

Dr. Thierry Mann, Waters, Beverly, MA,  
Dr. Marian Twohig, Dr. Nicholas Ellor, Waters, Beverly MA,  
Jim Krol, Waters, Milford Mass

### The Concerning News About French Fries

The acrylamide cancer concern was identified and reported by:

Tareke, et.al. at the Dept of Environmental Chemistry Stockholm University,

"Analysis of Acrylamide, a Carcinogen Formed in Heated Foodstuffs" J. Agric. Food Chem., 50, p4998-5006, 2002,  
and

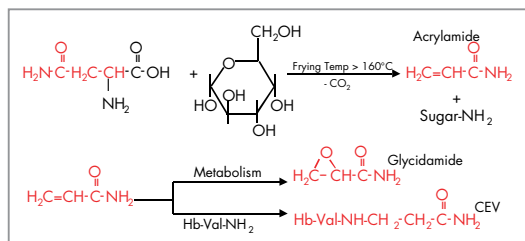
"Acrylamide: A Cooking Carcinogen?"

Chem. Res. Toxicol., 13, p517-522, 2000.

### Formation of Acrylamide

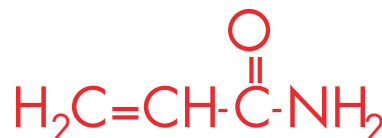
Acrylamide is formed by the reaction of 2 naturally occurring GRAS food ingredients fried at high temperatures

### Asparagine + Dextrose (Starch) at >180°C → Acrylamide



### Physiological Effects and Warning

- Acrylamide is probably the main cause of neurotoxic effects
- Acrylamide reacts with Hemoglobin (Hb)-N terminal Valine to form CEV (a Hb adduct); this Hb adduct also found in cigarette smokers
- Glycidamide, the acrylamide metabolite, reacts with DNA and is assumed to be the mutagenic and cancer-initiating species
- Sigma-Aldrich MSDS states:
  - Toxic, May Cause Cancer
  - May Cause Inheritable Genetic Damage, and Impair Fertility
  - Readily Absorbed through the Skin; Targets Nerves and Kidney
  - This product is or contains a component that has been reported to be probably carcinogenic



### Acrylamide Findings and Action Limits

- Acrylamide was reported by Tareke, et.al., 2002
  - French fries at 424 µg/kg; or 0.42 ppm (µg/g)
  - An average serving size = (Small, Medium, Large, Biggie) grams
  - Potato chips at 1739 µg/kg; or 1.74 ppm (µg/g)
  - A small bag is ~42 g
  - Hamburger at 18 µg/kg
  - Beer at < 5 µg/kg; or <0.005 µg/g; or < 5 µg/L
- WHO and EPA drinking water guideline is 0.5 µg/L corresponding to 1 µg / day acrylamide intake
- In 2003, European Union will adapt 0.1 µg/L limit

### Acrylamide Analysis Method Choices

- Modified EPA GC/MS Method
  - Bromination of acrylamide using KBr, HBr, Br<sub>2</sub>•H<sub>2</sub>O.
  - Laborious sample prep, time consuming
- or
- LC/MS/MS is the method of choice by Tareke
  - Direct injection using +ElectroSpray ionization
  - No derivatization required; simpler
  - Equivalent to LC/MS/MS method adopted by Swedish National Food Administration
  - Monitors the acrylamide [M+H]<sup>+</sup> at m/z 72
  - Fragmentation to [H<sub>2</sub>C=CH-C=O]<sup>+</sup> at m/z 55 for identification and quantification

*FDA scientists have developed a method to measure acrylamide levels in foods. The FDA is posting this method on its website to provide other researchers the opportunity to review and use the method.*

**Waters® Proposed Acrylamide Method**

- Choice of: Single Quadrupole, Waters Micromass® ZQ™ 2000 or
- Triple Quadrupole, Waters Micromass Quattro *micro*™, for enhanced sensitivity and specificity
- Uses Waters Oasis® SPE cartridges for sample preparation
  - Oasis HLB to remove non-hydrophilic neutral organics, lipid and oils
    - The strongly retained material
  - Oasis MAX to remove anionic organics and salts
    - The matrix components eluting in the void volume
- Uses Waters Atlantis™ column, new column technology; reverse phase columns for use with 100% Aqueous mobile phases

**Acrylamide Sample Extraction\***

- Weigh 1 gram sample into a 50 mL plastic centrifuge tube
- Add 1 mL internal standard and 9 mL DI
- Mix for 10 mins on a rotating shaker. DO NOT heat or sonicate because of fine particulate generation)
  - Forms viscous, “pasty” solution
- Centrifuge a 9,000-12,000 rpm for 30 mins
  - Difficult to obtain 2 mL clear supernatant
- Take 5 mL aliquot beneath the oil layer into Maxi-Spin filter tube, 0.45 µm PVDF, and centrifuge at 9000 rpm for 4 mins
  - Or filter through a 0.45 µm filter
  - Critical step affecting SPE recovery
- Use 2 mL of filtrate for SPE

**Solid Phase Extraction Protocol\***

- Condition Waters Oasis HLB (6 mL, 200 mg packing) with 5 mL MeOH followed by 5 mL water
- Load 2 mL sample filtrate onto cartridge and allow to pass through
  - Do not use vacuum assisted flow
- Wash with 2 mL water and collect the effluent 1
- Condition a Varian Bond Elut (200 mg mixed C8, SAX, SCX, 3 mL) with 3 mL MeOH followed by 3 mL water
  - or an Oasis MAX (alternative)
- Pass Oasis HLB effluent 1 (2 mL, from above) onto the Bond Elut cartridge, and collect the effluent 2
- Use this effluent 2 for LC/MS/MS analysis

**Waters LC/MS/MS or LC/MS Systems***This method developed on:*

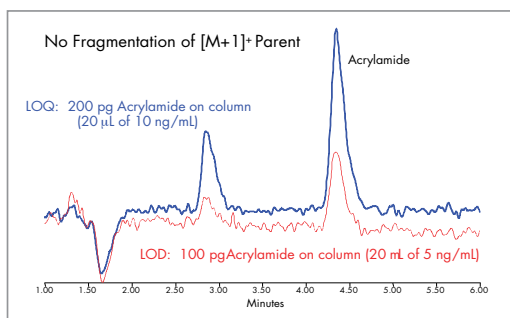
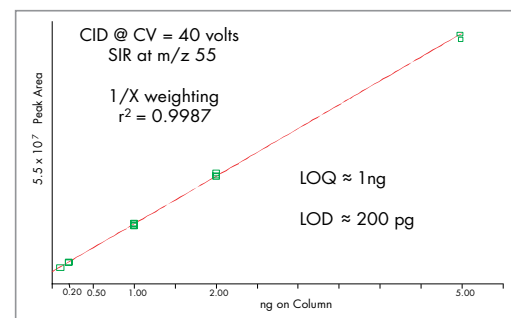
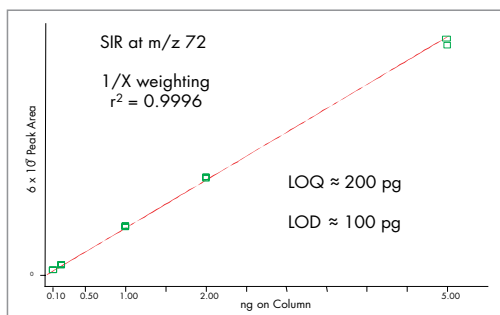
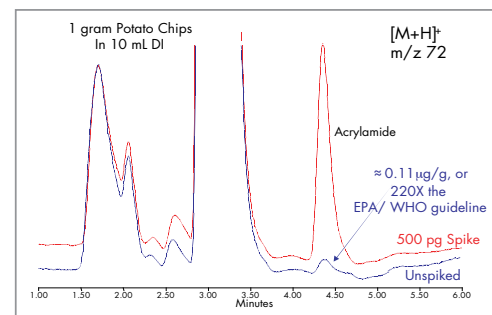
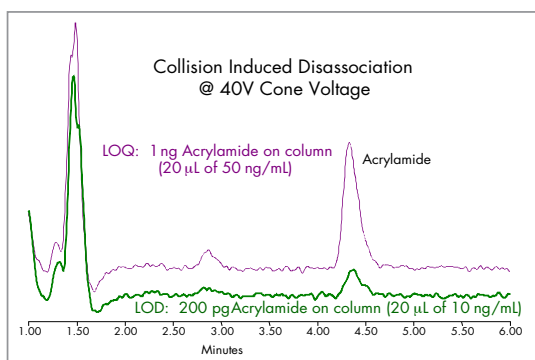
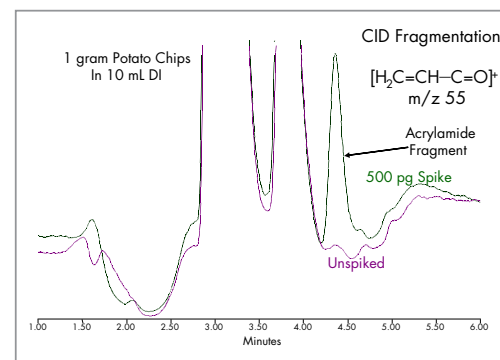
- Waters Alliance® System with Column Heater
- Quattro *micro* Triple Quadrupole Mass Spectrometer, or ZQ 2000 Single Quad Mass Spectrometer
- Waters 2996 PhotoDiode Array Detector (optional)
- Waters Masslynx (ver4.0) Data Management and LC Control
  - Data files imported into Waters Empower™ Software for presentation
- Waters Atlantis dC<sub>18</sub> Column (2.1 x 150 mm)
- Waters Oasis HLB and MAX Solid Phase Extraction Cartridges

*\*Sample prep taken and modified from the FDA method.*

**Acrylamide Chromatographic and MS Conditions**

- Waters Atlantis Column, 2.1 x 150 mm, 3  $\mu$ m
- 0.5% MeOH in 0.1% Acetic Acid
- 200  $\mu$ L/min at 26°C; backpressure  $\approx$ 1500 psi
- 10-20  $\mu$ L
- Detection with Single or Triple Quadrupole Mass Spec
- ZQ 2000 Single Quadrupole MS Tune Conditions
  - Capillary (kV) = 3.5
  - Source Temp (C°) = 125
  - Cone (V) = 22
  - Cone Temp (C°) = 20

- Extractor (V) = 3
- Desolvation Temp (C°) = 350
- RF Lens (V) = 0.5
- Desolvation Gas Flow (L/hr) = 500
- Cone Gas Flow (L/hr) = 25
- MS Acquisition for simultaneous parent ion and CID fragment ion
  - SIR @ m/z 72
    - dwell time = 0.3
    - Cone Voltage = 22
  - SIR @ m/z 55
    - dwell time = 0.3
    - Cone Voltage = 40

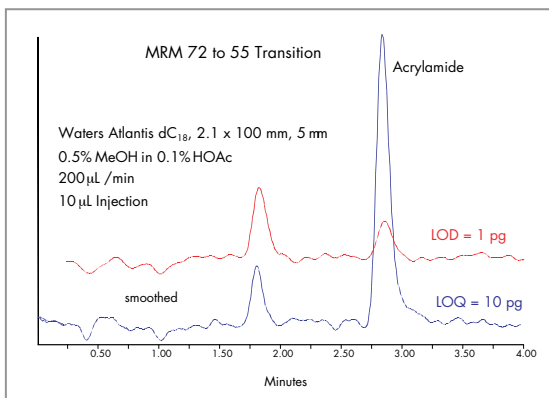
**SIR @ m/z 72****Linearity at m/z 55 (Fragment Ion)****Linearity at m/z 72 (Parent Ion)****Potato Chips No Sample Prep****SIR @ m/z 55 for CID Fragmentation****Potato Chips No Sample Prep**

### Need for High Sensitivity and Specificity

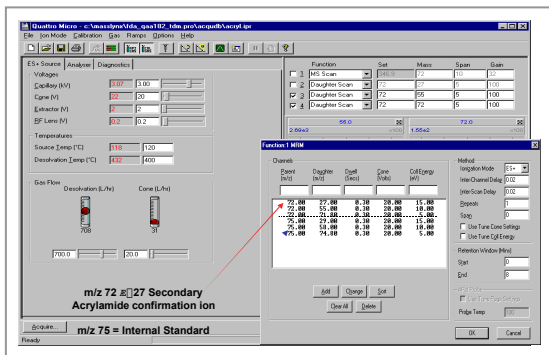
- Based upon single quadrupole performance, 20X additional sensitivity is required to meet EPA / WHO guidelines
  - 0.5 µg/L, or 5 pg on column
- Limited retention of the highly water soluble acrylamide suggests that sample preparation is required for method reproducibility and ruggedness
- Complexity of the prepared sample matrix and acrylamide retention may lead to interferences with m/z 72 and 55, requiring Triple Quadrupole Mass Spectrometry for optimal identification and quantification

### Triple Quadrupole MS/MS

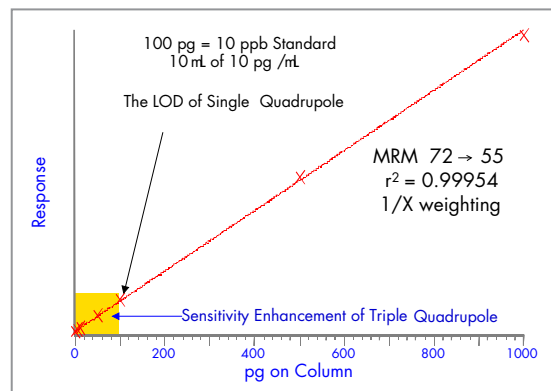
#### Detection & Quantification Limits



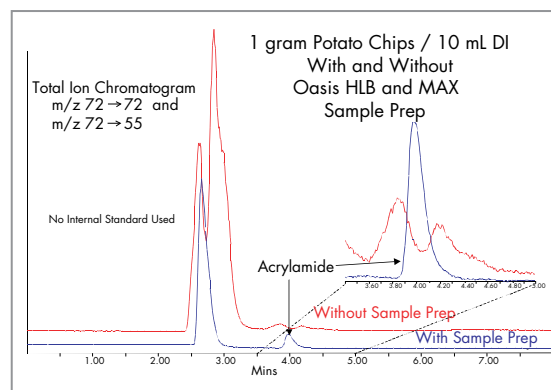
### MS/MS Tune Conditions



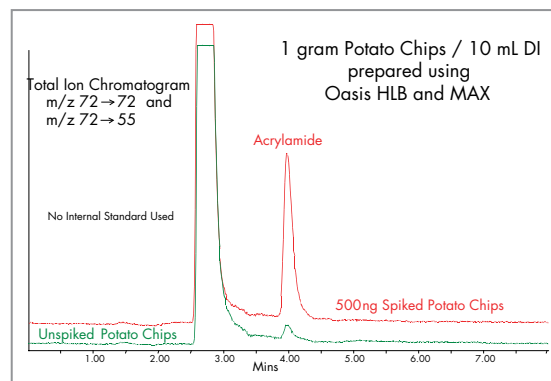
### MRM 72 \_ 55 Linearity



### MS/MS of Potato Chips Before and After Sample Prep



### MS/MS of Spiked & Unspiked Potato Chips



**Acrylamide LC/MS/MS Method Summary**

- This method approach is in the development process and has not been inter-laboratory validated
- Requires dual stage sample preparation
  - to remove hydrophobic components that give strong chromatographic retention, and
  - hydrophilic components that elute at the void volume
  - mandates use of ( $^{13}\text{C}_3$ ) Acrylamide internal standard
- Uses reverse phase columns with acidic 99.5% aqueous mobile phase
  - quality of the DI water (<10 ppb TOC content)
  - wash the column with MeOH after each batch of samples
- Requires validation for specific food stuffs

**Waters LC/MS/MS Solution**

- This presentation demonstrates the utility of liquid chromatography and mass spectrometry for the analysis of acrylamide in food products
- Waters provides products for a single vendor solution
  - Oasis HLB and Oasis MAX for sample preparation
  - Atlantis dC<sub>18</sub> columns
  - Alliance HPLC Systems
  - Mass Spectrometers, single or triple quadrupole
  - Triple Quadrupole, Quattro micro gives best sensitivity
    - LOD of 1 pg on column
    - MRM monitoring for specificity
  - Single Quadrupole, ZQ 2000
    - LOD of 100 pg on column
    - CID fragmentation

WATERS CORPORATION  
34 Maple St.  
Milford, MA 01757 U.S.A.  
T: 508 478 2000  
F: 508 872 1990  
[www.waters.com](http://www.waters.com)

Waters, Micromass, Alliance, Empower, ZQ, Quattro micro, Atlantis and Oasis are trademarks of Waters Corporation.  
©2002 Waters Corporation LW-PDF5

**Original poster created October 2002**

