

**Acrylamide in food: Kits, columns and tubes for sensitive GC-MS and LC-MS analyses and solid phase extraction .....** Acrylamide is a commonly analyzed food-borne carcinogen. Sigma-Aldrich has convenient kits, LC and GC columns and sample prep methods for fast and reliable analysis of acrylamide by both analytical techniques.

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### Formation and health risks of acrylamide in food

Acrylamide analysis has been a very hot topic since the chemical was identified in food in 2002 by researchers at the Swedish National Food Administration.<sup>1</sup> Since then, alarmingly high concentrations of acrylamide have been found in many popular processed foods including French fries, potato chips, breakfast cereals, coffee, chocolate, peanut butter, crisp bread and pastries. Researchers at the Department of Environmental Chemistry, Stockholm University, measured moderate levels of acrylamide (5-50 µg/kg) in heated protein-rich foods and higher levels (150-4,000 µg/kg) in carbohydrate-rich foods, such as potatoes and bread. Acrylamide could not be detected in unheated control or boiled foods (<5 µg/kg). Based on average consumption levels, consuming certain heated foods could lead to a daily intake of a few tens of micrograms of acrylamide.<sup>2</sup>

Acrylamide ends up in food via two routes: as a leachate from packaging materials and as an unintentional consequence of the cooking process when high carbohydrate, low protein foods are fried, roasted or baked at high temperatures. A proposed mechanism of its formation is the reaction of asparagine and a carbonyl-containing compound. The mechanism involves formation of a Schiff base followed by decarboxylation and elimination of either ammonia or a substituted imine to yield acrylamide.<sup>3</sup> Temperature plays an important role in the reaction.

In February 2005, the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA) met to consider the possible health risks associated with acrylamide and other food contaminants.<sup>4</sup> Although early reports suggested that acrylamide is a neurotoxin, reproductive toxin and teratogen, the Committee concluded that cancer is the most important toxic effect of acrylamide and that consumption of foods with acrylamide at levels currently found in some processed foods poses a public health concern. The low threshold of toxicity coupled with the high degree of activity surrounding acrylamide research necessitates reliable, fast and sensitive analytical methods.

### Analytical methods to measure acrylamide in food

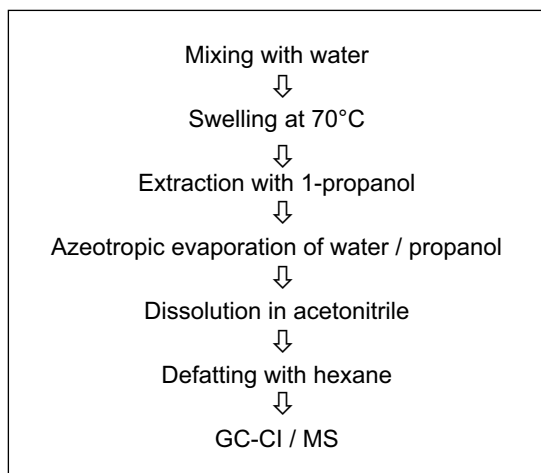
A review of the scientific literature shows that many different analytical approaches have been applied to the analysis of acrylamide in food, with GC-MS and LC-MS accounting for the majority of the citations. Analysis of acrylamide in food is difficult because of the high level of endogenous matrix interferences inherent to starchy foods. Sample clean-up is necessary to prevent starches and oils from interfering with GC-MS or LC-MS analyses.

**Table 1** ..... Acrylamide Kit and components

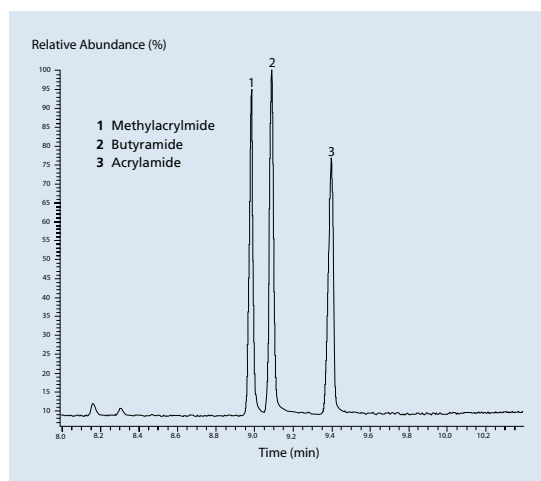
Cat. No.	Brand	Description*	Package Size
72615	Fluka	Acrylamide Kit (for 12 determinations according to the Grob method)	1 Kit

#### \*Contents of Acrylamide Kit (Cat. No. 72615):

Product	Description	Package Size and Type
Acrylamide Standard Solution	500ppm in acetonitrile	5mL (Certan® vial)
Acrylamide-D <sub>3</sub> Standard Solution	500ppm in acetonitrile	5mL (Certan® vial)
Methacrylamide Standard Solution	500ppm in acetonitrile	5mL (Certan® vial)
Butyramide Standard Solution	25ppm in acetonitrile	5mL (Certan® vial)
1-Propanol	puriss., p.a., >99.5% (GC)	250mL glass bottle
n-Hexane	puriss., >99.0% (GC)	100mL glass bottle
Acetonitrile	For residue analysis, >99.9% (GC)	50mL glass bottle
Oil	Suitable for acrylamide determination	50mL glass bottle



**Figure 1** ..... Summary of Grob GC-Cl/MS method



**Figure 2** ..... GC-Cl/MS chromatogram of methacrylamide, butyramide and acrylamide. (Column: SUPELCOWAX™ 10, 30m x 0.25mm ID x 0.25µm df. Oven: Gradient starting at 70°C (1 min.) to 220°C at 15° C/min, hold 2 min. Injection volume: 1µL. Carrier gas: He, 20 cm/sec. Injection: on column.)

### Food analysis GC-Cl/MS method

A common GC-MS technique for acrylamide analysis in food is based on a method developed by Grob, et al.<sup>5</sup> The sample preparation involves several steps (**Figure 1**): swelling the sample in water, centrifugation, addition of small amount of oil, evaporation, reconstitution in acetonitrile and removal of fats and lipids by hexane extraction. Internal standards are added both before and after extraction. The acetonitrile extract containing the acrylamide is analyzed by GC-MS on a Carbowax 20M capillary column with positive ion chemical ionization mass spectroscopic detection. Supelco SUPELCOWAX™ 10 capillary columns are ideally suited for the separation and MS detection.

The Grob method requires several high quality reagents and standards that initially were not available from a single supplier. To address this, scientists at Sigma-Aldrich's Fluka and Riedel-de Haën divisions developed a ready-to-use kit that makes acrylamide determination faster and easier. The Acrylamide Kit (**Table 1**) contains all the necessary standards and solvents for twelve determinations of acrylamide using the Grob method. The concentrations and specifications of the acrylamide, D<sub>3</sub>-acrylamide and methacrylamide standard solutions were designed specifically for this method. Each Kit also contains all sample preparation protocols and the GC test chromatogram and MS spectra of the standards (**Figure 2**). More information on the Acrylamide Kit can be found in the Product Listing (**Table 2**) and on the web site:

[www.sigma-aldrich.com/kit\\_gc\\_acrylamide](http://www.sigma-aldrich.com/kit_gc_acrylamide)

**Table 2** ..... Product List: Solvents, SPE Tubes and HPLC and GC Columns for acrylamide detection

Cat. No.	Brand	Description	Packaging Size
24079	Supelco	SUPELCOWAX™ 10 Capillary GC column	1 Unit
52784-U	Supelco	Discovery DSC-MCAX SPE tubes 3mL, 300mg bed weight	Pack of 54
52606-U	Supelco	Discovery DSC-C18 SPE tubes 6mL, 1gram bed weight	Pack of 30
57020-U	Supelco	SPE Tube Adapter for 1, 3 & 6mL tubes	Pack of 12
567507-U	Supelco	Discovery F5 HPLC column, 15cm x 4.6mm, 3µm particles	1 Unit
34967	Riedel-de Haën	Acetonitrile (LC-MS CHROMASOLV®)	1L, 2.5L
39253	Riedel-de Haën	Water (LC-MS CHROMASOLV®)	1L

### SPE-LC-ESI/MS method

Although the Grob GC-MS method gives excellent results and is widely accepted, the extensive sample preparation may be a drawback for some analysts, especially those who prefer LC methods. In the SPE-LC-MS method developed by researchers at Sigma-Aldrich's Supelco division, a mixed-mode cation-exchange SPE phase (Discovery DSC-MCAX) in series with a C18 SPE phase (Discovery DSC-18) is used to extract acrylamide from food prior to LC-MS analysis (Figure 3). The two SPE tubes are connected in series via an SPE tube adapter. The MCAX cartridge has both hydrophobic and ionic interactions and retains starch and oily interferences. The C18 cartridge retains the acrylamide by a weak hydrophobic mechanism. The eluate from the C18 cartridge is analyzed by LC-MS using a fluorinated reversed phase column, Discovery F5. Unlike standard C18 HPLC phases, the F5 column gives enhanced retention of the polar acrylamide and excellent retention and peak shape using a completely aqueous mobile phase (Figure 4). Complete details on the SPE-LC-ESI/MS method is described in the Supelco Reporter, Volume 22.5 (October 2004) which is available by contacting us or by visiting the website:

[www.sigma-aldrich.com/spe\\_acrylamide](http://www.sigma-aldrich.com/spe_acrylamide)

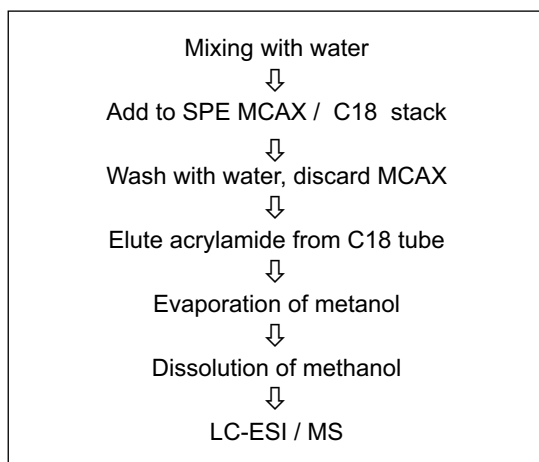


Figure 3 ..... Summary of SPE-LC-ESI/MS method

### Conclusions

Sigma-Aldrich offers the complete solution for acrylamide analysis, no matter which analytical method you prefer. For GC-MS, we offer the Acrylamide Kit and SUPELCOWAX™ 10 GC capillary columns. By choosing the Kit, time consuming standard preparation is avoided and detailed protocols take away any method development time. For LC-MS, the Discovery line of SPE and HPLC phases provides the necessary retention, efficiency and selectivity. This method requires no derivatization to produce sufficient retention and detection, and sample prep is fast and easy with high recoveries of acrylamide from complex matrices.

An excellent resource for up-to-date information on acrylamide research and regulations is the website [www.acrylamide-food.org](http://www.acrylamide-food.org). The site is maintained jointly by the FAO (UN), JIFSAN (US FDA and University of Maryland) and the WHO's Food Safety Department.

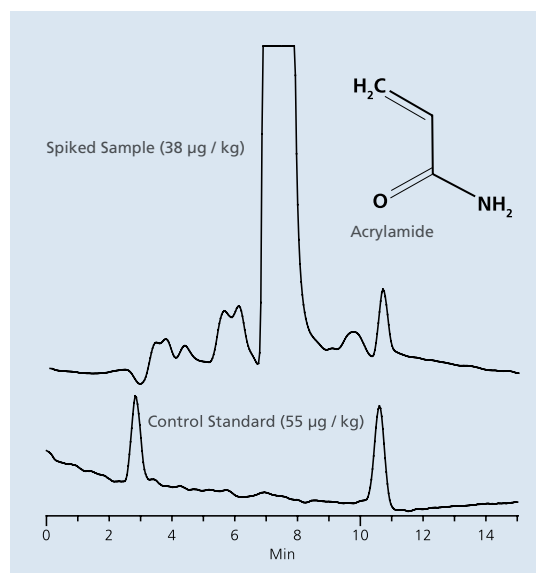


Figure 4 ..... LC-ESI/MS chromatogram of control and acrylamide-spiked potato chip extract. (Column: Discovery F5, 15cm x 4.6mm, 3µm. Mobile phase: 100% Water (LC-MS CHROMASOLV™). 0.3mL/min, 35°C. Injection volume: 5µL. Standard: 48µg/mL. MS conditions: +ESI, SIM 72 m/z.)

### References

- [1] Swedish National Food Administration: Information about acrylamide in food. [www.slv.se/Download/Document/approvedDocs/enginformatiakravl.htm](http://www.slv.se/Download/Document/approvedDocs/enginformatiakravl.htm), 24 April 2002.
- [2] E. Tareke, P. Rydberg, P. Karlsson, S. Eriksson, M. Tornqvist; J. Agric. Food Chem. 2002; 50(17); 4998-5006.
- [3] D. Zyzak, R. Sanders, M. Stojanovic, D. Tallmadge, B. Eberhart, D. Ewald, D. Gruber, T. Morsch, M. Strothers, G. Rizzi, M. Villagran; J. Agric. Food Chem. 2003; 51(16); 4782-7.
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- [5] M. Biedermann, S. Biedermann-Brem, A. Noti and K. Grob, P. Egli, H. Mändli; Mitt. Lebensm Hyg. 2002; 93; 638-652.