

72615 Acrylamide – Kit

On 24 April 2002 the Swedish National Food Administration published Data and comments on disturbingly high concentrations of acrylamide in some foods (1).

Acrylamide causes tumors in laboratory animal. The discovery was considered particularly alarming as the acrylamide-containing foods include products regularly consumed in rather large quantities, such as potato chips, French fries, roast potatoes, breakfast cereals, and crisp bread.

There are primarily two approaches for the analysis of acrylamide in food, based either on GC-MS or on HPLC-MS-MS

The method for which this kit has been put together is the GC-MS one, developed by K.Grob and co-workers at the Official Food Control Authority of the Canton of Zurich, Switzerland¹.

Kit components:

69188 Acrylamide standard solution
(500mg/l in acetonitrile)

72834 Acrylamide-d₃ standard solution
(500mg/l in acetonitrile)

51677 Methacrylamide standard solution
(500mg/l in acetonitrile)

66467 Butyramide standard solution
(25mg/l in acetonitrile)

82090 1-Propanole

00689 Acetonitrile

52765 n-Hexane

68464 Oil

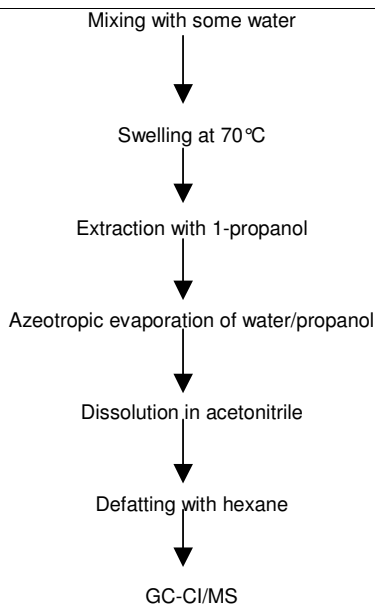
The chemicals can be used for 12 samples.

All standard solutions come in Certan® flasks. To extract the solutions needles 0.8x80mm (or smaller diameter) are recommended.

The steps of the method are listed in figure 1

A minimum of water is added for the swelling of solid parts. Extraction is performed with 1-propanol and the propanol/water evaporated in order to avoid the difficult extraction of acrylamide from an aqueous phase.

Acetonitrile enables a simple clean up by restricted solubility of the salts and the defatting with hexane.



Procedure:

To 25g of a sample 75ml of deionised water are added (more water for exceptionally dry samples) into a 150ml beaker glass. With a syringe (needle: gauge 22 [0.72x0.41mm], length 70mm or smaller diameter) 100µl of the methacrylamide standard solutions (51677) and the acrylamide-d₃ standard solution are added to the sample mixture (i.e. 500 ppb of the two internal standards, 1µl per 1g of total sample weight). Depending on the applied GC method, we recommend not adding the acrylamide-d₃ solutions (pl. refer to the section GC-MS analysis).

After mixing the homogenate is allowed to swell during 30 min at 70°C in a water bath. The beaker glass is covered by aluminium foil to prevent evaporation of water.

10g of the homogenate is weighed into a 50ml centrifuge glass with a screw cap and thoroughly mixed with 40ml of 1-propanol (82090). To improve the extraction of the acrylamide one should at least leave the mixture for ½ h. The mixture is centrifuged for 3min at 3600 rpm. Then 10ml of the clear supernatant is transferred to a 50ml pointed flask and fifteen droplets of the oil (68464) are added. The addition of oil shall retain the acrylamide on the wall of the flask while evaporating the 1-propanol/water mixture. The evaporation is done in a rotary evaporator starting at about 100 Torr and going down to 50 Torr as soon as the bumping of the solution slows down and 60-70° C water bath. Evaporation is stopped as soon as no liquid is left.

The residue from the evaporation, consisting of fat/added oil and often much salt, is extracted with acetonitrile (00689) and defatted with n-hexane (52765). 3ml acetonitrile and 20 ml n-hexane are added and mixed with the help of an ultrasonic bath with the sample (2 min). The acetonitrile (lower phase) is transferred into a 10ml reagent glass with screw cap by means of a micropipette. The acetonitrile phase is again extracted by another 5ml n-hexane (ultrasonic bath, 2 min), now transferring 1.5ml of the acetonitrile phase (assumed to be half) into a 2ml auto sampler vial. 100µl butyramide standard solution (66467) is added to the vial.

GC-MS analysis:

Five calibrating solutions are prepared, containing an increasing amount of acrylamide.

	+ ppb Acryl.	+ ppb d3- Acryl.	+ ppb Methacryl.	+ ppb Butyramide
Cal.sol. I	50	50	50	50
Cal.sol. II	100	100	100	100
Cal.sol. III	200	200	200	200
Cal.sol. IV	500	500	500	500
Cal.sol. V	1000	1000	1000	1000

Reference:

¹M. Biedermann, S. Biedermann-Brem, A. Noti and K. Grob; P. Egli and H. Mändli, Mitt. Lebensm. Hyg. 2002, 93, 638-652

Remarks on GC/MS analysis

GC-MS involved a Trace-GC 2000 gas chromatograph with on-column injector and a PolarisQ ion trap mass spectrometer (ThermoFinnigan). 1 µl of the sample was injected on-column into a 30 m x 0.25 mm i.d. polyethylene-glycol coated separation column (e.g. Supelcowax 10). The latter was equipped with a 1 m x 0.53 mm i.d. pre-column (fused silica, uncoated). The carrier gas (helium) flow was kept constant at 2 ml/min throughout the analysis and the oven temperature was programmed at 15 °/min from 70 °C (1 min) to 220 °C (2 min).

Mass spectrometry involved positive ion chemical ionisation (CI) with methane as reagent gas. The ion source was at 150 °C. To increase sensitivity only the mass range from m/z 72 to 88 was scanned. Selected ion monitoring (SIM) usually used with quadrupole instruments to increase sensitivity is not applicable for ion trap instruments; we therefore also recommend to omit the d3-Acrylamide IST with ion trap instruments.

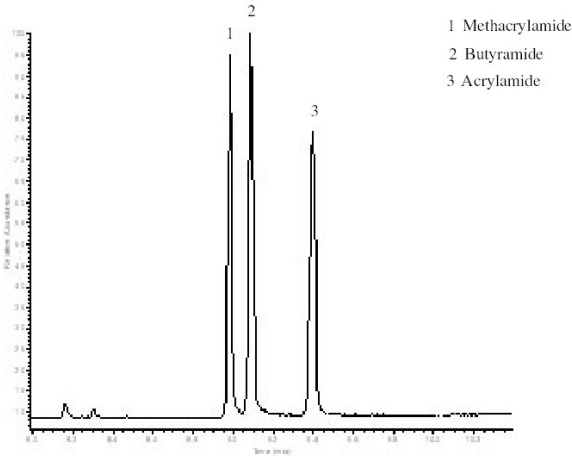


Figure 1: Chromatogram of Methacrylamide, Butyramide and Acrylamide
Column : Carbowax CW 20 M 30mx0.25mm ID, 0.25µm
Oven: 70 °C (1min) to 220 °C at 15 °C/min, hold 2 min
Injection volume: 1 µl
Carrier : helium, 20 cm/sec
Inj: on column
Carrier Gas: Helium, 2ml/min

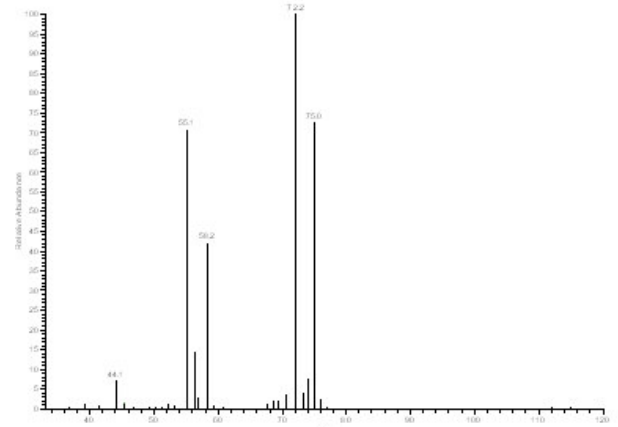


Figure 2: MS Spectra of d₃-Acrylamide
CI-Gas: Methane, 2.0 ml/min
Source-Temperature: 150 °C
full scan (33-120 m/z)

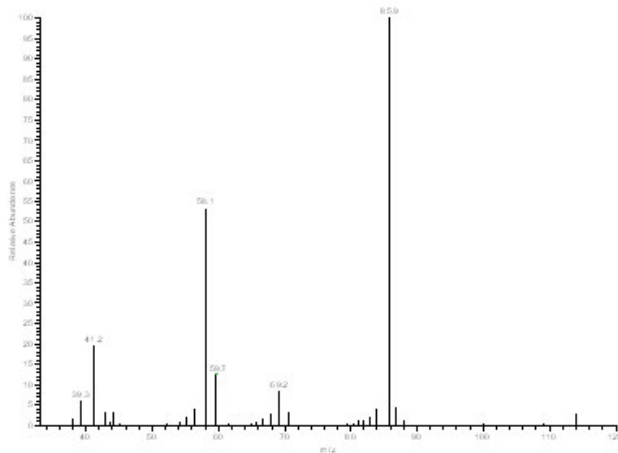


Figure 3: MS Spectra of Methacrylamide
CI-Gas: Methane, 2.0 ml/min
Source-Temperature: 150 °C
full scan (33-120 m/z)

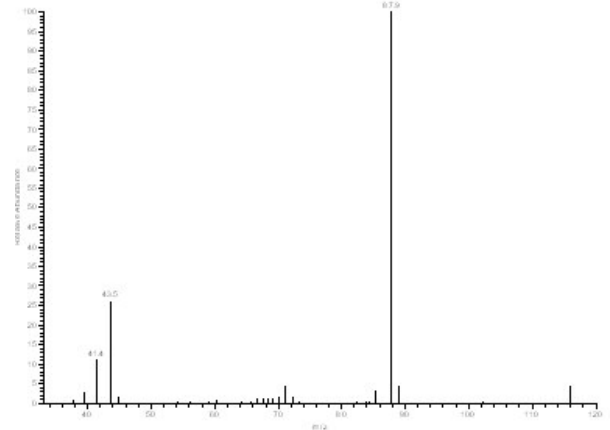


Figure 4: MS Spectra of Butyramide
CI-Gas: Methane, 2.0 ml/min
Source-Temperature: 150 °C
full scan (33-120 m/z)