



Can DBS stabilize ester pro-drugs and glucuronide metabolites?

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Background

- Enzymes naturally present in biological samples degrade unstable pro-drugs and metabolites.
- Accurate determination is necessary for pharmacokinetic / toxicokinetic studies in Drug Discovery & Development or later in therapeutic drug monitoring.
- Conventional analyses need the addition of inhibitors or cooling the samples.
- Can DBS be a solution? Can new treated cards containing agents which degrade enzymes stop the degradation?

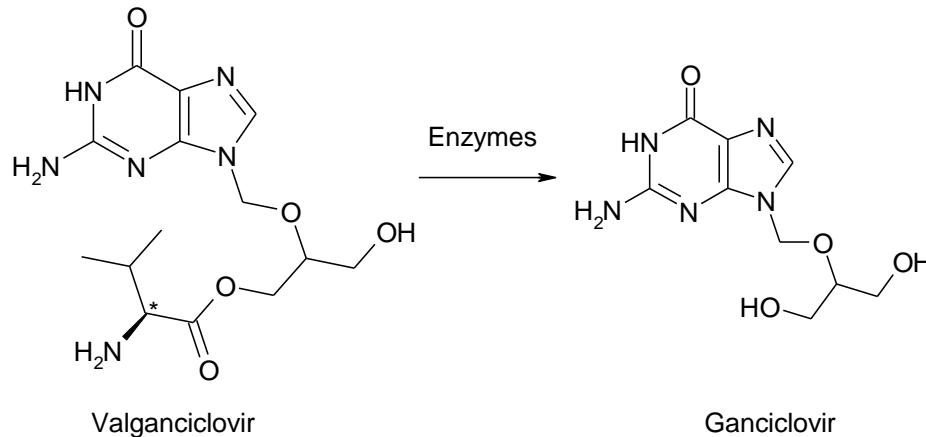
Compounds under study

- Two ester pro-drugs: Valganciclovir (Valcyte), and Oseltamivir (Tamiflu), both anti-viral drugs.
- One glucuronide: Mycophenolic acid acylglucuronide a metabolite from Cellcept, an immunosuppressant drug.
- One dihydropyrimidinone drug, sensitive to hydrolysis.
- LC-MS/MS was used for compound quantification.
- Experiments were performed in duplicate or triplicate.
- At least partial method validation was carried out for Valcyte, Tamiflu, and Cellcept.

Valcyte



- The pro-drug “Valganciclovir” is rapidly converted into an active metabolite “Ganciclovir” by intestinal and hepatic esterases.

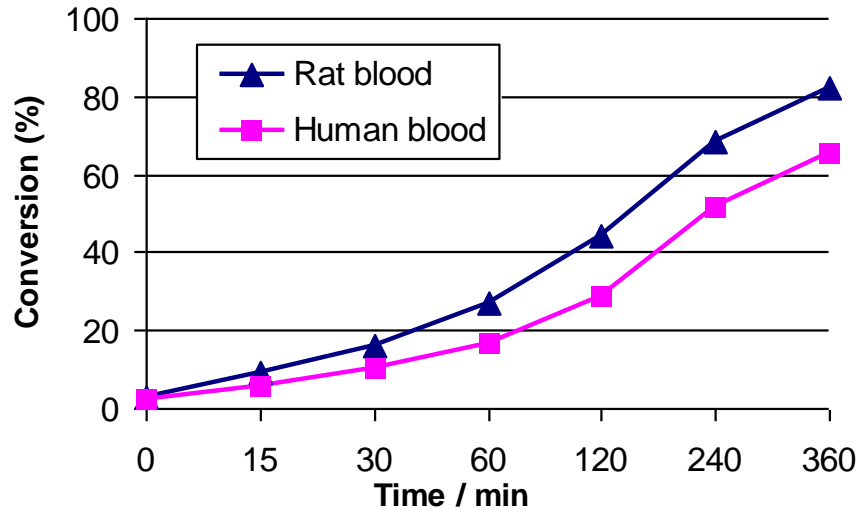


- Conventional analysis implies working on ice.
- What about DBS?

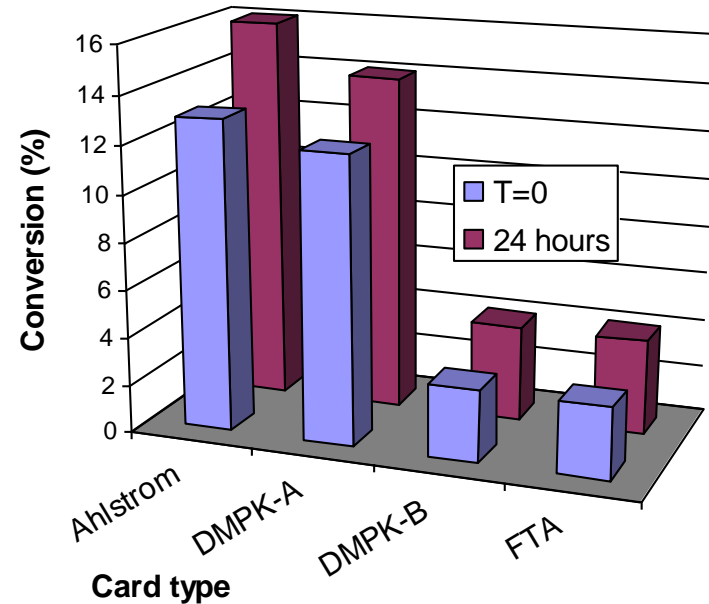
Stability in blood versus DBS



Stability of Valganciclovir



DBS Stability in rat blood

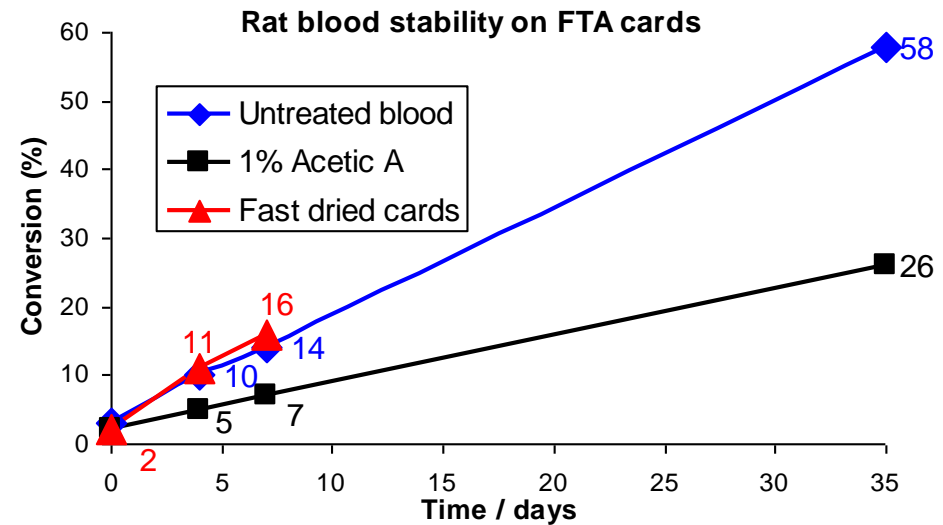
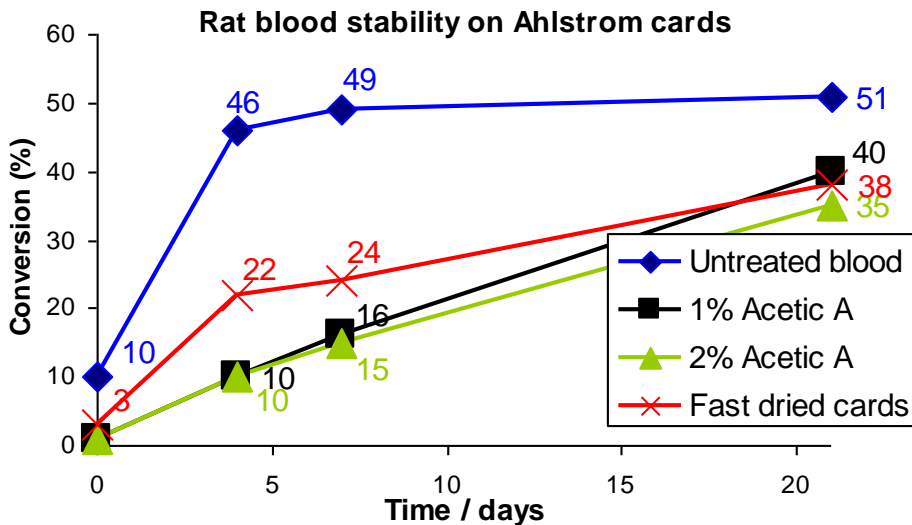


- Valganciclovir is not stable in whole blood.
- Not stable on Ahlstrom & DMPK-A.
- Good stability in FTA Elute & DMPK-B during 24h.
- Treated cards clearly improve short-term stability.

Long-term stability



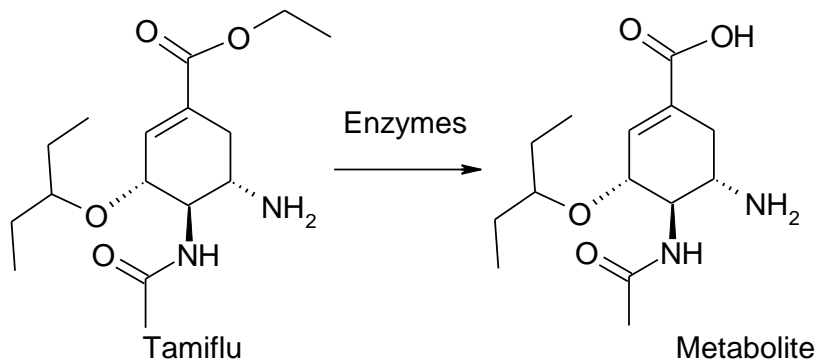
- Valganciclovir is not stable over a long term on treated or untreated cards in rat blood.
- Do “fast drying” (cool air stream) or additives improve stability?
- Either the addition of acid or speeding-up the drying process reduces the instability of the pro-drug in a long-term.
- Nevertheless the turn-over remains significant due to chemical hydrolysis.



Tamiflu



- Tamiflu, an ethyl ester pro-drug, degrades rapidly into its carboxylic metabolite in rat blood.

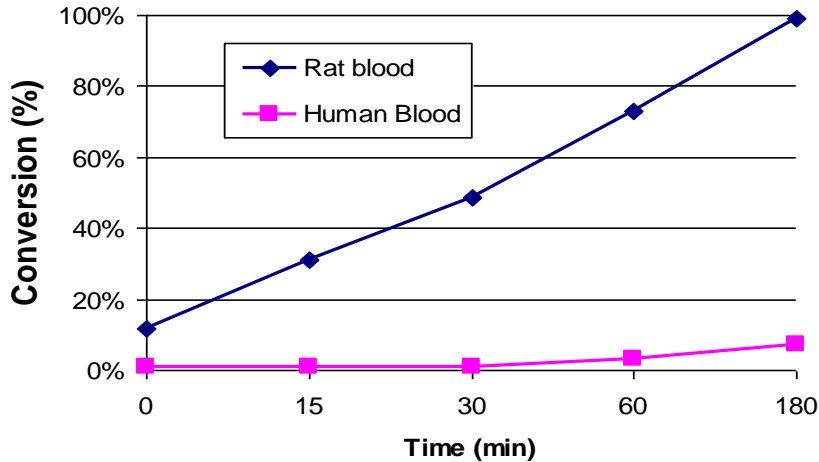


- Conventional analysis needs the addition of dichlorvos.
- Is DBS a solution?

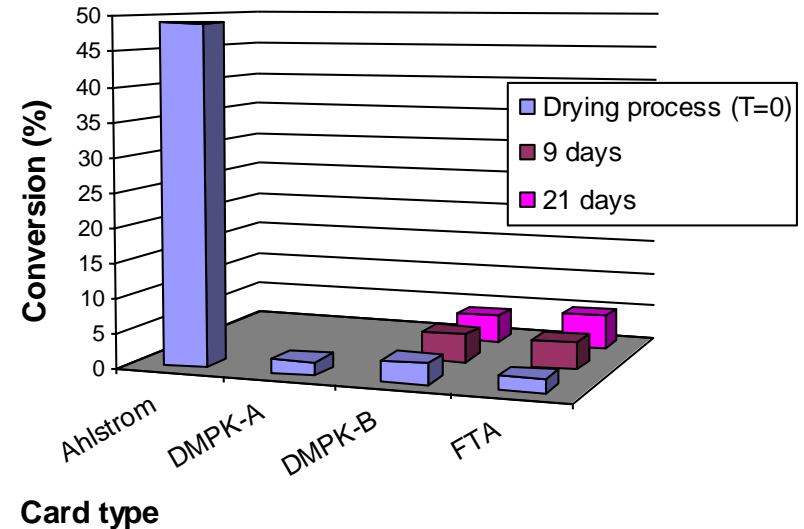
Stability in blood versus DBS



Stability of Tamiflu



Short & long term DBS stability in rat blood

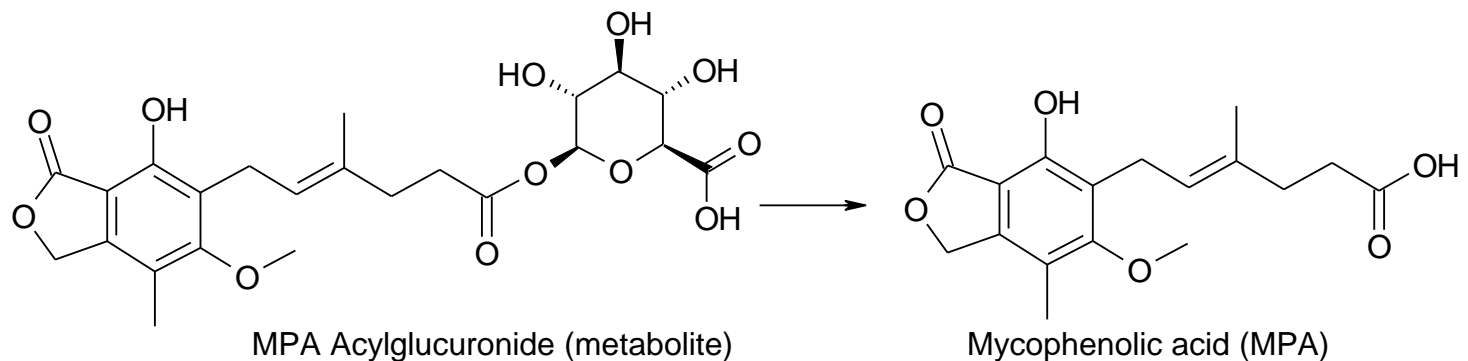


- Tamiflu is not stable in rat blood.
- Not stable on Ahlstrom. Good stability during the drying process on FTA Elute & DMPK-B & A.
- Tamiflu remains stable in FTA & DMPK-B cards for at least 3 weeks, less than 5% conversion.

MPA Acyl-glucuronide

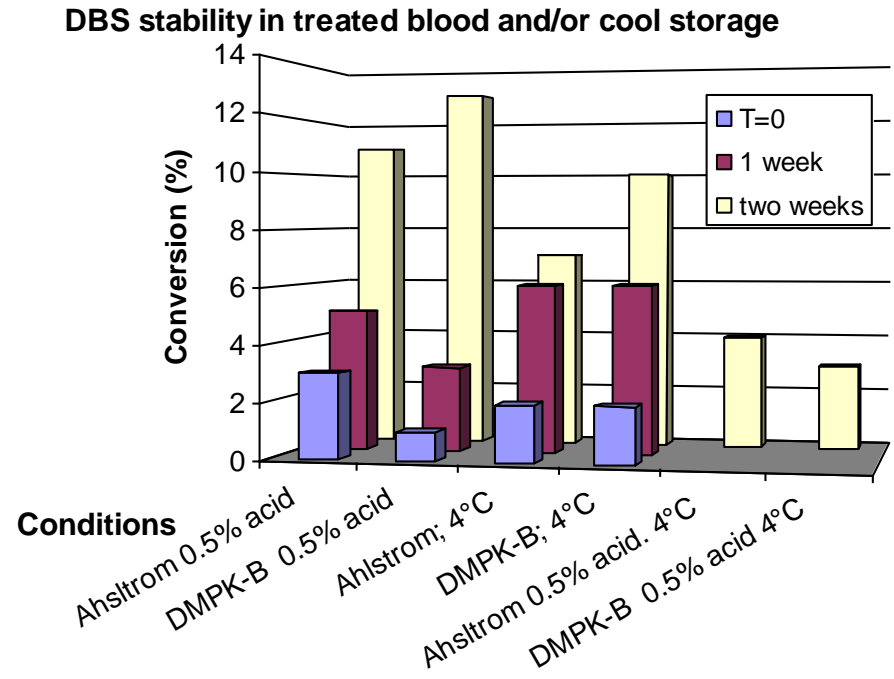
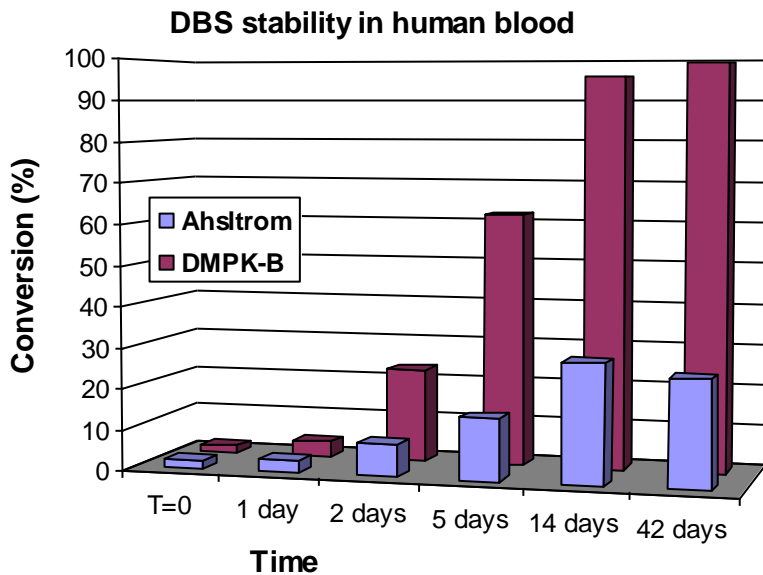


- Acyl-glucuronide metabolite converts back into the parent drug MPA. Critical when the metabolite is formed above 15% of MPA.
- Cooling the samples or the addition of acid is necessary to avoid its back –conversion to MPA.



Stability in blood versus DBS

- The metabolite is unstable in human blood. After 15 min the response decreases by 12%.
- On DBS it is stable for only 1 day on treated cards and up to 5 days on plain cards.
- Significant instability after 6 weeks: 25% conversion on untreated and almost complete conversion on treated cards.

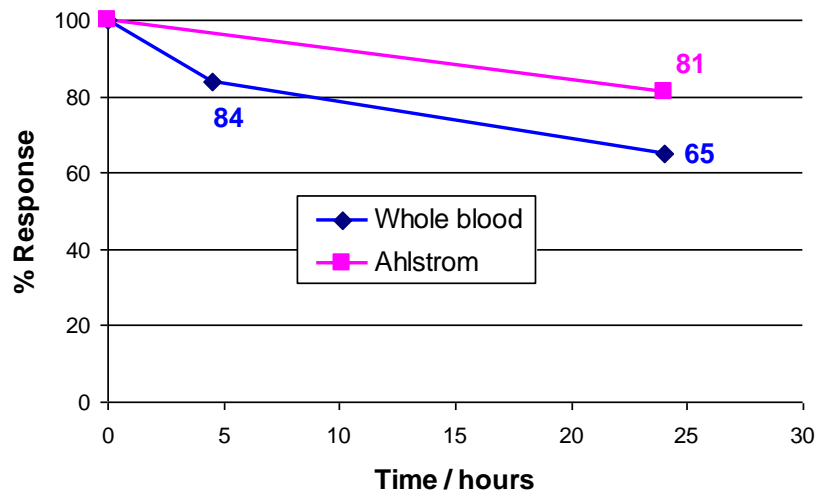


Reactive drug

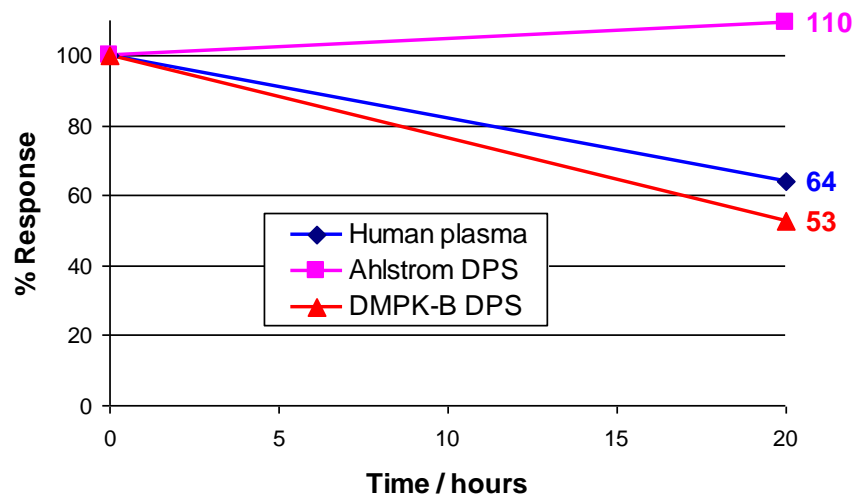


- The hydrolysis of a dihydropyrimidinone drug is studied in human / mouse blood and in human plasma
- Not stable in mouse blood, stable in DBS?
- Stable in human blood but not in plasma, stable in DPS?

Stability in mouse blood versus DBS



Stability in human plasma versus DPS



✓ Stability is enhanced on Ahlstrom cards.

✓ Stable on untreated cards.

✓ Unstable on treated cards.

Conclusions

- DBS technique generally improves stability of compounds over a short term. A long-term stability was proven to be compound dependant.
- Stability of reactive compounds cannot be taken for granted on DBS and each case should be studied independently.
- Recommendations in case of stability issues: select appropriated card type, use fast drying and consider low temperature storage or the addition of stabilization agents.
- The coating of treated cards may stop enzymatic activity but may not stop chemical degradation.
- Fast drying and additives improves stability, but does it nullify DBS advantages?

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