

Salbutamol Assay for Micro-volume Samples of Blood using GC-MS



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Salbutamol Screening

Salbutamol is a medication to relief symptoms of asthma, bronchospasm and chronic obstructive pulmonary disease (COPD).



- First line therapy: inhalation.
- Next step: intravenous administration.
- Excessive administration: lactic acidosis, which can precipitate respiratory failure¹.

At present there are insufficient data to recommend a dosage regime for children and extrapolation of data from one age group to another is recognized to be unsafe².

- Research gap: dose finding studies to determine optimal doses for paediatric asthma applications.
- Challenge: measuring blood concentrations of a drug in children.
- Research aim: development of a novel assay to detect and quantify salbutamol from micro-volumes of blood.

Blood Collection

- Dried Blood Spots (DBS): spotting and drying micro-volumes blood onto filter paper³.
- Sampling issues using filter paper: haematocrit inhomogeneity and unknown sample volumes.
- Comparison with another collection device: Volumetric Absorptive MicroSampling (VAMS), also named Mitra⁴.



- Research samples: 10 µl blood samples spiked with salbutamol, left to dry, extracted with methanol, spiked with d3-salbutamol as internal standard, evaporated to dryness, derivatised with BSTFA with 1% TMCS.
- Concentration range: therapeutic levels (10 to 20 ng/ml) to toxic levels (100 to 150 ng/ml).

Assay Performance

- Limit of detection (LOD): 5 ng/ml (below the therapeutic range)
- Accuracy and precision: <20% for LOD samples and <15% for other samples, meeting the guidelines for bioanalytical method validation of the United States Food and Drug Administration (US FDA)⁵.
- Recovery: Mitra tip samples result in chromatograms with less interfering peaks compared to DBS samples (Fig 1).
- Stability: DBS samples show a decreasing recovery with increasing drying time compared to Mitra tip samples (Fig 2).

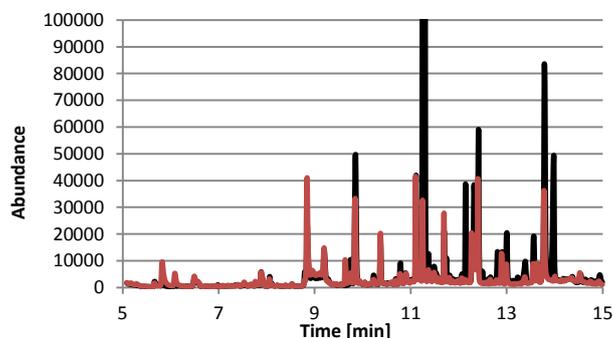


Figure 1: Chromatogram (m/z 369) of 20 ng/ml salbutamol recovered from DBS (black) and from blood sampled with Mitra tips (red).

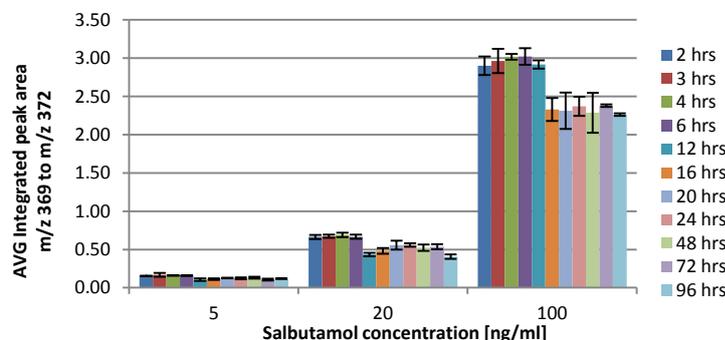


Figure 2: Decreasing salbutamol recovery from DBS with increasing drying/storage times.

Conclusion

- An assay to detect and quantify salbutamol from micro-volumes of blood has been developed, optimized and validated according to US FDA guidelines, especially valuable for paediatric asthma applications.
- Two blood collection devices were tested and compared for this study; the novel developed VAMS devices have sampling, stability and analysis advantages compared to the conventional filter cards used for DBS research and applications.
- As a final method evaluation, three healthy volunteers will be administered with 1 mg of salbutamol via a 100 µg per dose inhaler and spacer in the near future.

References

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