of interest.

For a method to be considered fully evaluated, individual results from at least three laboratories should be reported. The specifications of performance generally relate to maximum tolerable values for total error (random and systematic errors) systematic error (bias) total standard deviation and limit of detection. Often, full evaluation is not possible and only limited performance data may be available.

In addition, good laboratory practice and analytical quality control are essential if satisfactory results are to be achieved.

Standing Committee of Analysts

The preparation of booklets within the series "Methods for the Examination of Waters and Associated Materials"

and their continuing revision is the responsibility of the Standing Committee of Analysts. This committee was established in 1972 by the Department of the Environment and is now managed by the Environment Agency. At present, there are nine working groups, each responsible for one section or aspect of water quality analysis. They are

- 1 General principles of sampling and accuracy of results
- 2 Microbiological methods
- 3 Empirical and physical methods
- 4 Metals and metalloids
- 5 General non-metallic substances
- 6 Organic impurities
- 7 Biological methods
- 8 Biodegradability and inhibition methods
- 9 Radiochemical methods

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, in co-operation with the working group and main committee. The names of those members principally associated with this booklet are listed at the back of the booklet.

Publication of new or revised methods will be notified to the technical press. If users wish to receive copies or advance notice of forthcoming publications, or obtain details of the index of methods then contact the Secretary on the Agency's internet web-page (<http://www.environment-agency.gov.uk/nls>) or by post.

Every effort is made to avoid errors appearing in the published text. If, however, any are found, please notify the Secretary. Users should ensure they are aware of the most recent version of the draft they seek.

Dr D Westwood
Secretary
December 2004

Warning to users

The analytical procedures described in this booklet should only be carried out under the proper supervision of competent, trained analysts in properly equipped laboratories.

All possible safety precautions should be followed and appropriate regulatory requirements complied with. This should include compliance with the Health and Safety at Work etc Act 1974 and regulations made under this Act, and the Control of Substances Hazardous to Health Regulations 2002 (SI 2002/2677). Where particular or exceptional hazards exist in carrying out the procedures described in this booklet, then specific attention is noted

Numerous publications are available giving practical details on first aid and laboratory safety. These should be consulted and be readily accessible to all analysts. Amongst such publications are; "Safe Practices in Chemical Laboratories" and "Hazards in the Chemical Laboratory", 1992, produced by the Royal Society of Chemistry; "Guidelines for Microbiological Safety", 1986, Portland Press, Colchester, produced by Member Societies of the Microbiological Consultative Committee; and "Safety Precautions, Notes for Guidance" produced by the Public Health Laboratory Service. Another useful publication is "Good Laboratory Practice" produced by the Department of Health.

A The determination of acrylamide in waters using gas chromatography with mass spectrometric detection

Introduction

Acrylamide, $\text{CH}_2\text{CHCONH}_2$, is the monomer compound used in the manufacture of polyacrylamide. Polyacrylamide flocculants are approved materials, prescribed under "The Water Supply (Water Quality) Regulations 2000", Statutory Instrument 2000 No 3184, as amended, and are used in drinking water treatment processes. The current limit for acrylamide concentrations in drinking water is based on the product specification for polyacrylamide used in the treatment process, and is 0.1 $\mu\text{g/l}$.

A1 Performance characteristics of the method

A1.1	Substances determined	Acrylamide.
A1.2	Type of sample	Raw waters, drinking waters and process waters.
A1.3	Basis of method	Acrylamide is converted to its dibromo derivative, and extracted from the sample using solid phase extraction cartridges. Extracts are determined using gas chromatography (GC) with mass spectrometric (MS) detection.
A1.4	Range of application	Typically up to 0.5 $\mu\text{g/l}$. The range may be extended (see section A7.14, note i).
A1.5	Standard deviation	See Table A1.
A1.6	Limit of detection	Typically, 0.008 $\mu\text{g/l}$.
A1.7	Bias	See Table A1.
A1.8	Interferences	Any co-extracted material which has a GC retention time similar to acrylamide and which gives a detector response at the monitored masses will interfere. However, none are known at the m/z values selected.

A2 Principle

Acrylamide is converted to its dibromo derivative and then extracted from the aqueous sample using a solid phase extraction cartridge. The extract is determined using gas chromatography with mass spectrometric detection using a large volume injection technique.

A3 Hazards

Acrylamide is toxic and a known carcinogen and bromine is toxic. Caution should therefore be exercised when handling and preparing stock standards and saturated

bromine water. Skin contact, ingestion and inhalation of these compounds should be avoided by using appropriate protective equipment and working within a fume cupboard, when appropriate. Bromine and hydrobromic acid are corrosive. Ethyl acetate and *iso*-octane are flammable; methanol is toxic and flammable.

A4 Reagents

All reagents should be of analytical grade quality and distilled, deionised or similar grade water should be used throughout. It may be advantageous to weigh the standard solutions before and after use to ensure any solvent (i.e. water) loss is accounted for. Any loss in mass (in excess of pre-defined amounts established by the laboratory) should then be replaced with the appropriate amount of water.

A4.1 *iso*-Octane.

A4.2 Methanol.

A4.3 Ethyl acetate.

A4.4 Sodium thiosulphate solution (9 % m/w). Add 9.0 ± 0.5 g of sodium thiosulphate pentahydrate to approximately 90 ml of water and mix well. Make to 100 ml with water. This solution may be stored at room temperature out of direct sunlight for up to 1 year.

A4.5 Hydrobromic acid solution. Carefully, add 1.0 ± 0.2 ml of concentrated hydrobromic acid (48 %w/v) to 500 ± 10 ml of water contained in an amber glass bottle. Mix well. The pH of this solution should be approximately 2. This reagent should be prepared on the day of use.

A4.6 Saturated bromine water. Add approximately 2 ml of bromine to approximately 230 ml of water. Swirl the contents gently and allow the mixture to stand. After allowing the mixture to stand a while, un-dissolved bromine should be observed. If this is not the case, additional bromine should be added. This reagent should be prepared on the day of use.

A4.7 Stock standard acrylamide solution (1000 mg/l). Add 50.0 ± 0.1 mg of acrylamide to approximately 40 ml of water contained in an amber glass container. Mix well. Make to 50 ml with water. An AQC standard solution should similarly be prepared using a different batch of acrylamide. These solutions may be stored at 5 ± 3 °C for up to 26 weeks.

A4.8 Stock internal standard solution (1000 mg/l). For example, add 50.0 ± 0.1 mg of acrylamide- d_3 to approximately 40 ml of water contained in an amber glass container. Mix well. Make to 50 ml with water. This solution may be stored at 6 ± 2 °C for up to 26 weeks.

A4.9 Stock working standard solution (500 µg/l). Add 25 ± 1 µl of stock standard acrylamide solution (A4.7) to approximately 40 ml of water contained in an amber glass container. Mix well. Make to 50 ml with water. An AQC working standard solution should similarly be prepared using a different batch of acrylamide. These solutions may be stored at 5 ± 3 °C for up to 4 weeks.

A4.10 Working internal standard solution (500 µg/l). Add 25 ± 1 µl of stock internal standard solution (A4.8) to approximately 40 ml of water contained in an amber glass container. Mix well. Make to 50 ml with water. This solution may be stored at 5 ± 3 °C for up to 4 weeks.

A4.11 Potassium bromide.

A4.12 Working standard solutions. For example, the following volumes of stock working standard acrylamide solution (A4.9) should be added to separate 250.0 ml amounts of water. This equates to 0.0125, 0.025, 0.05 and 0.125 µg of acrylamide in each 250 ml of water. Each solution should be mixed well. These reagents should be prepared on the day of use.

Volume of stock working standard solution (A4.9) added to 250 ml of water (µl)	Concentration (µg/l)
25 ± 1	0.05
50 ± 1	0.10
100 ± 2	0.20
250 ± 10	0.50

A5 Apparatus

In addition to normal laboratory glassware the following may be required.

A5.1 SDB2 SPE cartridges. SDB2 (styrene divinyl benzene) SPE cartridges have been used in generating the performance data. Equivalent cartridges may also be used.

A5.2 C18 SPE cartridges. C18 (end capped octadecyl silane) SPE cartridges have been used in generating the performance data. Equivalent cartridges may also be used.

A5.3 Evaporator A Zymark evaporator has been used in generating the performance data. Equivalent systems may be used.

A5.4 GC-MS equipment. The suitability of the equipment will need to be evaluated.

The following conditions have been used in generating performance data.

Column:	HP5-MS, 15 m x 0.25 mm diameter, 0.25 µm film thickness. with 1 m HP5-MS 0.25 mm diameter, 0.25 µm film thickness guard column
Carrier gas:	Helium, constant flow at 12.25 psi.
Injection volume:	12 µl (3 x 4 µl).
Temperature programmes:	
Oven	Initial temperature at 40 °C for 1.5 minutes, then 35 °C per minute to 130 °C, hold time for 2 minutes, then 100 °C per minute to 290 °C, hold time for 2 minutes.
Injector	Initial temperature at 40 °C, then 720 °C per minute to 400 °C, hold time for 5 minutes, then cool at 100 °C per minute to 250 °C, hold time for 4 minutes.

Vent time: 1 minute.
 Vent flow: 25 ml per minute.
 Vent pressure: 4 psi.
 Purge flow: 50 ml per minute.
 Purge time: 3 minutes.
 Total flow: 55 ml per minute.

Using these conditions, the following apply

Compound	Approximate retention time (minutes)	Ions monitored	
		Target	Qualifier
acrylamide-d ₃	5.96	155	111
acrylamide	5.98	150	106

Equivalent equipment and conditions may be used. See Figure A1 for typical chromatogram.

A6 Sample collection and preparation

Samples should be collected in amber glass bottles. Sodium thiosulphate preservative should not be present. Samples should be transported and stored under refrigerated conditions. Samples should be analysed as soon as possible. The bottles should not be opened until the extraction process begins.

A7 Analytical procedure

Step	Procedure	Notes
A7.1	To V _s ml (typically, 250.0 ± 2.5 ml) of sample contained in a clear glass bottle (note a) add 250 ± 1 µl (note b) of working internal standard solution (A4.10). Mix well. Add 20.0 ± 0.5 g of potassium bromide and mix well. To this solution, add 0.5 ± 0.1 ml of concentrated hydrobromic acid and mix well. To this solution, add 4.00 ± 0.25 ml of saturated bromine water (A4.6). Mix well. Cap the bottle and place in a refrigerator for at least 3 hours but no more than 24 hours.	(a) This enables the removal of excess bromine to be easily observed. (b) This equates to 0.125 µg of internal standard.
A7.2	Remove the bottle from the refrigerator and allow the treated sample to equilibrate to room temperature. To the treated sample, add 500 µl of sodium thiosulphate solution (A4.4). Mix well. See note c.	(c) The addition of sodium thiosulphate should remove all traces of excess bromine. If the solution still remains yellow in colour, add a further 500 µl of sodium thiosulphate solution (A4.4) and mix well.

SDB2 SPE conditioning

- A7.3 Attach an SDB2 SPE cartridge (A5.1) to a vacuum manifold and set the vacuum to 5 psi. Add 6 ml of ethyl acetate (A4.3) to the cartridge and elute the cartridge, see note d. Discard the eluate. (d) Do not allow the cartridge to dry out at this stage, i.e. do not allow the meniscus of the solvent to go below the level of the cartridge packing material.
- A7.4 Add 6 ml of methanol (A4.2) to the cartridge and elute the cartridge, discarding the eluate. See note d.
- A7.5 Add 6 ml of hydrobromic acid solution (A4.5) to the cartridge, but do not allow the solution to elute through the cartridge.

C18 SPE conditioning

- A7.6 Attach a C18 SPE cartridge (A5.2) to a vacuum manifold and set the vacuum to 5 psi. Add 6 ml of methanol (A4.2) to the cartridge and elute the solvent. See note c. Discard the eluate.
- A7.7 Add 6 ml of hydrobromic acid solution (A4.5) to the cartridge and elute the solvent (at a vacuum set at 15 psi). See note d. Add a further 6 ml quantity of hydrobromic acid solution (A4.5) to the cartridge, but do not allow the solution to elute through the cartridge.

Sample extraction

- A7.8 Fix the conditioned C18 cartridge (A5.2) above a conditioned SDB2 cartridge (A5.1). Elute the treated sample from section A7.2 (at a vacuum set at 5 psi) through both cartridges. See note e. Discard the eluate. When all the treated sample has been eluted, discard the C18 cartridge (A5.2). Add 6 ml of hydrobromic acid solution (A4.5) to the SDB2 cartridge (A5.1) and elute the acid solution through the cartridge. Discard the acid solution. Dry the cartridge by continuing the vacuum process (note f). (e) This usually takes about 60 minutes. (f) The drying process can be terminated when the colour of the cartridge changes from brown to orange.

Sample elution

- A7.9 When dry, add 1.0 ± 0.2 ml of ethyl acetate (A4.3) to the SDB2 cartridge (A5.1). Leave the cartridge to stand for at least 2 minutes,

then elute the solvent through the cartridge and collect the eluate, note d. Repeat the elution process with a further 1.0 ± 0.2 ml of ethyl acetate (A4.3) combining the eluate fractions.

- A7.10 Evaporate (A5.3) the eluate fraction at $40\text{ }^{\circ}\text{C}$ to $50 \pm 10\text{ }\mu\text{l}$. To this volume, add $150 \pm 1\text{ }\mu\text{l}$ of *iso*-octane (A4.1). Mix well. Quantitatively, transfer this solution to a GC vial. The solution is now ready for GC-MS determination. See note g.
- (g) At this stage, the solution may be stored in a refrigerator for up to 1 week before the GC-MS determination begins.

Calibration

- A7.11 Using the working standard solutions (A4.12) see note h, in place of the volume of sample, and the procedures described in sections A7.1 - A7.10, calibrate the GC system, monitoring the ion fragments given in section A5.4.
- (h) This equates to 0.0125, 0.025, 0.05 and $0.125\text{ }\mu\text{g}$ of acrylamide and $0.125\text{ }\mu\text{g}$ of internal standard.
- A7.12 Set up the GC-MS system according to manufacturer's instructions and construct a calibration graph of response versus amount of acrylamide in the vial.
- A7.13 Analyse blank, sample and AQC extract solutions using the entire procedure described above.
- A7.14 From the calibration graph, obtain the amount, A_v , of acrylamide in the vial and then calculate the concentration, C_s , of acrylamide in the sample. See note i.
- (i) If the response exceeds the calibration range, the analysis may be repeated using a smaller amount of sample (A7.1) and making the volume to 250 ml with water.

A8 Calculation

From the calibration graph determine the amount, A_v , of acrylamide in the vial and determine the concentration, C_s , in the sample using the equation:

$$C_s = (A_v \times 1000) / V_s \quad \mu\text{g/l}$$

Where

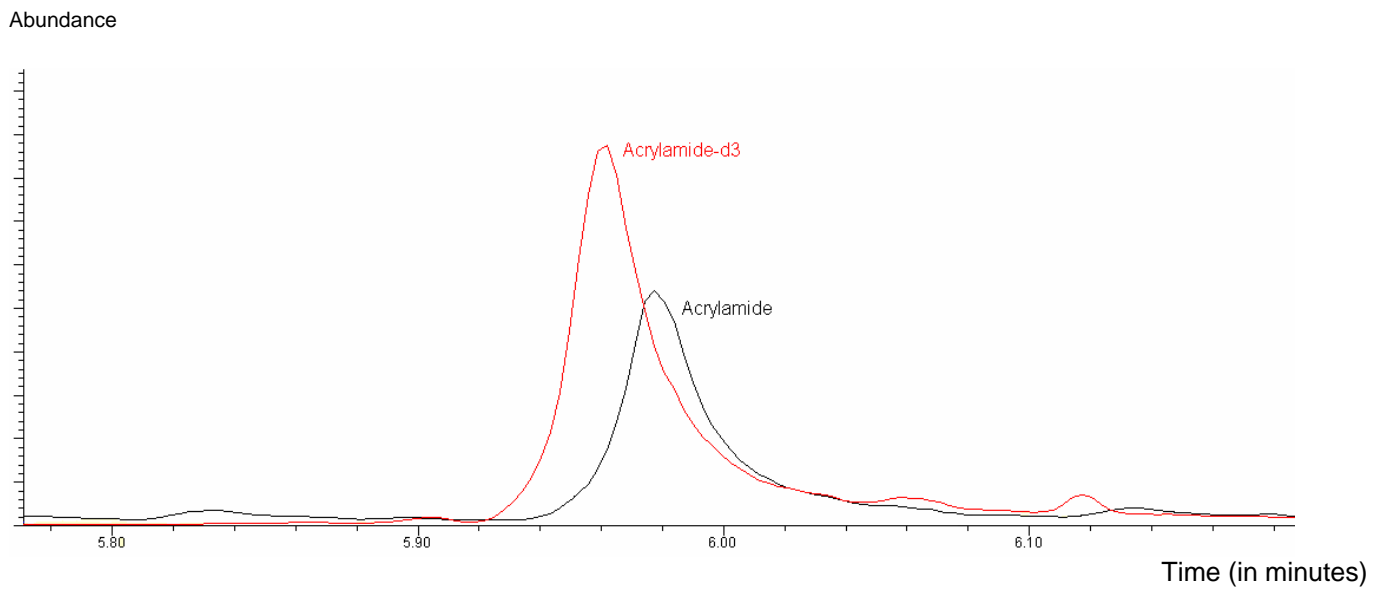
C_s is the concentration ($\mu\text{g/l}$) of acrylamide in the sample;
 A_v is the amount (μg) of acrylamide obtained from the graph;
 V_s is the volume (ml) of sample analysed (A7.1);

Table A1 Summary performance data for acrylamide

Concentration (µg/l)	Total standard deviation (µg/l)	Bias (%)	Recovery (%)	Degrees of freedom
0.03	0.002	0		14
0.1	0.005	-11		13
0.4	0.019	2		12
Sample spiked at 0.1 µg/l	0.006		97	15

Performance data provided by Three Valleys Water.

Figure A1 Typical chromatogram of standard solution (concentration of 0.1 µg/l)



B A note on the determination of acrylamide using liquid chromatography with mass spectrometric detection

The following conditions have been used to determine acrylamide concentrations using liquid chromatography with mass spectrometric detection with direct injection of the sample containing an internal standard.

Analytical conditions:-

Column:	Luna C18(2) 2.0 x 250xmm x 5 µm.
Temperature:	60 °C.
Isocratic elution:	Pump A - 0.1 % formic acid:water mixture (97:3 v/v). Pump B - methanol:water mixture (3:97 v/v).
Flow rate:	0.5 ml per minute.
Injection volume:	100 µl (direct aqueous injection)
Run time:	5 minutes.

Mass spectrometric conditions

APCI Positive ion mode:	SIM monitoring at 72 m/z
Drying gas temperature:	300 °C.
Drying gas flow rate:	8.0 litres per minute.
Nebulizer pressure:	40 psig
Vaporizer temperature:	425 °C.
Capillary voltage:	3000 volts.
Corona current	4.0 µA.
Fragmentor voltage:	100 volts.

Typical limit of detection calculated as approximately 0.1 µg/l.

Information on the routine use of this method is welcomed to assess its full capability, as the statutory maximum limit for acrylamide in drinking water is 0.1 µg/l.

Address for correspondence

However well procedures may be tested, there is always the possibility of discovering hitherto unknown problems. Analysts with such information are requested to contact the Secretary of the Standing Committee of Analysts at the address given below. In addition, if users would like to receive advanced notice of forthcoming publications please contact the Secretary on the Agency's web-page.

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Standing Committee of Analysts Members assisting with this booklet

Without the good will and support given by these individuals and their respective organisations SCA would not be able to continue and produce the highly valued and respected blue book methods.

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