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METHOD AT A GLANCE

FLOMASS® METHOTREXATE AND ITS METABOLITES IN SERUM

Methotrexate is a competitive antagonist of folic acid, necessary for the synthesis of thymidine nucleoside and purine bases. Thus, its cytotoxic action is closely linked to the cell cycle due to inhibition of DNA, RNA and protein synthesis.

For this reason, the drug has a great toxic effect on cells with a high replication rate, as malignant tumor cells. However, the inhibition of the development and proliferation of non-cancerous cells also leads to several undesirable effects.

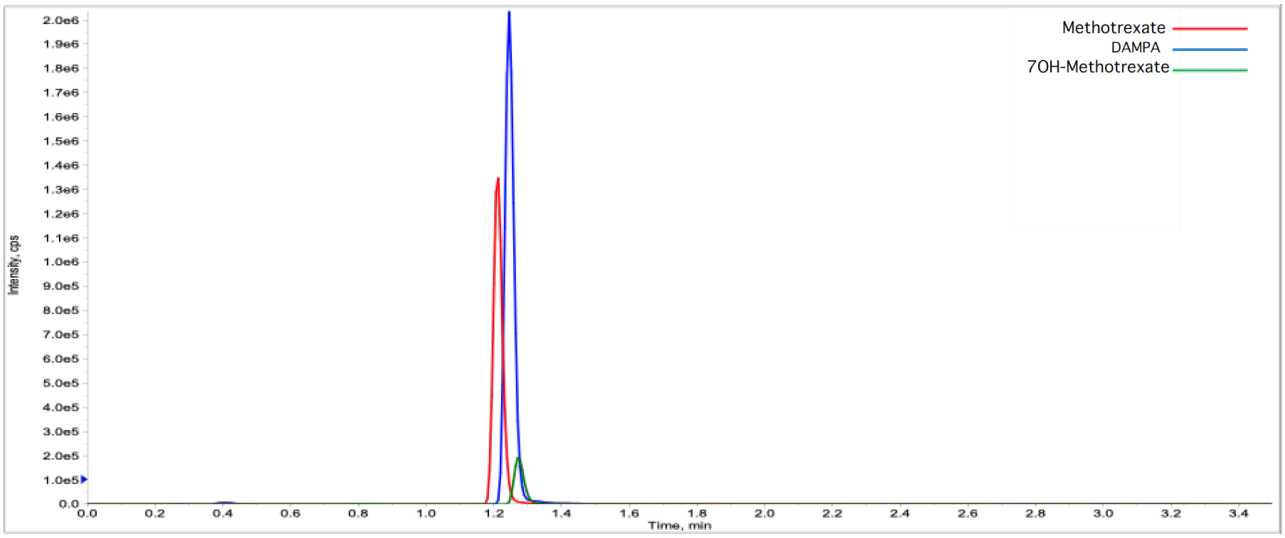
Low-dose methotrexate has also been shown to be effective against rheumatoid arthritis, Crohn's disease and psoriasis.

For an optimal pharmacokinetic monitoring, it is important to be able to measure the two main metabolites: 7-OH-methotrexate and Diamino-methylpteroic acid (DAMPA).

7-OH-MTX is the main metabolite, not pharmacologically active, but has been recognized as the main cause of nephrotoxicity. 7-OH-MTX is much less soluble than MTX and tends to precipitate in the renal tubules thus leading to kidney damage.

On the other hand, DAMPA, also inactive, is clinically less interesting, but it is an analytical problem due to its cross-reactivity with MTX by immunochemical method, leading to an overestimation of the latter.

Our LC-MS/MS method allows the simultaneous quantification of Methotrexate, 7-OH-Methotrexate and DAMPA in human serum in a fast, highly sensitive and specific way.



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LC-MS/MS system conditions

HPLC: gradient
Ionization: positive ESI
MS/MS: specific MRM
Injection volume: 5-20 µl

Running time: 3.5 min
Column heater: 50°C

Sample preparation

- Considering the number of assays to perform, prepare a mix of Internal Standard and Precipitant at a ratio of 1+9.
- Add 50 µl of serum (sample, control or calibrator) to 100 µl of Mix prepared at step 1.
- Vortex for 30 sec.
- Centrifuge at 10000-12000 rpm for 5 min.
- Transfer 20 µl of supernatant in a vial and add 80 µl of Mobile Phase A.
- Inject 5-20 µl and analyze by LC-MS/MS technique.

Performance

Analyte	Linearity (ng/ml)	LOD (ng/ml)	LOQ (ng/ml)	Intra-assay (CV%)	Inter-assay (CV%)
Methotrexate	0.16 - 500	0.05	0.16	2.00 – 6.10	6.00 – 6.30
7(OH)Methotrexate	1.38 - 5000	0.41	1.38	5.60 – 6.20	5.90 – 6.80
DAMPA	0.29 - 4000	0.09	0.29	2.40 - 3.80	3.10 – 4.30

Expected values and results interpretation

From clinical literature, reference values for evaluating toxicity after administration are:
Methotrexate > 4500 ng/mL at 24 h
> 450 ng/mL at 48 h
> 45 ng/mL at 72 h

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