ASMS 2013

WP-291

Determination of Metabolically Unstable Drugs in Blood by Heat-Stabilized DBS and LC-MS/MS

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Introduction

Objective

To investigate drug stability prior to, during and after blood spot sampling, using procaine, prulofloxacine, esmolol, and acetylsalisylic acid as examples of drug compounds with limited stability.

To compare conventional DBS sampling procedure with DBS sampling including heat stabilization.

Stabilizor™ T1 (Denator AB)
Instrument for heat stabilization of biological samples, used together with newly designed sample cards; Maintainor DBS; for treatment of blood spots.

Experimental

Preparation of blood spots

Whole blood kept at room temperature was spiked with drugs at a concentration of 1-10 ng/mL.

Blood was mixed for two minutes before any sampling was started. Blood spots (20 µL) were placed on Ahlstrom grade 226 filter, heat treated for 30s @ 95 C or not heat treated.

Heat treatment using Stabilizor T1 (Denator) set at 95 °C, for 30 s. Spotted cards were kept in open air or placed in plastic bags with/without silica gel for drying. Samples were analyzed same day or after ten day's storage at room temperature

Experimental

DBS sample extraction and cleanup:

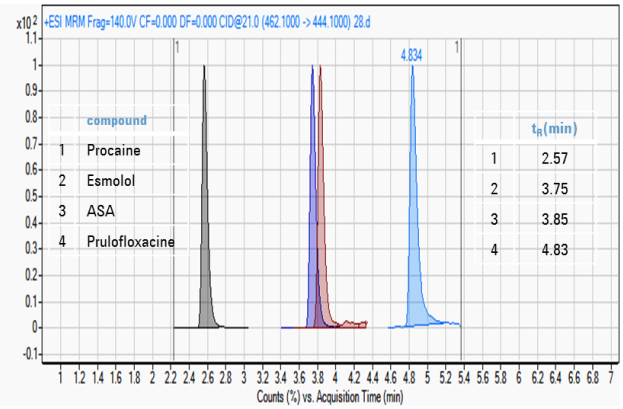
Whole spot removed for analysis using 1.0 mL extraction solvent (25% acetonitrile, 0.1% formic acid). Vortex mixing before and after 20 min sonication. Ultrafiltration 30 KDa (Amicon Ultra, Millipore). 3 µL for LC/MS analysis

LC/MS analysis

Model 1290 UHPLC and 6460 triple quad MS with Jet Stream ion source (Agilent Technologies). LC column: Atlantis T3 C18, 2.1x100mm, 3µm (Waters). Gradient elution: 10mM ammonium formate, 0.1% formic acid, 0-100% MeOH (0.4 mL/min).

Optimization of MRM conditions using MassHunter Optimizer B.06.00 (Agilent). Data processing using MassHunter B.05.02 software (Agilent). Calibration samples did not contain biological sample matrix. Correlation coefficient r^2

>0.998. Matrix effect on ionization was assessed for each analyte and for stabilized/not-stabilized blood spots by spiking blank extracts and was used to calculate final concentrations for DBS samples.



MRM traces from DBS sample analyzed by LC/MS

Results and Discussion

Esmolol

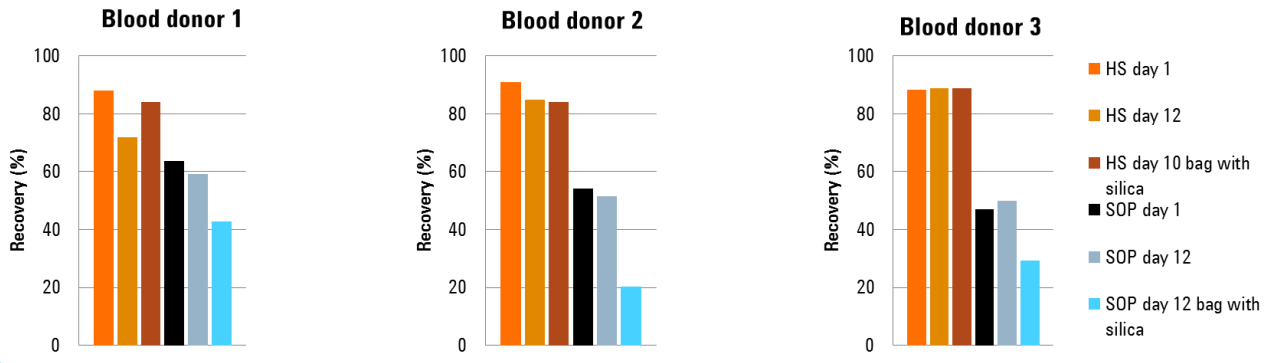


Figure 1
Recoveries* (mean, n=4) of esmolol from dried blood samples analyzed same day or after 12 days of storage. HS: heat treated. SOP: no heat treatment. Blood spot samples were allowed to dry in ambient air or in plastic bags with silica

Findings
- Heat stabilization provided high recoveries
- No stabilization => significantly lower recoveries
- Drying blood spot samples in plastic bag with silica was OK for HS samples but not for SOP samples

* Analytical recoveries may be affected by:
- Compound degradation prior to spotting, during drying and during storage
- Incomplete extraction of the drug from the DBS sample

Prulifloxacin

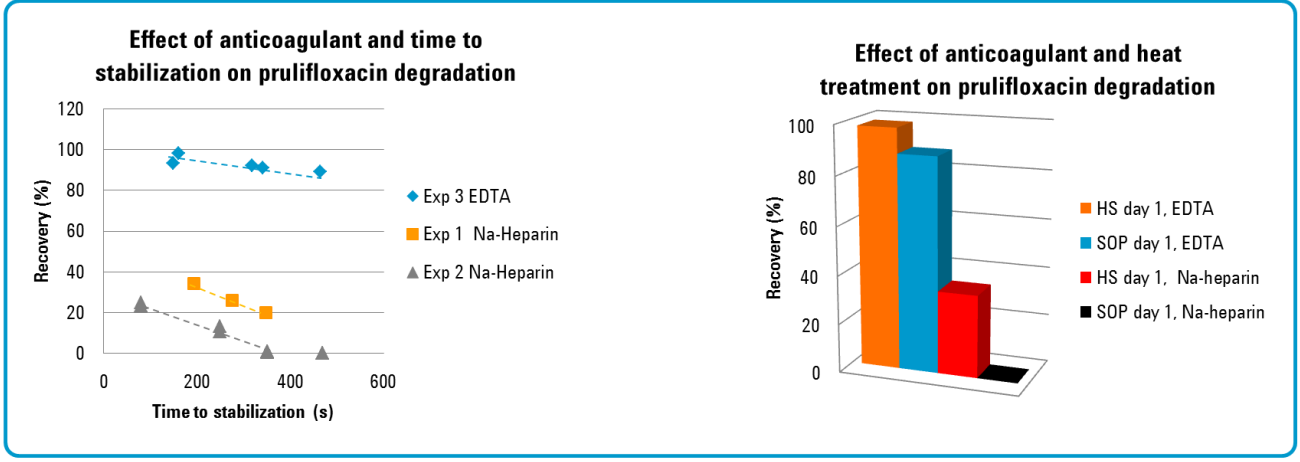


Figure 2, left
Recoveries of prulofloxacin in three different experiments (different blood donors). Samples were heat stabilized at different times after spiking blood

Figure 2, right
Recoveries of prolifluxacin in two different experiments, with either Na-heparin or EDTA blood

Findings
- Rapid compound degradation in Na-heparin blood
- Slow degradation in EDTA blood
- Heat treatment stopped degradation

Results and Discussion

Procaine

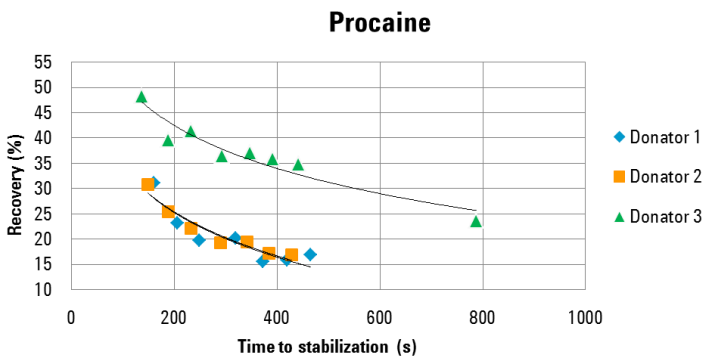


Figure 3, left
Recovery of procaine from blood stabilized at different time points after spiking blood from three different donors

Figure 3, right
Recovery of procaine from spot samples placed directly in plastic bags without drying agent

Findings
- Degradation rate varied between blood donors
- Heat treatment stopped degradation
- Degradation continued in SOP samples which were not allowed to dry in open air

Acetylsalicylic acid (ASA)

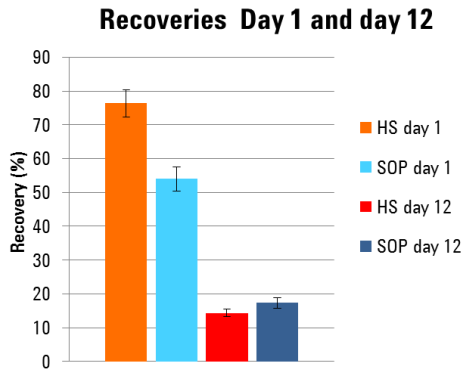


Figure 4
Recoveries of ASA from blood spot samples from three different donors. Mean recoveries (n=4) was calculated for each donor. As well as standard deviations between these mean values.

Findings
- When samples were analyzed same day, heat stabilization gave higher recovery
- When samples were analyzed after 12 day's storage, recoveries were low and similar with/without heat treatment, indicating non-enzymatic degradation

Conclusions

- Heat stabilization can be used to stop enzymatic degradation which may occur during the drying of samples on DBS cards
- Heat stabilization can provide information on whether losses of analyte are due to enzymatic degradation or due to other, non-enzymatic processes