

Environmental

Analysis of Nitro Compounds in Sheep Rumen Fluid Using UHPLC/SQ MS



Chemically synthesized nitro aromatic compounds including pesticides, explosives, pharmaceuticals, etc. can enter the environment and cause both short- and long-term damage to living organisms. It is important to understand the biological fate of these compounds so that appropriate remedial measures can be adopted to clean up the environment.

One innovative and cost-effective cleanup of explosive nitro compounds – such as TNT, HMX and RDX – involves the use of ruminant animals, including sheep, which possess a highly anaerobic intestinal environment. The anaerobic bacteria in these ruminant animals can reduce the highly toxic compounds to more benign, non-toxic molecules. We present a study of the analysis of HMX spiked in sheep rumen fluid using UHPLC/SQ MS.

Experimental Conditions

Target Analyte: Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocane (HMX)

Sample Preparation Conditions

A standard solution of HMX was prepared by diluting mobile phase A. A calibration curve was set up in the range of 5 to 200 ng/mL. Sheep rumen fluid spiked with HMX (6 µg/mL) was diluted 1:100 in mobile phase A prior to analysis.

Liquid Chromatography Conditions

Pump Type:	PerkinElmer® Flexar™ FX-15		
Column:	PerkinElmer Brownlee™ Supra™ Aqueous C18 column (2.1 mm x 50 mm, 1.9 µm)		
Mobile Phase:	A: water containing 0.1% acetic acid B: 80/20 methanol/acetonitrile containing 0.1% acetic acid		
Flow Rate:	0.4 mL/min		
Injection Volume:	2 µL in partial fill mode		
Gradient:	Time (min)	%A	%B
	0	80	20
	1	80	20
	2	50	50
	1	50	50

Mass Spectrometer Conditions

Ionization:	Ultraspray™ ESI – Negative mode
Scan Range:	40-365 m/z
Scan Rate:	1000 u/sec
Selected Ion Monitoring (SIM):	SIM ion 355.0 for HMX; dwell time of 200 ms
Capillary Exit Voltage:	-50 V

Results

The mass spectrum of the target analyte has been acquired by initial experiments in Full Scan UHPLC/SQ MS. Figure 1 shows both the acetate adduct and the chloride adduct of the HMX molecule.

Subsequently, using SIM mode, a calibration curve was established for the acetate adduct of HMX (355 m/z). Excellent linearity was observed in the range of 5 to 320 ng/mL of HMX ($r^2 = 0.999$, Figure 2). Figure 3 shows the SIM chromatogram of the HMX acetate adduct spiked in sheep rumen fluid. The HMX was separated on column in less than 4 minutes using UHPLC with a Flexar SQ 300 MS detector at pg sensitivity.

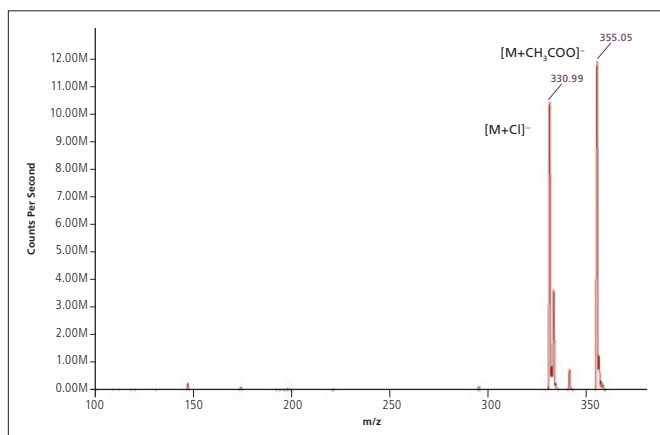


Figure 1. Mass spectrum of HMX showing acetate and chloride adducts of analyte.

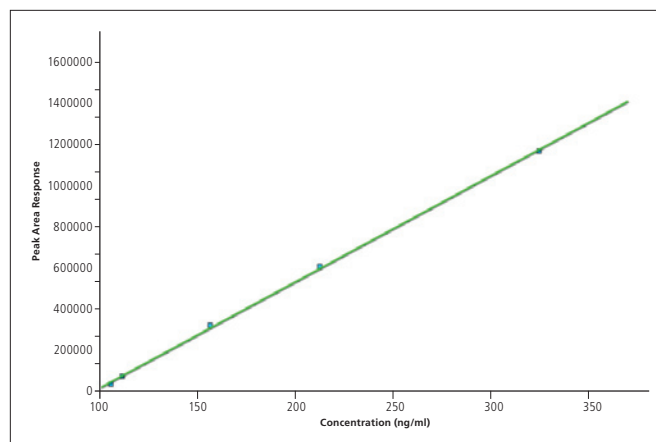


Figure 2. Calibration curve of HMX (range 5 to 320 ng/mL, $r^2 = 0.999$).

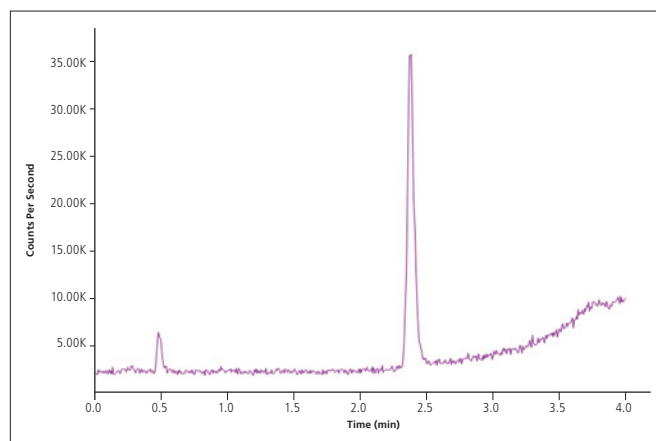


Figure 3. Shows the 355.0 m/z, SIM ion of HMX spiked in sheep rumen fluid.