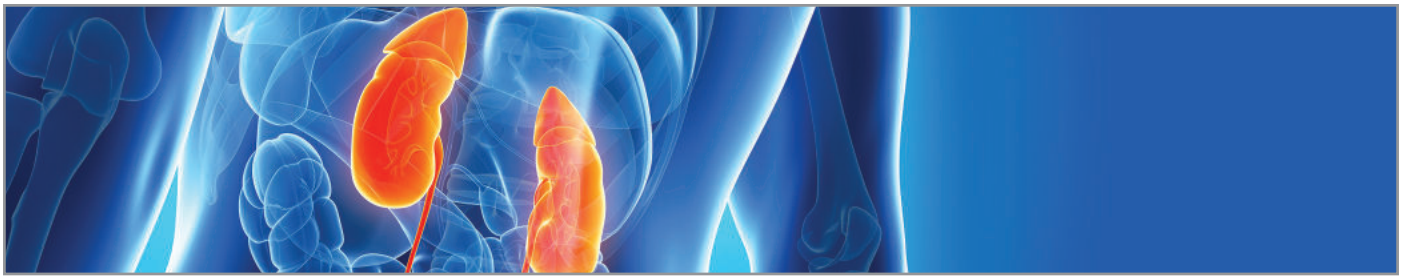


Featured Application: *Creatine and Creatinine in Plasma and Urine on Raptor HILIC-Si*

Fast, Simple LC-MS/MS Analysis of Creatine and Creatinine

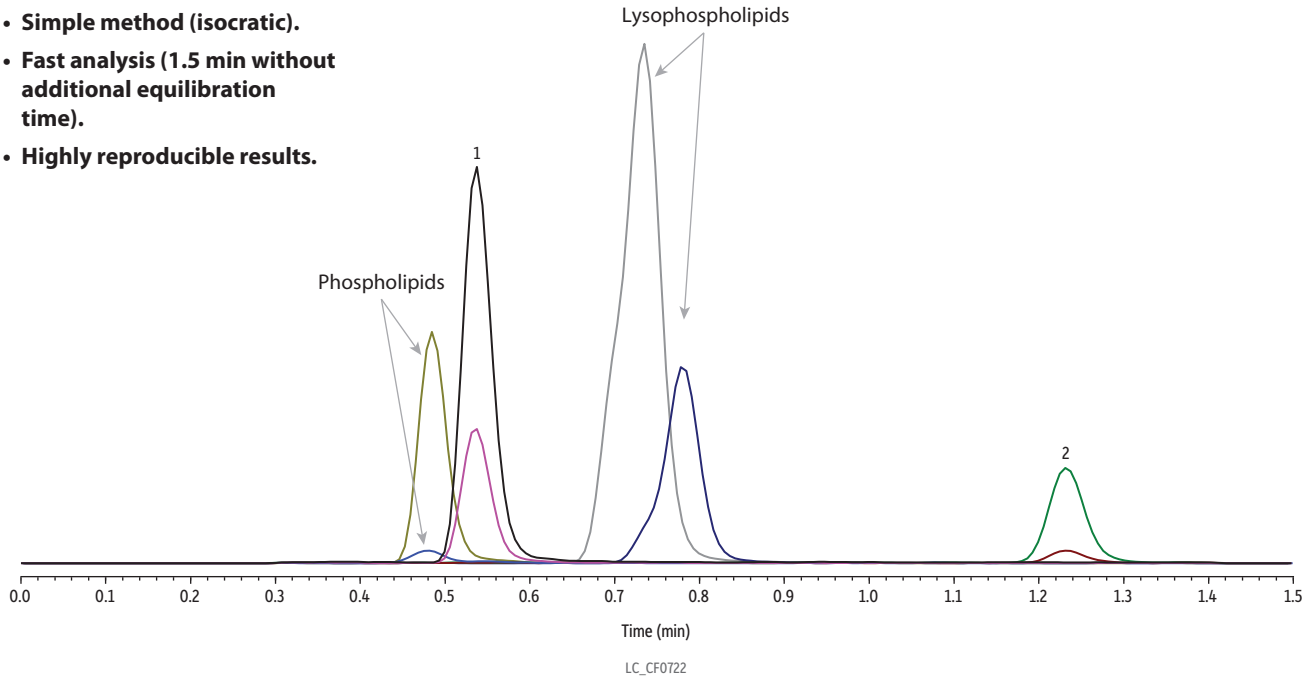
- Simple isocratic method, no complex mobile phases.
- Fast, 1.5 min analysis without additional equilibration time.
- Highly reproducible results.

Creatine is a compound that is made primarily in the liver and then transported to muscles where it is used as an energy source. Once in the muscle, some of the creatine is spontaneously converted to creatinine. It is important to monitor creatine and creatinine because these compounds can be used as biomarkers of renal, liver, and heart health. Historically, colorimetric and enzymatic assays have been used, but LC-MS/MS is a better technique because it reduces interferences. Although LC-MS/MS analysis of creatine and creatinine using an alkyl-bonded (e.g., C18) column is the usual approach, these highly polar analytes are very difficult to retain and separate from each other using a reversed-phase column. When a reversed-phase method is employed, complex ion-pairing reagents (e.g., tetrabutylammonium hydroxide) are generally required to increase analyte hydrophobicity.

Using HILIC, ion-exchange, or other newer LC phases can be a better alternative to reversed-phase methods because these phases employ different mechanisms of interaction. Some non-C18 columns do provide increased retention, but analysis times can be long (e.g., 10 min) and may require the use of complex mobile phases and gradients. Labs needing faster LC-MS/MS analysis of creatine and creatinine should consider adopting the HILIC method shown here. Good separations in both plasma and urine samples were obtained in just 1.5 min using a Raptor HILIC-Si (2.7 µm, 50 mm × 2.1 mm) column and a simple, isocratic mobile phase. Results during in-house testing were highly reproducible, making this fast, simple LC-MS/MS analysis a good procedure for high-throughput labs.

Human Plasma

- **Simple method (isocratic).**
- **Fast analysis (1.5 min without additional equilibration time).**
- **Highly reproducible results.**



Peaks	t_R (min)	Precursor Ion	Product Ion	Product Ion
1. Creatinine	0.537	114.0	44.3	86.0
2. Creatine	1.232	132.1	43.3	90.2

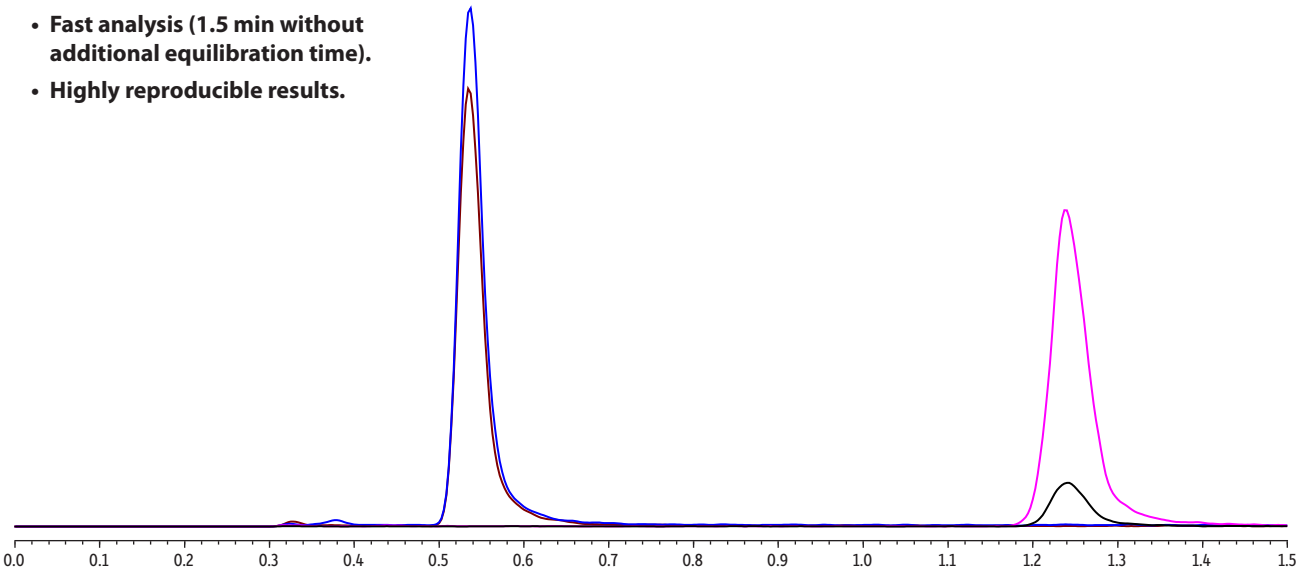
Column Raptor HILIC-Si (cat.# 9310A52)
Dimensions: 50 mm x 2.1 mm ID
Particle Size: 2.7 µm
Pore Size: 90 Å
Guard Column: UltraShield UHPLC precolumn filter 0.2 µm (cat.# 25810)
Temp.: 40 °C
Sample
Diluent: 20:80 Water:acetonitrile
Conc.: Endogenous levels
Inj. Vol.: 0.2 µL
Mobile Phase
A: 5 mM Ammonium formate in 20:80 water:acetonitrile

Time (min)	Flow (mL/min)	%A
0.00	0.5	100
1.5	Stop	

Detector MS/MS
Ion Mode: ESI+
Mode: MRM
Instrument UHPLC
Notes Sample Preparation:
Endogenous levels of creatinine and creatine in human plasma were determined using a single protein precipitation step followed by LC-MS/MS analysis. A 50 µL aliquot of human plasma (K2EDTA) was mixed with 950 µL acetonitrile. After vortexing and centrifuging at 4,300 rpm for 10 min, 200 µL of the supernatant was transferred to a new vial and mixed with 50 µL of water. Centrifugation was performed again before injection. Endogenous peaks for phospholipids and lysophospholipids are displayed as common matrix interferences known to suppress ionization efficiency.

Human Urine

- **Simple method (isocratic).**
- **Fast analysis (1.5 min without additional equilibration time).**
- **Highly reproducible results.**



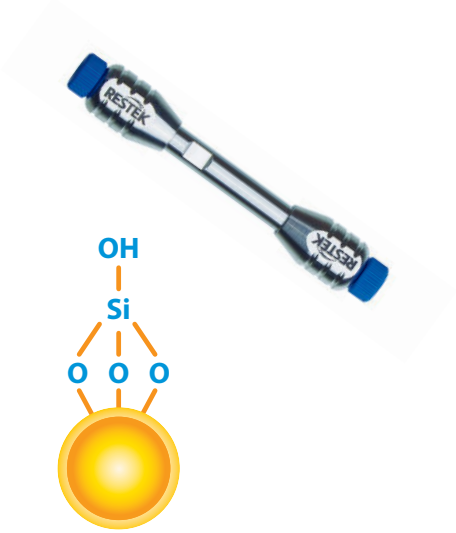
Peaks	t_R (min)	Precursor Ion	Product Ion	Product Ion
1. Creatinine	0.537	114.0	44.3	86.0
2. Creatine	1.240	132.1	43.3	90.2

Column Raptor HILIC-Si (cat.# 9310A52)
Dimensions: 50 mm x 2.1 mm ID
Particle Size: 2.7 µm
Pore Size: 90 Å
Guard Column: UltraShield UHPLC precolumn filter 0.2 µm (cat.# 25810)
Temp.: 40 °C
Sample
Diluent: 20:80 Water:acetonitrile
Conc.: Endogenous levels
Inj. Vol.: 0.2 µL
Mobile Phase
A: 5 mM Ammonium formate in 20:80 water:acetonitrile

Time (min)	Flow (mL/min)	%A
0.00	0.5	100
1.5	Stop	

Detector MS/MS
Ion Mode: ESI+
Mode: MRM
Instrument UHPLC
Notes Sample Preparation:
Endogenous levels of creatinine and creatine in human urine were determined using a simple dilute-and-shoot method. A 50 µL aliquot of human urine was mixed with 950 µL acetonitrile. After vortexing and centrifuging at 4,300 rpm for 10 min, 10 µL of the supernatant was added to the 1,490 µL of 80% acetonitrile in water. Centrifugation was performed again before injection.

Raptor HILIC-Si LC Columns (USP L3)



Length	2.1 mm cat.#	3.0 mm cat.#	4.6 mm cat.#
2.7 µm Columns			
30 mm	9310A32		
50 mm	9310A52	9310A5E	9310A55
100 mm	9310A12	9310A1E	9310A15
150 mm	9310A62	9310A6E	9310A65

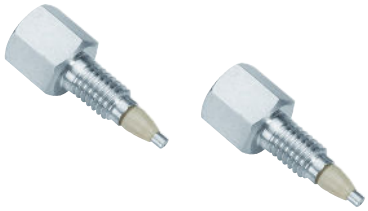
Column Characteristics:

Stationary Phase Category: bare silica (L3)
Ligand Type: none
Particle: 2.7 µm superficially porous particle (SPP or "core-shell" particle) silica
Pore Size: 90 Å
Carbon Load: n/a
End-Cap: n/a
Surface Area: 130 m^2/g
Recommended Usage:
pH Range: 1.0–8.0
Maximum Temperature: 80 °C
Maximum Pressure: 600 bar/8700 psi (2.7 µm)

Properties:
- Compatible with both HPLC and UHPLC instruments.
- Restek's 2.7 µm core-shell particles provide the speed of SPP and the performance of Raptor.

Switch to a Raptor HILIC-Si LC column when:
- Increased retention of small polar compounds is needed.
- You want to avoid using ion-pairing reagents.
- You want retention and sensitivity for hydrophilic compounds by LC-MS.

UltraShield UHPLC PreColumn Filter



Specifications:
Inlet/Outlet: Female/Male 10-32
Port Geometry: Parker ($^1/_{16}$ CPI)
Material: stainless steel, PEEK ferrule
Filter: 0.5 µm or 0.2 µm stainless steel
Pressure Rating: 15,000 psig (1034 bar)
Wrench Flat: $^5/_{16}$"

- Cost-effective protection for UHPLC systems.
- Reliable way to filter out particulates and extend column lifetime.
- Minimize extra column volume and maximize UHPLC sample throughput vs. guard cartridges.
- Connects easily to any column with Parker-style ports; not compatible with Waters columns.
- Leak tight to 15,000 psi (1034 bar).
- 0.5 µm or 0.2 µm stainless steel frit in a stainless steel body with PEEK ferrule.

Description	Filter Porosity	qty.	cat.#
UltraShield UHPLC PreColumn Filter	0.2 µm frit	ea.	25809
		5-pk.	25810
		10-pk.	25811

RESTEK