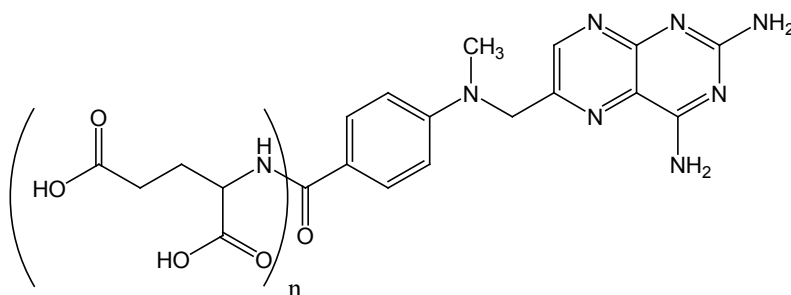


## Analysis of Methotrexate and its Polyglutamate Metabolites in Plasma by LC-MS/MS

Methotrexate (MTX) is an antifolate drug widely used as an anticancer drug and antirheumatic agent. MTX has a molecular structure similar to that of folic acid, with a glutamic acid structure on the inside. Known metabolites include 7-OH-MTX and polyglutamate-MTX. Here, as a method for analyzing MTX that is not bound to protein, MTX and 7-OH-MTX were simultaneously analyzed using reversed phase chromatography (RPC).

Also shown here is an example of simultaneous analysis of MTX and its polyglutamate metabolites in a plasma sample using hydrophilic interaction liquid chromatography (HILIC). In analyses of standard sample solutions, the calibration curve was linear in a concentration range of 0.1 to 50 $\mu$ g/L. In standard samples with added plasma (final concentration: 0.25 $\mu$ g/L), results showed recovery rates of  $\geq 78\%$ , with good reproducibility of  $\leq 3\%$  (n=6).

Figure 1. Structural formula

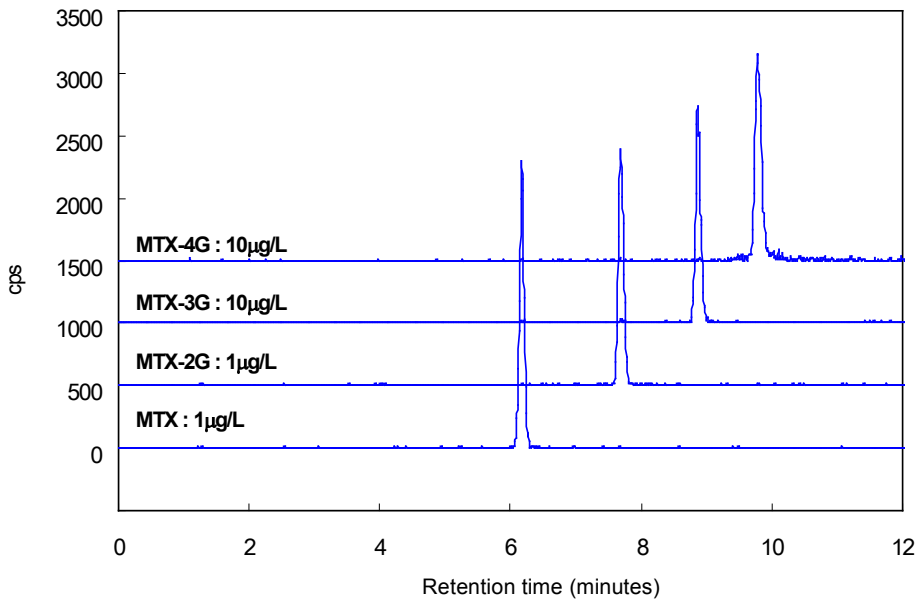


n= 1 : 4-Amino-10-methylfolic acid	(Methotrexate)
n= 2 : 4-Amino-10-methylpteroyl-di- $\gamma$ -glutamic acid	(Methotrexate-2G)
n= 3 : 4-Amino-10-methylpteroyl-tri- $\gamma$ -glutamic acid	(Methotrexate-3G)
n= 4 : 4-Amino-10-methylpteroyl-tetra- $\gamma$ -glutamic acid	(Methotrexate-4G)

**Table 1. Analytical conditions**

Column:	<b>TSKgel Amide-80, 3<math>\mu</math>m, 2.0mm ID x 15cm</b>	
Mobile phase:	A: 0.1% TFA in water B: 0.1% TFA in acetonitrile	
Gradient:	0min (95%B) $\rightarrow$ 15min (50%B) $\rightarrow$ 16min (50%B)	
Flow rate:	0.2mL/min	
Temperature:	40°C	
Injection vol.:	5 $\mu$ L	
Instrument:	Agilent 1200SL series QTRAP® (AB SCIEX)	
Ion Source:	ESI (Positive)	
m/z = 455.2>308.3 (MTX)	m/z = 584.2>308.3 (MTX-2G)	
m/z = 713.5>308.3 (MTX-3G)	m/z = 842.3>308.3 (MTX-4G)	

**Figure 2. Chromatograms of standard samples**



**Figure 3. Pretreatment of samples**

Plasma + 0.1 % TFA (acetonitrile solution) = 1 / 3 (V/V)  
↓  
5000 rpm, 5min  
↓  
Filter supernatant (pore size: 0.5µm)

**Figure 4. Chromatograms of samples with added plasma (0.25µg/L each)**

