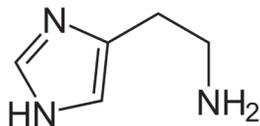
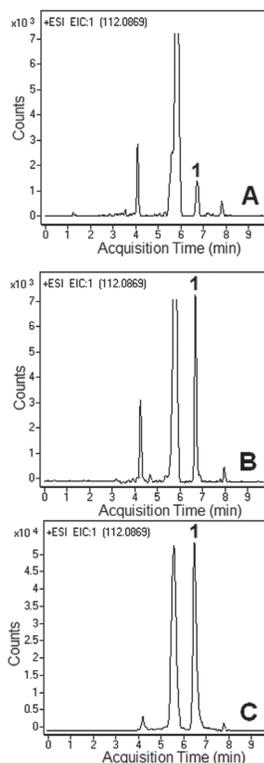


Histamine in Cheese by LC-MS

No derivatization required



Sample Preparation: Grated parmesan cheese was purchased from a local supermarket. Three cheese samples were prepared. The unspiked SPE sample was prepared by homogenizing 5g cheese and 50 mL DI H₂O / 0.1% FA in a Waring blender for 10 min at 13,500 rpm. The mixture was then centrifuged at 4000 g for 20 min. The supernatant was refrigerated (20°C) for 10 min, treated by adding dropwise 3 M ammonia to a pH of 11.0, and then centrifuged at 1000 g for 5 min. The resulting supernatant was purified by solid phase extraction (SPE) on a conditioned C18 sorbent and eluted with 2 mL of methanol. After removal of the methanol by nitrogen gas, the extracted sample was re-dissolved in 2.0 mL of DI H₂O / 0.1% FA for direct analyses. The spiked SPE samples were prepared by homogenizing 5.0 g of cheese, 50 mL of DI H₂O / 0.1% FA, and an appropriate amount of 1 mg/mL histamine stock solution in a Waring blender for 10 min at 13,500 rpm. Afterwards, the sample preparation was completed by following procedures for the unspiked SPE samples (i.e. centrifuge, SPE, etc.).

Method Conditions

Column: Cogent Diamond Hydride™, 4µm, 100Å

Catalog No.: 70000-15P-2

Dimensions: 2.1 x 150 mm

Mobile Phase: A: DI H₂O / 50% 2-propanol / 0.1% formic acid
B: Acetonitrile / 0.1% formic acid

Gradient:	time (min.)	%B
	0	80
	5	10
	7	10
	8	80

Post Time: 5 min

Injection vol.: 1 µL

Flow rate: 0.4 mL/min

Detection: ESI - POS - Agilent 6210 MSD TOF mass spectrometer

Peaks: 1. Histamine 112.0869 m/z [M+H]⁺

t₀: 0.9 min

Discussion

A small but measurable amount of histamine was found in a cheese sample (Figure A) after an extraction procedure and analysis using the Cogent Diamond Hydride column with MS detection. Two spiked cheese samples were also analyzed. In Figure B, the cheese sample was spiked before the extraction procedure at a level of 0.5 mg/L and Figure C shows the histamine peak in a spiked extract from the cheese sample at a level of 8.0 mg/L. From the figures, it is obvious that the identification of histamine by mass or retention time is not affected by the cheese matrix or the extracted material. The histamine content in the cheese sample was determined based on a calibration curve and it was calculated to be 500 ± 5 ng/grams of cheese (with a %RSD of 0.2 for n=5).