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Evaluation of Dried Blood Spot technology in preclinical and clinical development

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BACKGROUND

Medical need

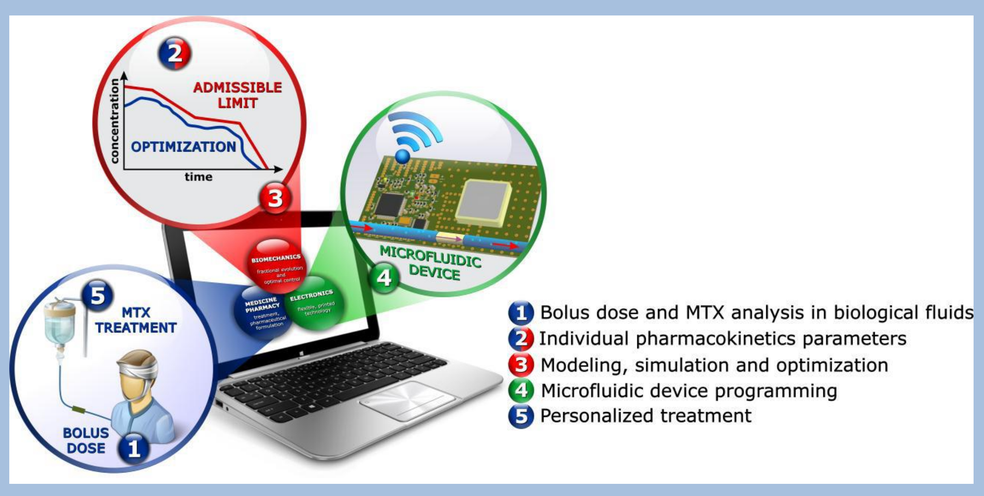
Leukemia is a group of cancers that starts in blood-forming tissues such as the bone marrow and causes large numbers of abnormal blood cells to be produced and enter the bloodstream. Worldwide over 250000 people are diagnosed with leukemia each year, accounting for 2.5% of all cancers. 75000 new patients are diagnosed in Europe each year (in USA around 40000). All age groups can be affected, but leukemias are the most common pediatric cancers accounting for 35% of cancers in children aged 0-14 years. The recent advances in diagnostics, therapy and improvements to therapy protocols lead to long-term curing, with an overall five-year survival rate of almost 80% in children with Acute Lymphoblastic Leukaemia (ALL). There is still an utmost need for new treatment options and new devices can potentially help in personalizing therapy of leukemias.

The MEDLEM consortium ** addresses this challenge. Namely, implementation of this project will help in both detection of high risk patients, especially children, and general improvement of the human condition during chemotherapy. Its overall goal is to increase five and ten-year survival rates of patients with leukemia. Methotrexate MTX is currently a standard treatment for Leukemia and the optimization of MTX administration is the central subject of MEDLEM studies.

MEDLEM project

The MEDLEM project aims at strengthening research collaboration for improved Leukemia treatment. This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No. 690876, bringing together 4 European organizations from **Serbia**, **Germany** and **France** as well as 2 non-European institutions from **Thailand** and **Australia**. The main technical goals of the joint research program are:

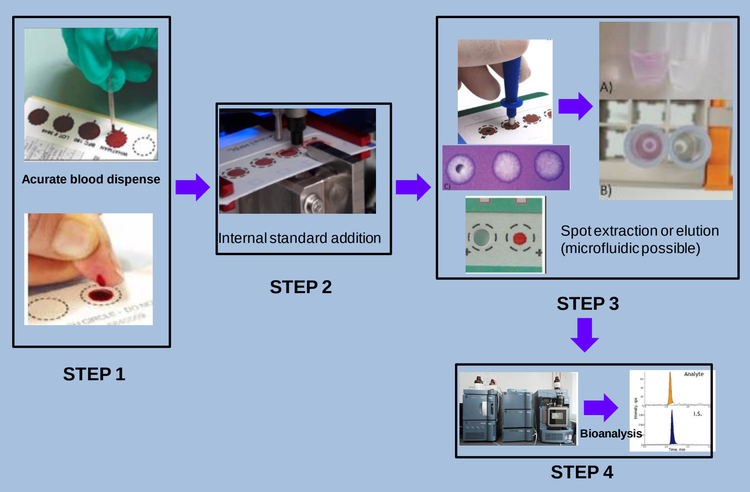
- Modeling of pharmacokinetics parameters of methotrexate using fractional calculus and optimal control
- Design and fabrication of cost-effective microfluidic device for optimal drug delivery
- Estimation of appropriate dosage regime for leukemia treatment



This poster presents the preliminary bioanalysis results which are the common part of the goals 1, 2 and 3.

Dried Blood Spot

Dried Blood Spot DBS ** is a technology for body fluid collection on paper. It was selected to facilitate the sample travelling between the MEDLEM labs for the analysis of blood and plasma samples after methotrexate administration to rat or human.



Benefits of DBS

- **More ethical** : low volume = multiple sampling from the same animal possible, so less animal needed
- **Cost benefits**: blood collection is simple and inexpensive
  Simple storage and transport = Multi-country collaboration friendly
- **Lower risk** of bacterial contaminations as preservative can be incorporated in paper

But: No regulatory guidelines for use of DBS in clinical development program may partly explain why DBS is under-used in pharmaceutical R&D

Objectives:

- **Evaluation** of DBS in preclinical/clinical applications : doability and efficiency
- **Optimisation** of DBS parameters for blood or plasma analysis of MTX: paper quality, extraction and recovery
- **Compatibility** of DBS with UPLC-MS/MS rapid analysis development

** References : http://medlemproject.com
Dried Blood Spot, Li and Lee, Wiley 2014

RESULTS

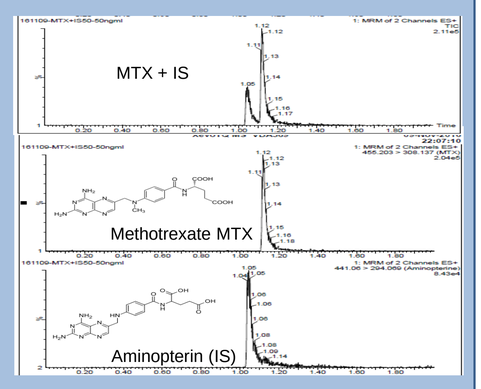
Bioanalysis

UPLC-MS/MS Waters Acquity – XEVO TQMS
Column Acquity BEH C18 (2.1x50mm, 1.7µm) at 40°C

Solvent A: H2O + 0,01% formic acid - Solvent B: MeCN + 0,01% formic acid
2 minute run - Flow 0.6mL/min
Gradient %A:
0 to 0,8mn : 100% to 60% - 0,8 to 1,1mn : 5% - 1,1 to 1,4mn : 5 to 100%

MSMS : MRM mode in electrospray ionization –p ositive ESI+

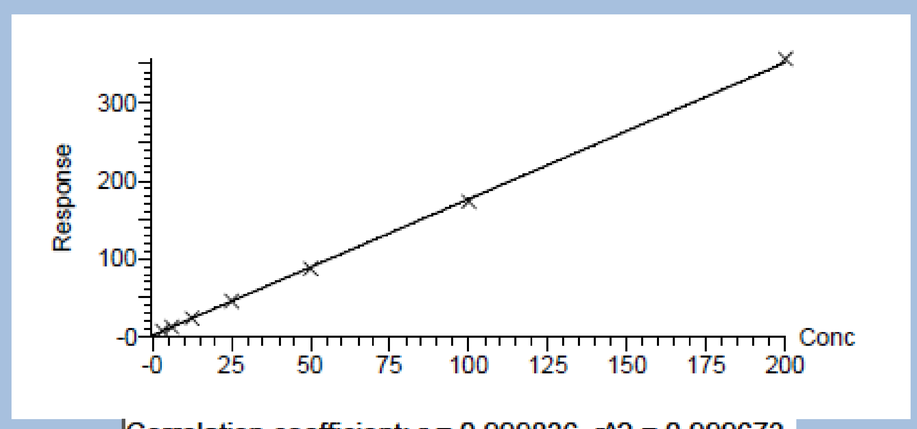
The precursor-product ion pair for MTX was: m/z 455,203 to 308,137
for Internal standard (Aminopterine) : m/z 441,06 to 294,069



Typical LCMSMS

CALIBRATION CURVE

MTX Working solutions : 200, 100, 50, 25, 12.5, 6.25, 3.175 ng/ml + IS = 50ng/ml (solvent : MeCN) – Injection : 1µl



Paper selection

Different grade of papers (Whatman filter paper, 903 Protein saver card, FTA Elute, FTA DMPK A and B) were evaluated on the following criteria : diameter of spot, recovery, amount of paper fibers extracted.

20µl of a solution of 50ng/ml of MTX was spotted on the papers with a pipette followed by 50ng/ml of IS. The solvent limit was circled with a pen at a distance of 1mm from the limit of the solvent front. The total spot was cut with scissors and cut in 6 pieces which were dropped in a borosilicate glass test tube (diameter 1cm). The scissors were cleaned with some lab tissue wetted with methanol after each cutting.

Whatman filter parper | Whatman 903 | FTA Elute | FTA DMPK A | FTA DMPK B

1ml of UPLC grade water was added and the test tube was sonicated for 30s, vortexed for 1mn then sonicated again for 30s. The supernatant was transferred into a centrifuge vial and centrifuged for 3mn at 14 000 x g. 500µl of supernatant was pipetted into glass vials for LCMSMS analysis.

The spot diameters by increasing size :

FTA Elute < FTA DMPK B < FTA DMPK A < Whatman 903 < Whatman filter paper

It was also noticed that the Whatman filter paper showed far less paper fibers in the extraction solution after sonication and vortex compared to the other grades.

The recovery for both MTX and IS were by increasing order (n=2) :

FTA DMPK B < FTA Elute < Whatman 903 < FTA DMPK A < Whatman filter paper

Extraction solution

1 ml of water gave the best recovery of both MTX and IS (>65%) compared to a 1/1 mixture of water/acetonitrile or 100% acetonitrile

As a conclusion the Whatman filter paper gave the best recovery although the spot was the larger. It was also the thinnest paper of all.

CONCLUSION

- ✓ A new and rapid method of LCMSMS analysis of Methotrexate was successfully developed from acetonitrile spotting on paper
- ✓ The Whatman filter paper showed the best recovery of MTX and IS after extraction in water
- ✓ The all spot cutting seemed quite tedious and labour intensive so punching will be assessed
- ✓ NEXT: Bioanalysis of human and rodent plasma and blood samples spotted on paper are currently ongoing

Thanks to GE Healthcare for the gift of DBS papers : 903 protein saver, FTA Elute, FTA DMPK A and B