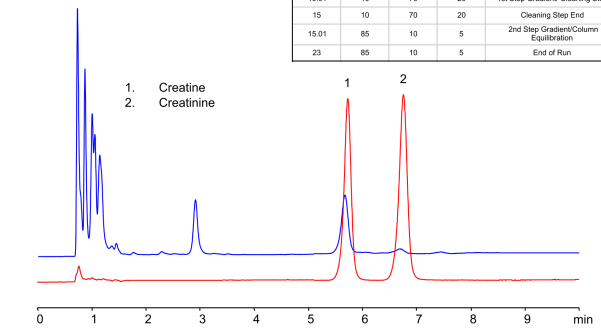
HPLC Method for Analysis of Creatine and Creatinine in Milk on Newcrom AH Column

Mobile Phase Conditions

Time (min)	A - H2O (%)	B - MeCN (%)	C - H3PO4 2% in H2O (%)	Notes
0	85	10	5	Starting Conditions/Isocratic
10	85	10	5	Isocratic End
10.01	10	70	20	1st Step Gradient/ Cleaning Step
15	10	70	20	Cleaning Step End
15.01	85	10	5	2nd Step Gradient/Column Equilibration
23	85	10	5	End of Run



Column:	Newcrom AH
Column size:	3.2 × 100 mm, 3 µm
Column part number:	NAH-32.100.0310
Mobile phase:	MeCN/H_2O
Buffer:	H_3PO_4
Flow rate	0.5 mL/min
Detection:	UV 200 nm
Sample:	Standard: 1. 0.02 mg/mL, 2. 0.02 mg/mL, Milk Sample
Injection Volume:	2 µL
Diluent:	MeCN/H2O - 10/90%
LOD:	1. 3.6 ppb, 2. 3.5 ppb

Creatine is a naturally occurring compound found in muscles and is used to produce energy during high-intensity exercise. It is synthesized in the body from amino acids and stored in muscles for quick energy release. Creatinine, on the other hand, is a waste product produced from the breakdown of creatine. It is filtered by the kidneys and excreted in urine. The measurement of creatinine levels in the blood and urine is commonly used to assess kidney function, as high levels may indicate impaired kidney function or other health issues. Creatine and creatinine are naturally occurring compounds found in milk, originating from muscle metabolism and dietary sources in lactating animals. While creatine contributes to energy metabolism, creatinine serves as a breakdown product and its levels in milk can be influenced by the animal's physiological state and kidney function.

Creatine and Creatinine can be analyzed and separated using a Newcrom AH mixed-mode stationary phase column. The analysis utilizes a step gradient method with a simple mobile phase consisting of water, acetonitrile (MeCN), and phosphoric acid as a buffer. Detection is carried out using UV.

Method Parameters

Column	Newcrom AH, 3.2 x 100 mm, 3 µm, 100 Å, dual ended
Mobile Phase	MeCN/H2O
Buffer	H3PO4
Flow Rate	0.5 mL/min
Detection	UV 200 nm
Sample	Standard: 1. 0.02 mg/mL, 2. 0.02 mg/mL. Milk Sample
Injection Volume	2 µl

Quelle: https://sielc.com/hplc-method-for-analysis-of-creatine-and-creatinine-in-milk-newcrom-ah