



Evaluation of Analyte Stability on Dried Blood Spot Cards

Authors

Suraj Dhungana, Michael S. Allen, David Minser, Robert S. Friley and D. Craig Sykes

Tandem Labs RTP

Abstract

The recent recognition of dried blood spot (DBS) technology, as an alternative to plasma as the matrix for systemic measurement of drug analytes in pharmacokinetic (PK) and toxicokinetic (TK) studies, has been fueled by a multitude of advantages DBS brings to an animal or clinical study. These advantages include: small sample volume, reduced number of study animals, sample collection ease, reduced sample shipping and storage costs, and analyte matrix stability. Most of these advantages are obvious results of small sample volume, while other advantages such as analyte matrix stability need further investigation before they can be fully recognized. Chemically coated DMPK sample collection cards are designed to inactivate enzymes in blood, which in turn is postulated to aid analyte stability for compounds susceptible to enzymatic degradation. However, the effectiveness of this stabilization remains to be investigated. The stability of two ester compounds, procaine and acetylsalicylic acid (Aspirin) is investigated on three different DBS sample collection cards (chemically coated DMPK A and DMPK B cards and uncoated Ahlstrom 226 card) using HPLC-MS/MS. The stability of these compounds on DBS cards was directly compared with their stability in K2EDTA human whole blood, both in the presence and in the absence of two chemical stabilizers, paraoxon and sodium fluoride (NaF). Similarly, stability of an acyl-glucuronide metabolite is evaluated on three different DBS sample collection cards. Our investigations suggest analyte stability on the DBS cards is compound specific, while the drying of the blood on the DBS sample collection card appears to be critical for analyte stabilization.



Introduction

Dried blood spot (DBS) has long been the preferred technique for the screening of newborn metabolic defects. The requirement of a very small sample volume has made DBS ideal for pediatric studies and with more recent development of sensitive mass spectrometers, has gained significant interest as a potentially powerful tool for quantitative analysis of small molecules for pharmaceutical drug development and toxicology. DBS is an attractive alternative to the plasma, the gold standard matrix for evaluating of pharmacokinetic studies or therapeutic drug monitoring, because of its multiple advantages.

Small sample volume (10-20 μ L) and the drying of the collected samples give DBS several advantages over the traditional plasma method. Small sample volume translates into reduced number of animals per study, ability to collect serial time points from a single animal, and ultimately, a reduction in the cost of a given animal study. For clinical studies, small sample volume and simple non-invasive sample collection techniques, like the finger prick, can potentially improve subject recruitment and retention. Once dry, the collected samples can be stored and shipped at ambient atmospheric conditions, which dramatically reduces processing, refrigeration and shipping costs. Certain coated sample collection cards, such as DMPK A and B cards, can lyse bacterial cells and deactivate virus and reduce health hazards.

In the process of inactivating bacteria and viruses, the coated DBS cards are also meant to inactivate the enzymes present in the blood and stop or slow down the enzymatic degradation of drug analytes. Drug analytes with ester functionality are particularly prone to ester-hydrolysis, both enzymatic and/or chemical. Prevention of such hydrolysis is critical to an accurate measurement of the drug and the robustness of a given bioanalytical assay. Similarly, metabolites such as acyl-glucuronide are susceptible to hydrolysis and need special consideration during analytical method development. An ability to prevent the hydrolysis of such analytes or metabolites with the use of DBS technology could be significant. Here we have investigated the stability to two ester compounds with different mechanisms of hydrolysis, procaine and acetylsalicylic acid (Aspirin), as well as an acyl-glucuronide metabolite on three different DBS cards. The hydrolysis of Aspirin is both enzymatic and non-enzymatic, while procaine hydrolysis primarily due to the action of butyrylcholinesterase. A comparative stability evaluation of multiple compounds with different hydrolysis mechanism has allowed for the identification of factors that contribute to the analyte stability on a DBS card over the whole blood.



Methods

SAMPLE PREPARATION

Analyte: Procaine, Acetylsalicylic acid (Aspirin), and EA913-acyl-glucuronide

Stability samples were prepared by spiking each of the analytes in fresh K₂EDTA human whole blood and gently mixing for 1 min.

Blood spots were generated on three different types of sample collection cards

- 1) DMPK-A card (GE Healthcare)
 - 2) DMPK-B card (GE Healthcare)
 - 3) Ahlstrom 226 card (ID Biological Systems)
- 5 uL of blood was used to generate each blood spot
 - Samples were taken from the DBS cards at 0, 1, 2, 4, 6, and 22 hrs.
 - At each time point entire blood spot was punched out using a 6 mm disc puncher for analysis
 - All blood spots were extracted with 100 uL 80:20 Acetonitrile: Water

Samples with stabilizer were prepared by first spiking the stabilizer to the fresh K₂EDTA human whole blood and then spiking each of the analytes into blood and gently mixing for 1 min.

- 5 uL of spiked blood was added to 100 uL 80:20 Acetonitrile:Water and extracted at 0, 1, 2, 4, 6, and 22 hrs

Extraction was achieved by vortexing the samples for 30 secs.

Extracts were centrifuged at 16,000 RCF for 4 mins and 4X diluted with HPLC grade water then transferred into autosampler vials for LC-MS/MS analysis

SAMPLE ANALYSIS

- Column: Phenomenex Synergi 4u Polar-RP (50x2.0 mm), Varian Monochrome CN (50x2.0 mm)
- Elution: Gradient flow, Mobile Phase A: 5 mM Ammonium Formate with 0.01% Formic Acid, Mobile Phase B: Acetonitrile

Or

Mobile Phase A: Water with 0.1% Formic Acid, Mobile Phase B: Acetonitrile with 0.1% Formic Acid

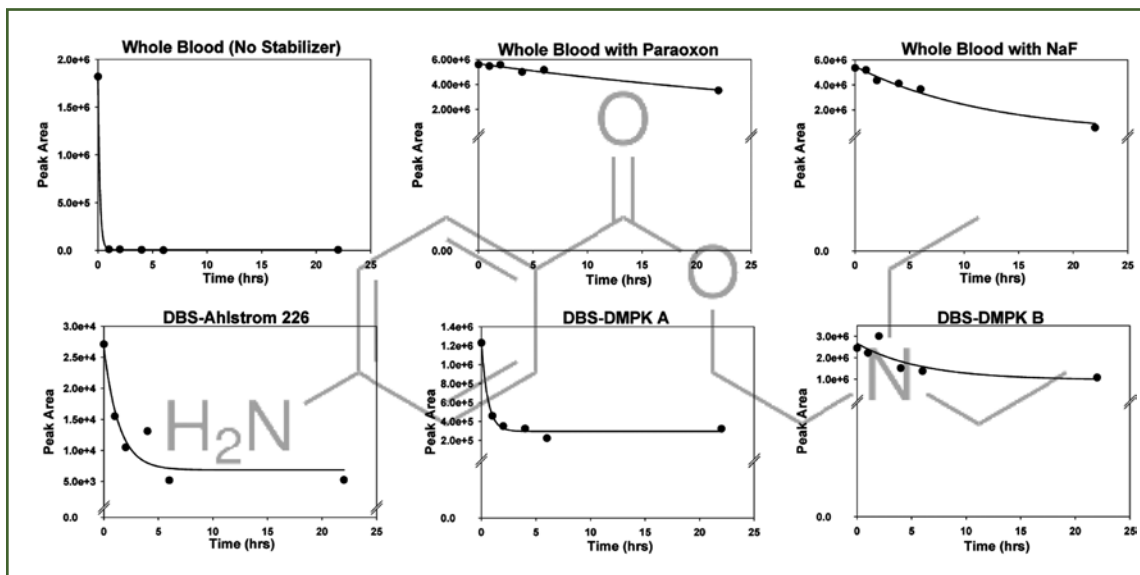
- Detector: Applied Biosystems/MDS Sciex API 4000 MS/MS

DATA ANALYSIS

- Chromatographic Peak Integrations: Applied Biosystems/MDS Sciex Analyst 1.4.2

Methods continued

PROCAINE STABILITY
Procaine hydrolysis is enzymatic

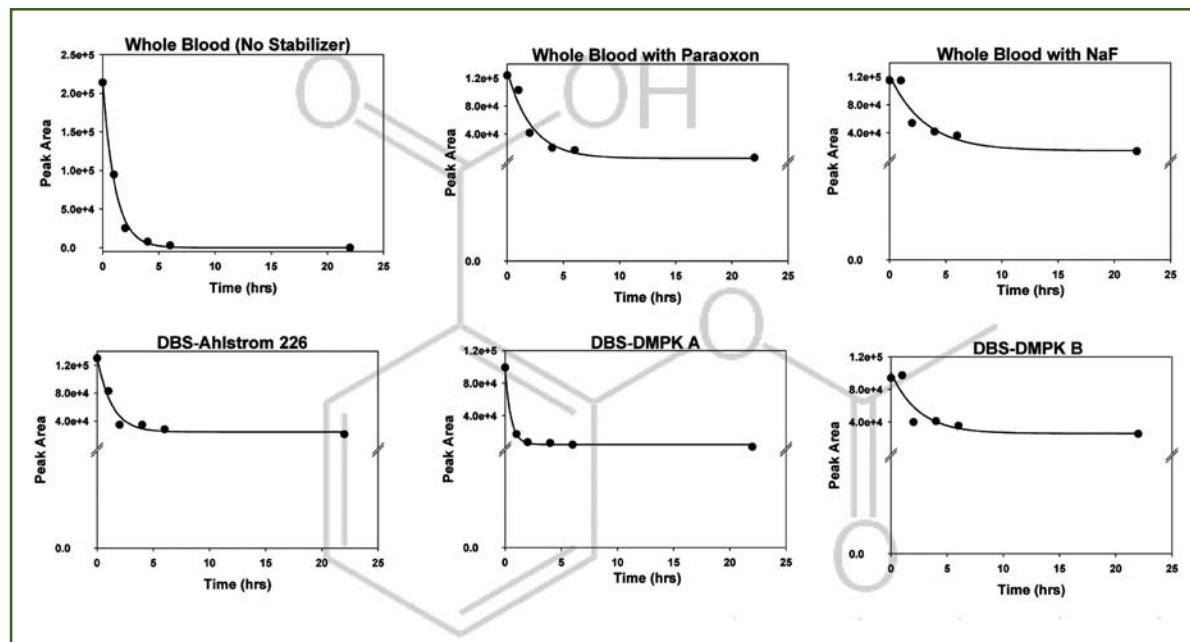


- Procaine undergoes enzymatic hydrolysis facilitated by butyrylcholinesterase
- Hydrolysis of procaine occurs rapidly in human whole blood
- Inhibitors, paraoxon and sodium fluoride significantly reduce the rate of procaine hydrolysis
- Procaine hydrolysis is reduced on DBS cards compared to that in the whole blood
- DMPK B card, a chemically coated card, is most effective in preserving procaine stability
- Uncoated card, Ahlstrom 226, also slows down procaine hydrolysis
- All cards show significant procaine stability after ~5 hrs from the time of blood spotting

Methods continued

ACETYLSALICYLIC ACID (ASPIRIN) STABILITY

Aspirin hydrolysis is both enzymatic and non-enzymatic



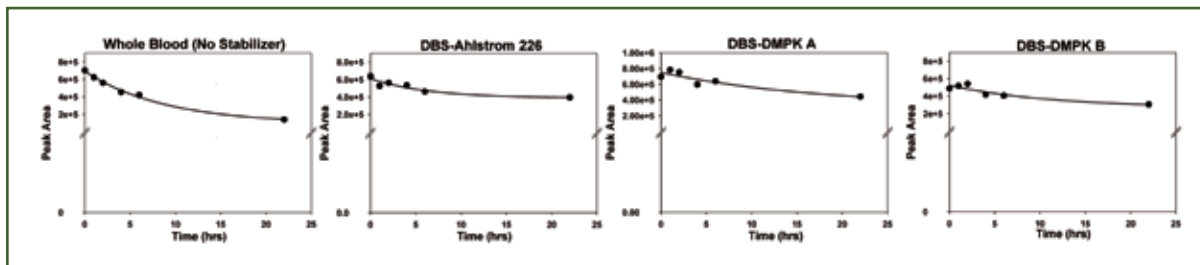
- Aspirin undergoes both enzymatic and non-enzymatic hydrolysis
- Ester-hydrolase inhibitors, paraoxon and sodium fluoride, are not very effective in eliminating hydrolysis
- Both chemically coated and uncoated cards showed similar aspirin hydrolysis profiles
- Approximately after 5 hrs from the time of blood spotting, analyte stability appeared to be maintained



Methods continued

ACYL-GLUCURONIDE STABILITY

Acyl-glucuronide hydrolysis is β -glucuronidase dependent or nonspecific



- Acyl-glucuronide hydrolysis is β -glucuronidase dependent or it can undergo non-enzymatic hydrolysis
- Hydrolysis of ENT913-acyl-glucuronide was observed in human whole blood and on Dried Blood Spots
- The rate of ENT913-acyl-glucuronide decomposition on DBS card showed some improvement over that in whole blood
- The gradual decomposition of ENT913-acyl-glucuronide continued on DBS card even after sample drying

FIRST ORDER RATES CONSTANTS FOR ANALYTE LOSS UNDER DIFFERENT CONDITIONS

Condition	Procaine	Aspirin	EA913 acyl-glucuronide
	$k (s^{-1}) \times 10^{-3}$	$k (s^{-1}) \times 10^{-4}$	$k (s^{-1}) \times 10^{-5}$
Whole Blood (No Stabilizer)	2	3	3
Whole Blood with Paraoxon	0.006	1	--
Whole Blood with NaF	0.02	0.7	--
DBS-Ahlstrom 226	0.2	1	0.5
DBS-DMPK A	0.5	5	0.7
DBS-DMPK B	0.05	1	0.7



Discussion

- DBS cards appear to aid the stability of ester compounds that undergo enzymatic ester-hydrolysis, eg. procaine.
- Procaine on DMPK B card is better preserved compared to that in DMPK A and uncoated Ahlstrom 226 card. Unlike aspirin, procaine primarily undergoes enzymatic hydrolysis, which suggests DMPK B card might be more effective in inactivating enzymes.
- All three DBS cards show a lag time of approximately 5 hrs before procaine stability is maintained. This lag-time most likely corresponds to the time required to attain complete dryness by the blood spots.
- One needs to be cognizant of the lag time required to attain stability on a DBS card, as it may not be apparent by the calibration curve and QC samples as they all will undergo the same rate of decomposition while drying.
- Short term (stability during drying of the blood spot) and long-term matrix stability need to be evaluated on DBS card before adopting this method for sample collection.
- For analytes and metabolites prone to hydrolysis, sample drying conditions of DBS cards most likely will be critical to data integrity.
- Both chemically coated cards and the uncoated card were able to slow down and further prevent procaine hydrolysis compared to the whole blood. This ability to stabilize ester by an uncoated Ahlstrom 226 card suggests that the change in the matrix state (going from liquid to DBS) also helps maintain the analyte stability.
- The stability of aspirin and ENT913-acyl-glucuronide was marginally preserved on DBS cards. This suggests that analyte stability on the DBS is not guaranteed and needs to be evaluated on a case by case basis.
- Combined use of chemical stabilizers along with DBS may potentially yield optimal condition to maximize analyte stability.



Reference

Guthrie, R.; Susi, A., *Pediatrics*, 1963, 32, 338-343.

Mei, J. V.; Alexander, J.R.; Adam, D. W.; Hannon, W.H., *J. Nutr.*, 2001, 131, 1631S.

Spooner, N.; Lad, R.; Barfield, M., *Anal. Chem.*, 2009, 81, 1557.

Barfield, M.; Spooner, N.; Lad, R.; Parry, S.; Fowles, S., *J. Chromatogr. B.*, 2008, 870, 32.

Liang, X.; Li, Y.; Barfield, M.; Ji, Q. C., *J. Chromatogr. B.*, 2009, 877, 799.

Williams, F. M., *Clin Pharmacokinet.*, 1985, 10, 392.

Jewell, C.; Ackermann, C.; Payne, N.A.; Fate, G.; Voorman, R.; Williams, F.M., *Drug Metab Dispos.*, 35, 2015.

Klimes, J.; Sochor, J.; Zahradníček, M.; Sedláček, J.,
J Chromatogr. 1992, 584, 221.

Acknowledgement

We thank ID Biological Systems for providing the Ahlstrom 226 Specimen Collection paper for bioanalysis.