Determination of Acrylamide in Food Simulants

WARNING: Acrylamide monomer is toxic and readily absorbed through the skin. The monomer should be handled in a fume cupboard using gloves.

1 SCOPE

This method describes an analytical procedure for the determination of acrylamide in the food simulants water, 3% w/v acetic acid, 15% v/v ethanol and fat simulant. The level of acrylamide monomer determined is expressed as mg acrylamide/kg of food simulant. The method is appropriate for the quantitative determination of acrylamide in approximate analyte concentration range of 0.01-0.1 mg/kg of food simulants.

The method should also be applicable to other fat simulants.

NOTE: The suitability of the fat simulant should be assessed prior to setting up migration tests - it may be found necessary to use sunflower oil or HB 307 if unacceptable interferences are found with olive oil.

2 PRINCIPLE

The level of acrylamide in food simulants is determined by direct injection of aqueous food simulants for analysis by high performance liquid chromatography with an ion exclusion column and UV detection. Fat simulants are extracted with water and the aqueous extracts than analysed by HPLC. Quantification is achieved using external standards.

Confirmation of the identity of acrylamide is established by means of reversed phase HPLC using a column of different polarity but the same detection as used in the quantitative determination.

3 REAGENTS

All reagents and solvents shall be of analytical quality, unless otherwise stated.

3.1 Analyte

3.1.1 Acrylamide, CH_2:CH $CONH_2$, MW 71.08, purity greater than 99%

3.2 Chemicals

3.2.1 Acetonitrile HPLC grade, suitable for low UV wavelength applications

3.2.2 Methanol HPLC grade

3.2.3 Water HPLC grade

3.2.4 Sulphuric acid 0.05 mol/l in water

3.3 Solutions

3.3.1 Stock solution of acrylamide in methanol (500 µg/ml)

Weigh to the nearest 0.1 mg approximately 0.05 g of acrylamide into a 100 ml volumetric flask. Dissolve the acrylamide in methanol and fill up to the mark with methanol. Close and mix thoroughly.

Calculate the actual concentration in µg acrylamide/ml solution.

Repeat the procedure to provide a second stock solution.

NOTE: The stock solutions may be stored at 5°C for up to 3 months protected from light in septum capped glass vials with minimum headspace.

3.3.2 Diluted stock solution (10 µg/ml)

Using a graduated pipette, transfer 1.0 ml of the acrylamide stock solution (3.3.1) to a 50 ml volumetric flask and fill to the mark with methanol. This solution contains nominally 10 µg/ml acrylamide.

Repeat the procedure using the second stock solution.

3.3.3 Intermediate standards

Using graduated pipettes transfer 0, 0.5, 1.0, 2.0, 3.0 and 4.0 ml of the 10 µg/ml diluted stock solution (3.3.2) to a series of 10 ml volumetric flasks. Dilute to the mark with methanol and mix. These standards correspond nominally to 0, 0.5, 1.0, 2.0, 3.0 and 4.0 µg/ml acrylamide.

3.3.4 HPLC mobile phase

Using a measuring cylinder transfer 70 ml of 0.05 mol/l sulphuric acid (3.2.4) to a 1 litre volumetric flask and dilute to about 500 ml with water (3.2 3). Add, using a measuring cylinder 70 ml of acetonitrile (3.2.1) and dilute to the mark with water (3.2.3). This solution may require de-gassing prior to use.

4 APPARATUS

NOTE: An instrument or item of apparatus is listed only where it is special, or made to a particular specification, usual laboratory glassware and equipment being assumed to be available.

4.1 High performance liquid chromatograph equipped with a ultraviolet detector (UV) and fitted with an injection valve with a 25 µl or 50 µl injection loop.

NOTES: The HPLC pump should be able to deliver a (almost) pulse free flow. The detector should be capable to give a stable baseline response after the column has been stabilised for a few hours with the mobile phase.

Appropriate operating conditions must be established for the specific equipment used for the determination.

The detector should preferably be capable of achieving a detection limit of 0.01 mg/kg acrylamide in food simulants, see 6.1.

4.2 Ion exclusion column, capable of the separation of acrylamide from substances present in the food simulants. The column packing is based upon a styrene divinylbenzene polymer with sulphonated (cationic ion-exchange) groupings.
The following chromatographic conditions have been found to be suitable:

Column	Dionex Ion Pac ICE-ASI 250 x 7.6 mm
Eluent	mobile phase as prepared in 3.3.4
Flow rate	1.5 ml/min
Detector	UV 202 nm
Injection	loop 25 µl
Temperature	stabilised at room temperature

4.3 Glass sample vials 120 ml with PTFE coated crimp top closures.

4.4 Graduated pipettes 1 ml, 2 ml, 5 ml.

4.5 HPLC disposable membrane filters 0.2 µm.

5 SAMPLES

Samples are to be kept refrigerated at 4°C in closed containers with the exclusion of light. Acrylamide-free simulants of the same type as those to be analysed are also required for calibration purposes.

NOTE: Depending on the available equipment it may not be possible to achieve the required detection limit of 0.01 mg/kg food simulant. In that case migration experiments should be performed using a different ratio of contact area / food simulant than 6 dm_/ kg. The high solubility of acrylamide in aqueous simulants justifies migration experiment to be carried out at ratios of 6 dm_/200 ml food simulant.

5.1 Test sample preparation

5.1.1 Aqueous food simulants

The aqueous food simulants require no pre-treatment except filtration, using an HPLC membrane filter, if cloudy.

5.1.2 Fat simulant

Weigh 50 ± 0.5 g of fat simulant obtained from the migration test into a 120 ml vial. Add 1.0 ml of the 0 µg/ml intermediate standard using a graduated pipette and mix well. Add 25 ± 0.5 ml of water and shake vigorously for 1 minute. Upturn the vial and allow the phases to separate for about 20 minutes. Withdraw about 4 ml of the lower aqueous phase using a syringe and filter using an HPLC membrane filter.

5.2 Blank sample preparation

Treat food simulants, free of acrylamide and which have not been in contact with packaging material in the same way as described in 5.1.1 and 5.1.2.

5.3 Calibration sample preparation

NOTE: Calibration solutions should be adapted to the ratio of contact area/food simulant in case a deviating ratio was used in the migration experiments.

5.3.1 Aqueous simulants

Into a series of 50 ml volumetric flasks add, by pipette, 1 ml of each of the intermediate standards (3.3.3) and dilute to the mark with the appropriate acrylamide-free aqueous food simulant. Mix thoroughly. These standards correspond nominally to approximately 0.0, 0.01, 0.02, 0.04, 0.06 and 0.08 µg acrylamide/ml of food simulant.

Calculate the exact concentration in µg acrylamide /ml food simulant.

Repeat the procedure for the second set of standard solutions.

5.3.2 Fat simulant

Weigh 50 ± 0.5g of acrylamide-free fat simulant into a series of 120 ml vials. Add 1.0 ml of each intermediate standard using a graduated pipette and mix well. Add 25 ± 0.5 ml of water and shake vigorously for 1 minute. Upturn the vial and allow the phases to separate for about 20 minutes. Withdraw about 4 ml of the lower aqueous phase using a syringe and filter using an HPLC membrane filter. The standards correspond nominally to approximately 0.0, 0.01, 0.02, 0.04, 0.06 and 0.08 µg acrylamide/g of fat simulant.

Calculate the exact concentration in µg acrylamide /g of fat simulant

Repeat the procedure for the second set of standard solutions.

6 PROCEDURE

6.1 HPLC analysis

NOTES: When starting measurements, baseline stability and response linearity of the detector should be examined, together with verification of the detection limit.

The same operating conditions of the HPLC system must be maintained throughout the measurements of all samples prepared in 5.1 to 5.3.

Each sample should be determined at least in duplicate, i.e. as a pair of measurements.

Under the conditions given in 4.2 the retention time of acrylamide was found to be approximately 14 minutes.

NOTES: The detection limit for the method should be verified as 0.01 mg/kg, or better, by injecting the 0.01 µg/ml and 0.01 µg/g standards prepared in 5.3 for analysis six times. Calculate the standard deviation Sd of the peak areas, and the concentration equivalent to 3 x Sd = detection limit. If this value is greater than 0.01 mg/kg change the chromatographic parameters to improve the peak sharpness and repeat the procedure.

If the migration experiments were carried out at a more favourable ratio than 6 dm_/kg simulant, then the detection limit may be increased with the same factor the ratio of contact area to simulant was increased. This means that if the migration experiment was carried out by bringing into contact 6 dm_ of plastic with 200 g of food simulant then the detection limit should be less than 0.05 mg/kg.

6.2 Sample treatment

The test samples, blanks, as well as calibration samples prepared in 5.1 and 5.2, are analysed as they are without further treatment using conditions described in 4.2.
Inject the aqueous food simulant samples , the aqueous extracts of the fat simulant as well as the simulant blanks onto the HPLC column.

Identify the acrylamide peak on the basis of the retention time and measure the respective peak area.

6.3 Calibration

Calibration samples (5.3) are measured according to 6.2. Construct or calculate the calibration curve, plotting peak area values against the concentration of acrylamide (dimension: mg) in the food simulant (dimension: kg).

NOTE: The calibration curves should be rectilinear and the correlation coefficient should be 0.996 or better.

The two sets of calibrant solutions made from independently prepared stock solutions should be cross-checked by generating two calibration curves which on the basis of peak area measurement should agree to ± 5% of one another.

6.4 Evaluation of data

NOTE: The following calculation assumes that, for all measurements, exactly the same weight or volume of food simulant has been used.

6.4.1 HPLC interferences

Following the method described before, for some batches of olive oil interferences have been observed in analysis of the olive oil extract. A suitable simulant should be sought which is found to be free from peaks interfering with the acrylamide peak or gives rise to insignificant interference equivalent to < 0.005 mg acrylamide/kg of food simulant.

6.4.2 Calculation of acrylamide concentration in the test samples

Graphical determination:

Calculate the average of peak area values obtained from the test samples according to 6.2 and read the acrylamide concentration of the test sample from the calibration graph (6.3).

Calculation from the regression parameters:

If the regression line equation is

y [peak area] = a * x [mg/kg] + b,

then the acrylamide concentration in the food simulant $C_{acrylamide, fs}$ is

$C_{acrylamide, fs} = (y - b)/a$

Both procedures yield directly the acrylamide concentration in the food simulant (in mg/kg).

NOTE: ***The method applying calculation from the regression parameters should be the preferred one. If relevant, correct values calculated for test samples with values calculated for blank simulants.***

6.4.3 Calculation of the specific acrylamide migration

Depending on the fill volume of the test material and on the surface area/food simulant ratio, the acrylamide concentration in the test sample as determined according to 6.4.2 may need mathematical transformation to calculate the specific migration value to be compared to the restriction criterion (SML-value).

7 CONFIRMATION

7.1 Requirement for confirmation

In cases where the specific migration of acrylamide into the food simulant (as determined according to 6.4.3) exceeds the restriction criterion set in Commission Directive 90/128/EEC, Annex II, list A which states SML = ND, DL = 0.01 mg/kg, the determination should be confirmed by the method described in 7.2.

NOTE: ***The confirmation is qualitative in the sense that it should demonstrate the correct identity of the measured analyte and the absence of interferences. For the purposes of quantification the result as calculated according to Section 6.3 shall be taken as the true value.***

The calibration procedure was found to be rectilinear while the detection limits calculated were only about two times higher than in the analytical method. Therefore the confirmation procedure is also suitable for the quantitative determination of acrylamide in the food simulants.

7.2 Confirmation by analysis using a column of different selectivity.

Food simulants, blanks and calibration samples are analysed by HPLC using a hexylsiloxane column and UV detection at 202 nm.

NOTE: ***The quantitative result for the analytical method and the confirmation method should agree to within ± 0.005 mg/l.***

7.2.1 Reagents (Analytical grade)

NOTE: Only additional chemicals required for the confirmation procedure are mentioned.

7.2.1.1 Sodium dihydrogen orthophosphate

7.2.1.2 Disodium hydrogen orthophosphate

7.2.2 Preparation of mobile phase

Dissolve 8.7g of disodium hydrogen orthophosphate and 6.1g of sodium dihydrogen orthophosphate in water and dilute with water to 1 litre. Take 10 ml of this solution and dilute to 500 ml with water.

7.2.3 HPLC conditions

column	250 x 4.6 mm, packed with hexyl bonded spherical silica, 5 µm particles
mobile phase	aqueous phosphate buffer see 7.2.2
flow rate	1.1 ml/min
detector	UV 202 nm
injection	loop 10 µl

NOTE: retention time observed for acrylamide: 4.2 min

7.2.4 Procedure for 15% ethanol simulant

Dilute 10 ml of each calibration standard and the samples in 15% ethanol simulant, prepared in 5.1.1, 5.2 and 5.3.1, to 50 ml with water. Inject 10 µl for HPLC analysis. Follow the instructions given in 6.2 to 6.4 to determine the specific acrylamide migration.

7.2.5 Procedure for water, 3% acetic acid and fat simulant

Re-inject the calibration standards and samples, prepared in 5.1.1/5.1.2, 5.2 and 5.3.1/5.3.2, for HPLC analysis. Follow the instructions given in 6.2 to 6.4 to determine the specific acrylamide migration.

NOTE: In some cases problems were encountered with the 3% acetic acid simulant. The injection of acetic acid may influence the pH of the mobile phase. Addition of a few drops of sodium hydroxide solution to neutralise the major part of the acetic acid may be useful to obtain a better separation between acrylamide and acetic acid.

8 PRECISION

8.1 Validation

This method was pre-validated by a within-laboratory precision experiment using the four official EC food simulants for establishment of precision data at the restriction criterion as well as by carrying out within-laboratory migration tests with an

acrylamide containing coated polymer film sample being in contact with water, 3% acetic acid, 15% ethanol and sunflower oil, respectively.

8.2 Repeatability

Evaluation (ISO 5725) of the within-laboratory precision experiment results at a concentration of 0.01 mg/kg at the 95% probability level yielded the following performance characteristics:

Repeatability	r = 0.0023 mg/kg in water
	r = 0.0022 mg/kg in 3% acetic acid
	r = 0.0019 mg/kg in 15% ethanol
	r = 0.0015 mg/kg in sunflower oil

8.3 Detection limit

The within-laboratory detection limits (DL), based on the calibration curve method according to DIN 32645, were found to be in the range 0.0014 - 0.0042 mg acrylamide /kg food simulant (depending on the type of the food simulant). Thus the method is capable of quantitative detection at a minimum value of 0.01 mg acrylamide/kg.

The detection limits found in the confirmation procedure were in the range of 0.002 - 0.010 mg acrylamide/kg food simulant.