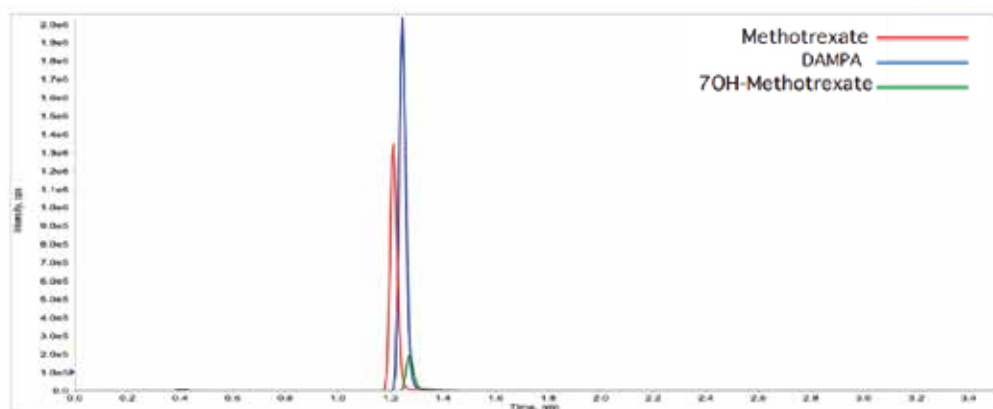


## SHORT METHOD DESCRIPTION

### FloMass® Methotrexate and Metabolites in Serum

The cytotoxic action of MTX is related to the cell cycle. It inhibits the synthesis of DNA, RNA, thymidylates and proteins by acting as a competitive antagonist of folic acid. For this reason, the drug has a greater toxic effect on cells with a high replication rate, such as malignant tumor cells. MTX compromises tumor growth without causing irreversible damage to normal tissues. However, the inhibition of the development and proliferation of non-cancerous cells can lead to a series of unexpected side effects. Low doses of MTX are effective against rheumatoid arthritis, Crohn's disease and psoriasis. Due to the narrow therapeutic index of this drug, it is necessary to determine the MTX concentration in serum of patient in order to avoid intoxication (if elevated) or therapeutic failure (if low). The determination of the two main inactive metabolism is useful to monitor the pharmacokinetic trend of the molecule. 7-OH-MTX, the main metabolite of the drug, has been recognized as the main cause of nephrotoxicity because of its precipitation in the renal tubules causing kidney damage. DAMPA has a lower toxicity than 7OH-MTX. Although clinically less interesting, DAMPA is to be considered as an analytical problem because of its cross-reactivity in the immunochemical dosage of MTX, leading to an overdose of the drug. Differently, HPLC-MS/MS methods are not affected by this problem, allowing to determine the two molecules individually.



#### HPLC-MS/MS system conditions

**Ionization:** ESI positive mode

**MS/MS:** specific MRM

**Injection volume:** 20  $\mu$ l (variable according to instrumental sensitivity)

**Running time:** 3.5 min

**Column heater:** 50°C

#### Performance

Analyte	Linearity (ng/mL)	LLOD (ng/mL)	LLOQ (ng/mL)	CV% Intra	CV% Inter
MTX	0.16 - 5000	0.05	0.16	2 - 6.1	6 - 6.3
7-OH-MTX	1.38 - 10000	0.41	1.38	5.6 - 6.2	5.9 - 6.8
DAMPA	0.29 - 4000	0.09	0.29	2.4 - 3.8	3.1 - 4.3

## ORDERING GUIDE

Order No.	Description	Quantity
<b>EUM16100</b>	<b>FloMass® MTX, 7(OH)MTX, DAMPA in Serum</b> LC-MS/MS detection	<b>100 assays</b>
	<b>Contents</b>	
	Mobile Phase A	1 x EUM16011
	Mobile Phase B	1 x EUM16012
	Precipitant Solution	1 x EUM16021
	Internal Standard	1 x EUM16031
	<b>Separately available components</b>	
EUM16011	Mobile Phase A	500 ml
EUM16012	Mobile Phase B	400 ml
EUM16021	Precipitant Solution	10 ml
EUM16031	Internal Standard	1.5 ml
	<b>Accessory</b>	
EUM00C16	Analytical Column with test chromatogram	1 pc
EUM16071	FloTuning MTX and 7(OH)MTX	1x2 ml
EUM16051	Control Set for Methotrexate and Metabolites in Serum, lyoph. (3 levels)	2x3x0.5 ml
EUM16041	Calibrator Set for Methotrexate and Metabolites in Serum, lyoph. (7 levels)	2x7x0.5 ml