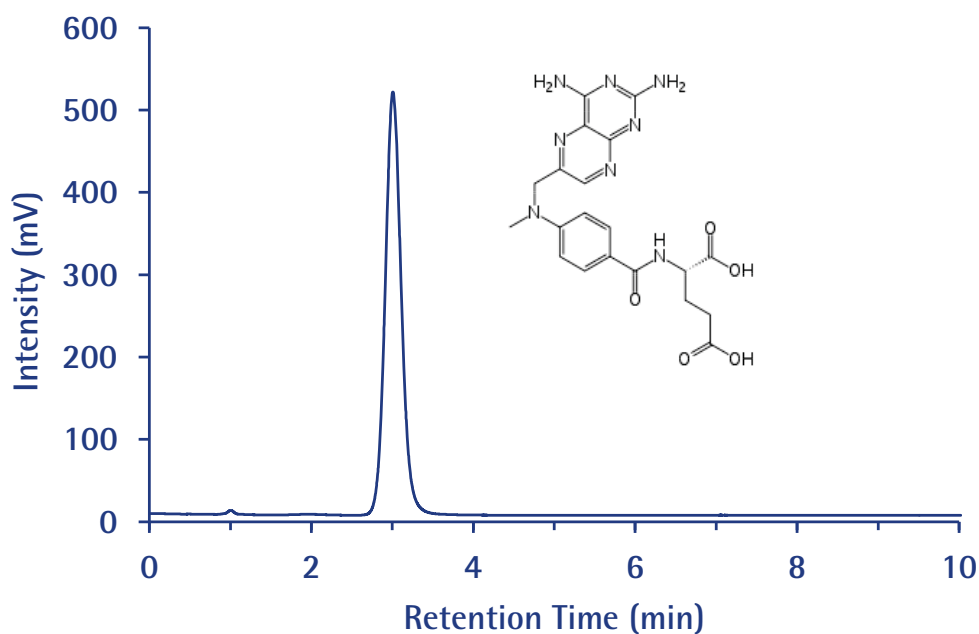


Methotrexate

SeQuant® ZIC®-pHILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-pHILIC (5 µm) 100x2.1 mm	1.50462.0001
Injection:	3 µL	
Detection:	Shimadzu UFLC XR, PDA 302 nm with 10 µL flow-cell	
Flow Rate:	0.5 mL/min.	
Mobile Phase:	Acetonitrile and 20mM ammonium acetate buffer pH 6.8 (80:20 v/v) Total ionic strength: 4 mM	
Temperature:	40 °C	
Diluent	Mobile phase	
Sample:	500 ppm of Methotrexate in mobile phase	
Pressure Drop:	103 Bar (1494 psi)	



Chromatographic Data

No	Compound	Time (min)	Resolution	Asymmetry
1	Impurity 1	2.4	-	1.0
2	Methotrexate	3.0	2.2	1.1
3	Impurity 2	5.5	4.5	0.9
4	Impurity 3	8.9	2.2	1.2