BSN Biological Sales Network

METHOD AT A GLANCE

FLOMASS® SERUM METHOTREXATE METABOLITES

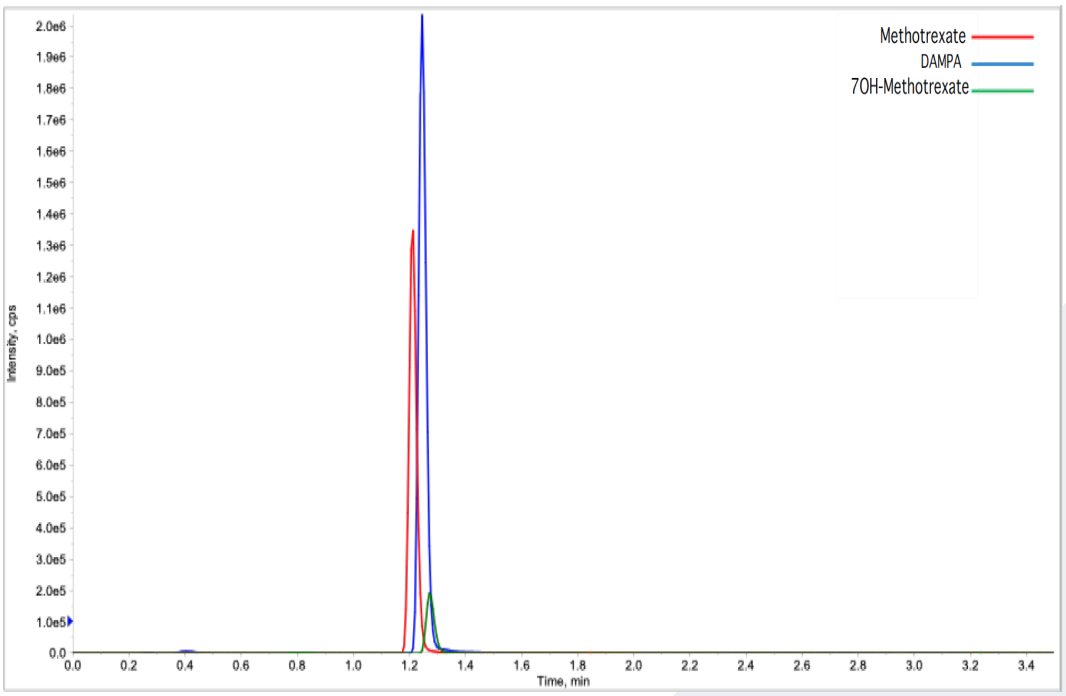
The cytotoxic action of MTX is related to the cell cycle. It inhibits the synthesis of DNA, RNA, thymidylates and proteins by acting as a competitive antagonist of folic acid. For this reason, the drug has a greater toxic effect on cells with a high replication rate, such as malignant tumor cells.

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.



PROVIDE PERSONAL SOLUTIONS

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HPLC-MS/MS system conditions

Ionization: ESI positive mode
MS/MS: specific MRM
Injection volume: 20 µL (variable according to instrumental sensitivity)
Running time: 3.5 min
Column heater: 50°C
Column conditioning: column should be conditioned for 5 min at chromatographic gradient initial condition. Then run 3 blank injections (MPA only) using the gradient as indicated in IFU

Sample preparation

- Prepare a mix with 90 µL of Precipitant Solution + 10 µL of Internal Standard sufficient for the number of samples to be analyzed
- Add 50 µL of serum in a vial
- Add 100 µL of Mix Solution obtained in the previous step of the procedure
- Vortex for 30 sec
- Centrifuge for 5 min at 10000-12000 rpm
- Transfer 20 µL of supernatant and dispense in vial
- Add 80 µL of Mobile Phase A in vial
- Inject 5-20 µL and analyze with HPLC-MS/MS technique

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

EUM16100	FloMass® Serum Methotrexate and Metabolites	100 assays
EUM16041	7-Levels Calibrators, lyophil.	2 x 7 x 0.5 mL
EUM16051	3-Levels Controls, lyophil.	2 x 3 x 0.5 mL
EUM00C16	Chromatographic Column	1 pc

CHR-01-20-REV.1

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