

## SPE Application Note for Extraction of Acrylamide from Foodstuffs

This method is recommended for the extraction of acrylamide from foodstuffs using an initial extraction with mixed-mode SPE column ISOLUTE Multimode, followed by clean-up with ISOLUTE ENV+ non-polar SPE columns.

**ISOLUTE**<sup>®</sup> **SPE Column:** ISOLUTE Multimode 1 g/3 mL, part # 904-0100-C ISOLUTE ENV+ 1 g/6 mL, part # 915-0100-C

Pre-treatment:	<ol> <li>Homogenize the sample (4 g) with water (40 mL) containing internal standard (400 uL of water solution containing deutering labelled acrylamide (1.0 ug/mL).</li> <li>Centrifuge (10 Celsius, 4000 rpm, 20 min.).</li> <li>Apply supernatant to a conditioned ISOLUTE Multimode color using the following method:</li> </ol>	g um- umn
	Column Conditioning: Rinse column with acetonitrile (3 mL). Column Equilibration: Rinse column with water (2 $\times$ 6 mL). Sample Application: Apply sample (10 mL). Collect eluent.	
	Apply to an ISOLUTE ENV+ column using the method detailed below.	
Solvation:	Condition the column with methanol (5 mL).	
Equilibration:	Equilibrate with water (5 mL).	
Sample application:	Apply the sample (~10 mL).	
Interference elution:	Rinse with water (2 x 5 mL).	
Analyte elution:	To elute analytes, apply first volume of elution solvent to extraction cartridge. Soak for two minutes. Add second volume of elution solvent to extraction cartridge and collect.	
	<ol> <li>Elute with methanol in water (60% v/v). Discard the first fraction (1.7 mL) and collect the eluate fraction (1.7 mL-3.7 m 2. Evaporate until 1 mL of the extract remains.</li> <li>Inject an aliquot into the LC-MS/MS system for analysis.</li> </ol>	nL).
Structure	Acrylamide shown.	H <sub>2</sub>

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Structural considerations	The analyte is polar and highly water soluble.		
Matrix considerations	The matrix is complex with a variety of interferences including sugars, salts, proteins, lipids etc.		
Analytical method	LC-MS/MS		
Reagents	<ol> <li>Deionized water</li> <li>Internal standard, deuterium-labelled acrylamide</li> <li>Acetonitrile</li> <li>Methanol</li> </ol>		
General comments	Reference: Fermentation Reduces Free Asparagine in Dough and Acrylamide content in Bread Fredriksson, -H; Tallving, -; Rosen, -J; Aman, -P Cereal Chem. 81(5): pp650-653		
ISOLUTE column part numbers re 96-well and alternative column co © 2006 Argonaut Technologies, n Technologies, now a Biotage com	present the product configuration of choice for use v onfigurations compatible with any SPE automation s ow Biotage company. All rights reserved. ISOLUTE i pany.	with a vacuum sample processing station. For ystem, please contact Biotage. s a registered trademark of Argonaut	
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