Heat stabilization can be used to stop enzymatic degradation. No stabilization leads to significantly lower recoveries. Slow degradation in EDTA blood can be observed.

Heat treatment using Stabilizer T1 (Denator AB, Sweden) killed DBS spots in open air or in plastic bags with no silica gel for drying. Samples were kept at 20°C for 2 days before storage.

DBS stabilization can provide information on whether enzymatic degradation prior to spotting, during drying and during storage was stopped by heat treatment. This is important for the analysis of metabolically unstable drugs in blood.

Determination of metabolically unstable drugs in blood by heat-stabilized DBS and LC-MS/MS

Introductions

- Heat stabilization can be used to stop enzymatic degradation occurring during drying of DBS samples.
- Heat stabilization can provide information on whether or not enzymatic degradation is occurring under storage conditions.

Experimental

Preparation of blood spots
Whole blood kept at room temperature was applied to the spots at a concentration of 1.10 mg/ml.

Blood spots were mixed for two minutes before any sampling was started. Blood spots (30 µl) were placed on an Alumina grade 220 filter, heated treated for 264 ± 5°C or not heat treated.

Heat treatment using Stabilizer T1 (Denator AB, Sweden) killed DBS spots in open air or in plastic bags with no silica gel for drying. Samples were analyzed immediately after the last day's storage at room temperature.

DBS sample extraction and cleanup:
Whole DBS spots taken for analysis 1 h after extraction contained 2.0% (w/w) acetylsalicylic acid. Weekly mixing before and after 24 min incubation. Ultrapure ethanol 99.5% (Amresco Ultra, USA) was used for LC-MS analysis.

LC/MS analysis
Model 1206 UPLC and 6430 triple quadrupole MS (Agilent Technologies) LC/MS Atlas. T3 C18, 2.1x10mm, 3µm (Waters). Gradient elution: 100% aceCN, (10% acetic acid, 90% 0.1% formic acid) to 100% aceCN (3.4 min./run).

Optimization of MRM conditions using MassHunter Optimizer 4.0 (Agilent). Data processing using MassHunter Qualitative software (Agilent). Calibration samples did not contain biological matrix samples. Correlation coefficients r > 0.98. Method effect on spiked samples was assessed for each analyte and for stabilized/reconstituted blood spots by spiking blank extracts and samples used to calculate final concentrations for DBS samples.

Results and Discussion

Esmolol

- First experiment 3 donors, 4 samples from each. Donors were heated at different times after spiking blood.
- Donors were heated for 2 days storage in bags with or without heat stabilization.
- Donors were heated at different temperatures. Heat stabilization can provide information on whether enzymatic degradation prior to spotting, during drying and during storage was stopped by heat treatment.
- Donors were heated at different temperatures. Heat stabilization can provide information on whether enzymatic degradation prior to spotting, during drying and during storage was stopped by heat treatment.

Propranolol

- Second experiment 3 donors, 4 samples from each.
- Donors were heated for 2 days storage in bags with or without heat stabilization.
- Donors were heated at different temperatures. Heat stabilization can provide information on whether enzymatic degradation prior to spotting, during drying and during storage was stopped by heat treatment.
- Donors were heated at different temperatures. Heat stabilization can provide information on whether enzymatic degradation prior to spotting, during drying and during storage was stopped by heat treatment.

Acetylsalicylic acid (ASA)

- Third experiment 3 donors, 4 samples from each.
- Donors were heated for 2 days storage in bags with or without heat stabilization.
- Donors were heated at different temperatures. Heat stabilization can provide information on whether enzymatic degradation prior to spotting, during drying and during storage was stopped by heat treatment.
- Donors were heated at different temperatures. Heat stabilization can provide information on whether enzymatic degradation prior to spotting, during drying and during storage was stopped by heat treatment.