Acrylamide in heated potatoe products
Analytics and formation routes

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Analytical properties of acrylamide (1)

- High polarity
- Very good soluble in water, alcohols, acetone and acetonitrile
- Slightly soluble in ethylacetate, CH$_2$Cl$_2$, dietyether
- Insoluble in Hexane and other alkanes
- Low, but significant volatility
Analytical properties of acrylamide (2)

- Very low retention on reversed phases like RP-18
- No significant UV-absorption above 220 nm
- Reactive C=C – double bond
- Fast addition of Br₂ or R–SH
- Amide-group is protonated by medium and strong acids
Acrylamide – detection methods

- LC-MS-MS (ESI) without derivatisation
- GC-MS after bromination
- GC-MS without derivatisation
  Ionisation by electron impact or chemical ionisation (CH₄, C₄H₁₀ or NH₃)
- LC-MS (ESI) after derivatisation with mercaptobenzoic acid
GC-MS-method with bromination (1)

\[
\text{HCN} + \text{Br}_2 \xrightarrow{\text{H}^+} \text{Br-HCN}
\]
GC-MS-method with bromination (2)

- Extraction with water (40°C –80°C, Ultra Turrax, Ultrasonic bath, mechanical stirring)
- Treatment with diastase (if necessary)
- Alternatively: Extraction with organic solvents and reextraction with water
- Clearing procedure with Carrez solutions I and II
GC-MS-method with bromination (3)

- Bromination with $\text{Br}_2$ or with $\text{KBr} + \text{KBrO}_3$
- Removing of excess bromine with sulfite
- Extraction of dibrompropionylamide with ethylacetate
- Evaporation of solvent = concentration step
GC-MS-method with bromination (4)
GC-MS-method with bromination (5)

- GC-MS equipment is available in much more laboratories than LC-MS-MS equipment (and it is much cheaper)
- Higher sensitivity and lower detection limits because of the concentration step
- Characteristic mass-spectrum with dibromine-pattern (m/z 133/135 and m/z 105/107)
- High separation power of capillary-GC
GC-MS-method with bromination (6)

- Less problems with interferences in complex matrices like coffee or cocoa
- Complex and time consuming clean-up procedure
- HBr-elimination can take place on active surfaces of injector and capillary
- During the bromination step an exchange $^2\text{D} \leftrightarrow ^1\text{H}$ is possible, if $D_3$-acrylamide is used as internal standard
Clean-up procedure for LC-MS-MS (1)

- **Recommendable for fatty matrices (e.g. potato chips):**
  
  *Partial defatting procedure by soaking the dry sample with hexane /butylmethylether*

- Extraction with water (e.g. ultrasonic bath at 40°C)

- Addition of internal standard ($^2$D$_3$-acrylamide)

- Clearing procedure with Carrez solutions I and II

- Filtration oder centrifugation
Clean-up procedure for LC-MS-MS (2)

- Removing of interfering compounds by passing a solid phase extraction (SPE) cartridge
- „Easy“ matrices (potato chips, french fries, crisp bread, butter cookies): RP-18-phase
- „Difficult matrices“ (coffee powder, cacao): multi functional phases (RP-18 + anion exchanger phase + cation exchanger phase)
Clean-up procedure for LC-MS-MS (3)

- Many interfering substances are retended on the cartridge, but not acrylamide → no concentration effect for acrylamide
- Enrichment of acrylamid is possible by retention on special phases (Oasis HLB, active charcoal, cation exchanger at pH < 2,5) but this methods are not robust.
- Enrichment of acrylamide also is possible by salting out into organic solvents followed by reextraction into water.
Recommended parameters of LC-MS-MS

- Column: Hypercarb (graphite) or suitable RP-18-phases
- Solvent water/acetonitrile/formic acid (99:1:0,05)
- Ionisation: Elektrospray positive (ESI)
- Quantifier-ion: m/z = 72 → 55 (ISTD: 75 → 58)
- Qualifier-ions: m/z = 72 → 54; 72 → 44
- Qualifier ions: very low intensity!
LC-MS-MS chromatograms

acrylamide-standard
44 ng/ml

Potato chips
(796 ng/kg)
Aspects of LC-MS-MS

- Easy to handle, high throughput is possible
- No concentration step, but dilution of the sample
- No characteristic mass spectrum, low intensity of qualifier ions, verification problem at low levels
- In „difficult matrices“ like coffee powder and cacao, extraction problems and interferences are frequent
- Matrix induced quenching effects are frequent, the use of an isotope-labelled internal standard is obligatory
GC-MS without derivatisation

- For GC-MS without derivatisation a very effective clean-up procedure is required.

- **Problem**: Formation of acrylamide in the injector, if traces of asparagine and sugars are present in the extract.

- Formation of acrylamide often very high in PTV-injectors (programmable temperature vaporizser)

- Check clean-up procedure and injection with a solution of asparagine and fructose
LC-MS / derivatisation with mercaptobenzoic acid

\[
\text{COOH} \quad \text{SH} \quad + \quad \text{O} \quad \text{NH}_2 \quad \rightarrow \quad \text{COOH} \quad \text{S} \quad \text{NH}_2
\]
LC-MS / derivatisation with mercaptobenzoic acid

- Higher retention on RP-phases, because of the lower polarity of the derivate leads to better separation from interfering substances.
- Less interferences, higher mass range and higher sensitivity in MS
- No LC-MS-MS is required, a benchtop LC-MS-system is satisfactory
- $^{13}$C-labelled internal stand is required
Limits of detection (simple matrices)

- GC-MS (with bromination): 2 – 20 µg/kg
- LC-MS-MS: 10 – 50 µg/kg
- GC-MS without derivatisation: 10 – 100 µg/kg
- LC-MS with derivatisation: 25 µg/kg
- (high price) LC-MS-MS with optimum sensitivity in the low mass-range: < 1 µg/kg
Presuppositions for acrylamide formation

- Free asparagine
- Free reducing sugar (glucose, fructose)
- Low water activity
- Product temperature > 100°C
Asparagine delivers the backbone of acrylamide
Acrylamide formation in instant potato powder (1)
Acrylamide formation in instant potato powder (2)

Heating at constant temperature (160°C)

ng/g acrylamide vs. minutes
Acrylamide formation – reducing sugars

Model experiments with different glucose/asparagine ratios
Acrylamide formation – pH value

Model experiments with glucose+asparagine at different pH

![Graph showing the relationship between pH of reaction system and micrograms of acrylamide. The graph indicates an increase in acrylamide formation as pH increases, peaking around pH 7.0.](image-url)
Mechanism of acrylamide formation

Reducing sugar + Asparagine

\[ \text{Schiff's base} \]

\[ \text{Acrylamide} \]
Alternative (less important) formation routes

Lactic acid \( \xrightarrow{\Delta T} \) Methylglyoxal \( \xleftarrow{\Delta T} \) Glucose / Fructose

Acrylic acid

Oxidation

Acrolein

\( \xrightarrow{\Delta T} \) Triacylglycerol

\( \xrightarrow{\Delta T} \) Monoacylglycerol

Acrylamide

Oxid. / RNH\(_2\)

proteine bound

Alanine
... Thank you very much for your friendly attention