Heat stabilization of DBS for metabolically unstable drugs

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In memory of Niklas Lindegårdh
Analyze the true *in vivo* content

Proteins and peptides and drugs degrade rapidly post sampling due to enzymatic activity.

Degradation makes it difficult or even impossible to identify labile peptides, proteins and their modifications.

**Crucial in:**
- Biomarker discovery
- Drug development
- Cancer research etc.

Denator’s Stabilizor system abolishes enzymatic activity which enable efficient analysis with mass spectrometry or antibodies techniques like Western Blot, Elisa and others.

– *see the true *in vivo* picture*

Peptide analysis with nano-LC-MS, visualized with DeCyder software

Sköld, et al., Proteomics 2007, 7(24), pp 4445
Degradation of proteins

“New” peptides fragments from high high-abundance proteins if not stabilized

Sköld, et al., Proteomics 2007, 7(24), pp 4445
Peptide intensity post sampling in brain tissue

Rapid decrease of peptides -> the result of enzymatic activity

Sköld et al, Proteomics 2007, 7 pp 4445–4456
MALDI Imaging: Stabilizing PEP-19

Snap frozen
Full length PEP-19
$m/z$ 6715
PEP-19 fragment
$m/z$ 1755
PEP-19 Fragment
$m/z$ 1393

Stabilized
Intact PEP-19 peptide
Ex vivo fragment of PEP-19
Ex vivo fragment of PEP-19

Goodwin et al., Journal of Proteomics, 2012
Effect of heat stabilization
Tissue stabilization workflow
Heat stabilized- DBS

New method for Dried Blood Spots
Heat stabilized DBS – Background

- Degradation in standard protocols during drying on DBS
- Stability after 45min is too slow (between 45min to 8h in field for DBS)
- The goal is to enhance the DBS technology by new level of reproducibility and stability in the workflow for DMPK
- Stable sample within one minute from sampling
Stability during the drying process
• Drying time from 45min up to 8h depending on temperature and humidity.
• Storage stability when sample are completely dry

Example of labile pharmaceuticals
• Prodrugs (e.g. oseltamivir, enalapril).
• Soft drugs (e.g. esmolol, remifentanil)
• Drugs with short life time in blood/plasma (e.g. acetylsalicylic acid, cefotaxime)
• Peptide and Protein pharmaceuticals (e.g. insulin, GLP-1)

Markers for diseases (e.g. BnP, TnI)
How do I know when the spot is dry?

Drying time depends on conditions.
Do not put in bag before dried.
Drying time in room temperature of 25ul stabilized (orange) and not stabilized (red) blood. End point blood dry matter about 20% of initial weight for both samples.
Oseltamivir (trade name Tamiflu) is an oral prodrug of oseltamivir carboxylate, a selective inhibitor of viral neuraminidase glycoprotein in influenza A. Oseltamivir undergoes fast bioconversion to oseltamivir carboxylate mostly by human carboxylesterase 1 (CES1). Dotted line describes the fragmentation of the compounds during MRM measurement.
Heat-stabilized DBS: Proof of Concept
Tamiflu in mouse blood (Whatman® FTA™ DMPK-C)

Recovery of Tamiflu and corresponding Metabolite

Pro-drug Tamiflu/ Oseltamivir

Metabolite Oseltamivir carboxylate
Oseltamivir
Metabolized Oseltamivir
Commercially available cellulose based and glass fiber based filters

Result after heat stabilization independent of carrier (DBS filter paper/matrix supplier)
Ribavirin

![Graph showing the extraction recovery of Ribavirin over time for different conditions: DBS stabilized, dried different times, DBS dried different times, stabilized, and wholeblood dried different times, stabilized. The graph indicates the percentage of extraction recovery (%N) over time (min) with error bars for each condition.](image-url)
Artemether (ARM) and dihydroartemisinin (DHA)

Artemether used in combination with lumefatrine and dihydroartemisinin in combination with piperaquine. Anti malarials, used to treat multi-drug resistant strains of *falciparum* malaria
Artemether (ARM) and dihydroartemisinin (DHA)
Cefotaxime is a third-generation cephalosporin antibiotic metabolized by enzymes in red blood cells.
Cefotaxime

- Blood spot frozen after drying
- Blood spot stabilized after drying then frozen
- Blood spot stabilized before drying then frozen
- Blood (5% hemolyzed) on foil stabilized after drying then frozen

Bioanalysis 5 (1) January 2013 (accepted)
Future workflow

Sample Collection

- Pre clinical DBS
- Clinical DBS

Heat stabilization within 60 sec.

Sample prep & Analysis

- Extraction
- LC-MS

Sample quality
Safe transport and storage
Future plans

• Setting up methods for more pharmaceuticals

• Launch of Maintainor DBS cards planned to Q2 2013

• Method paper will be published in Bioanalysis 2013

• First clinical trial “Oseltamivir treatment in children under one year of age with moderate or severe influenza lower respiratory tract infection – a clinical and pharmacokinetic study“ with heat stabilization.

• Future work include direct packing of DBS cards

• Continuously looking for collaborations
Heat-stabilized DBS Summary

Optimized DBS systems for Bioanalysis

• Exact, irreversible and reproducible inactivation of all enzymes
• Stable sample within one minute from collection
• Safer and faster workflow, no risk of further degradation during transport or storage due to re-moisturing
• No edge or halo effects induced by chemical coatings
• No additives or biohazards – user/environment friendly

Thank you for listening!