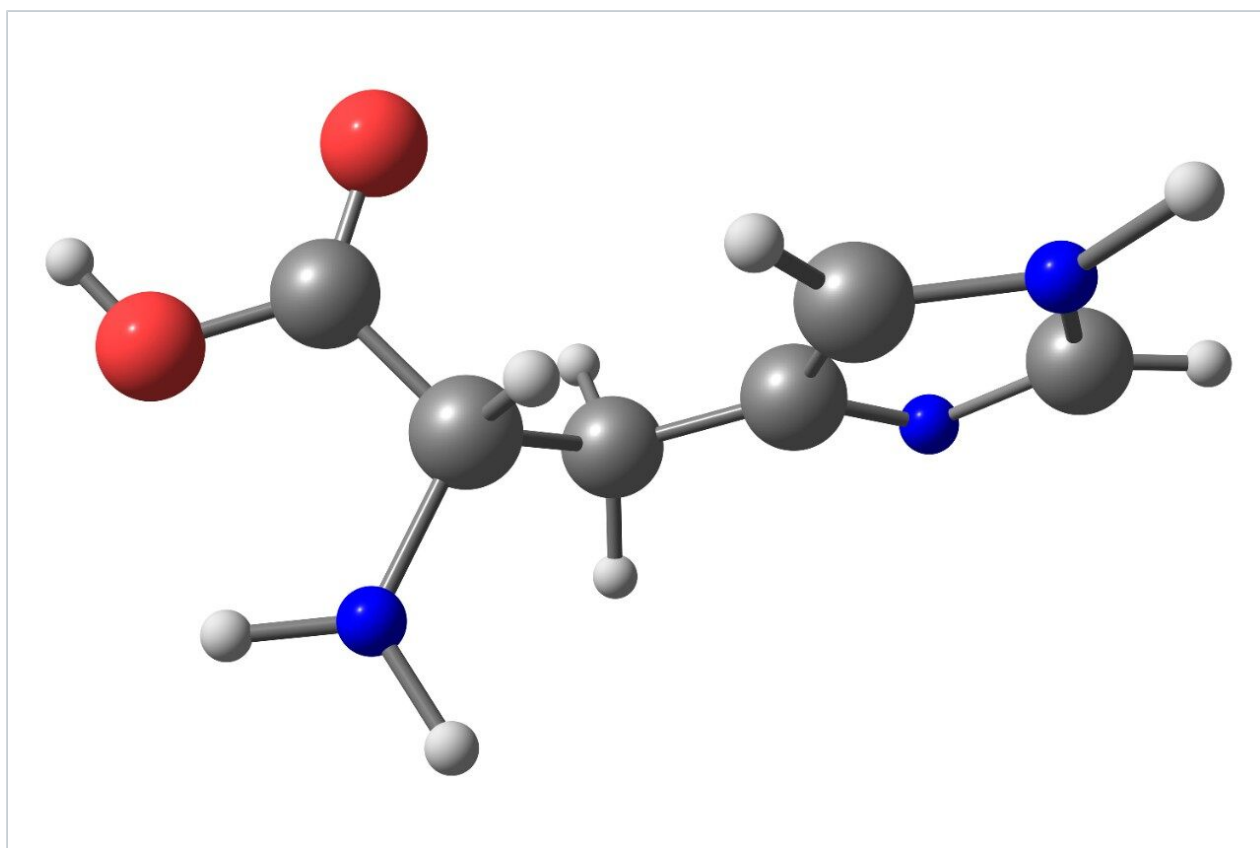


Application Note

Gradient Separation of Histidine Dipeptides on ACQUITY UPLC BEH HILIC

Waters Corporation



This is an Application Brief and does not contain a detailed Experimental section.

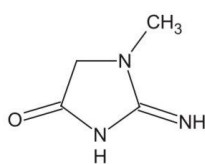
Abstract

This application brief demonstrates the gradient separation of histidine dipeptides on ACQUITY UPLC BEH HILIC Columns.

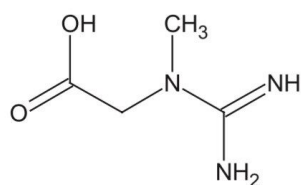
Introduction

The compounds used in this study are:

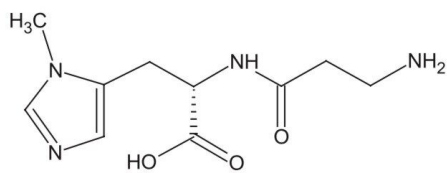
1. Creatinine
2. Creatine
3. Anserine
4. Carnosine



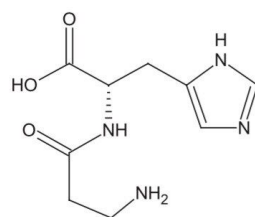
Creatinine



Creatine



Anserine



Carnosine

Experimental

Test Conditions

Column:	ACQUITY UPLC BEH HILIC, 2.1 x 50 mm, 1.7 μ m
Part Number:	186003460
Mobile Phase A:	50/50 ACN/10 mM ammonium formate, w/ 0.125% HCOOH, pH 3.0
Mobile Phase B:	95/5 ACN/10 mM ammonium formate, w/ 0.125% HCOOH, pH 3.0
Flow Rate:	0.5 mL/min
Injection Volume:	5.0 μ L
Sample Diluent:	75:25 ACN:MeOH
Sample Concentration:	Creatinine 1 μ g/mL; Carnosine 5 μ g/mL; Anserine 5 μ g/mL; Creatine 5 μ g/mL
Column Temperature:	30 $^{\circ}$ C
Weak Needle Wash:	ACN/H ₂ O 95/5
Instrument:	Waters ACQUITY UPLC with ACQUITY SQD

Gradient

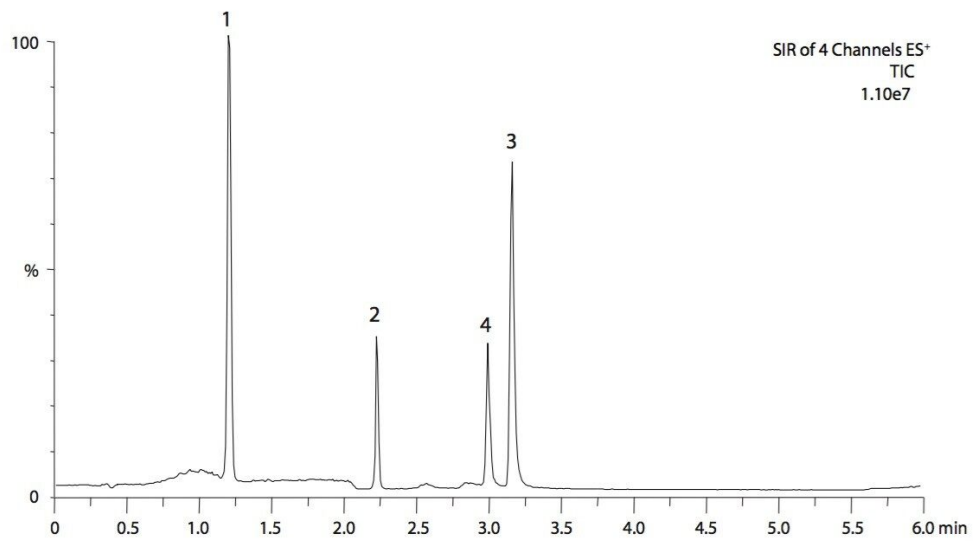
Time (min)	Profile
	%A
0.00	0.1

Time (min)	Profile
5.00	99.9
5.01	0.1
6.00	0.1

MS Conditions

Ionization Mode:	ES+
Capillary:	2.5 kV
Cone:	20 V (Carnosine; Creatinine, Anserine); 25 V (Creatine)
Source Temperature:	120 °C
Desolvation Temperature:	400 °C
Desolvation Gas Flow:	800 L/Hr
Cone gas Flow:	5 L/Hr
SIR <i>m/z</i> :	227.1 <i>m/z</i> (Carnosine); 132.1 <i>m/z</i> (Creatine); 114.05 <i>m/z</i> (Creatinine); 241.1 <i>m/z</i> (Anserine)
Dwell Time:	0.1 s

Results and Discussion



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[ACQUITY UPLC System <https://www.waters.com/514207>](https://www.waters.com/514207)

WA60139, August 2009