Dried Matrix Spot Analysis – A Novel Dye Technology to Analyze Drugs from Clear Fluids: Tobramycin from Tears

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Overview
- **Purpose** – Develop a dried matrix spot (DMS) extraction method and an HPLC/MS/MS method to determine concentrations of Tobramycin from tears
- **Methods** – DMS methanol extraction and HPLC/MS/MS (API4000)
- **Results** – Range from 0.5 to 15 µg/mL with accuracies and precision better than ± 15%

Introduction
The analysis of dry blood spots (DBS) for the determination of drug concentrations in whole blood is well known.1,2 Recently, novel methods for visual indication of clear fluids (e.g. synovial fluid and CSF) on the collection paper using the DBS technique have been developed.3 This novel technique is called dried matrix spot (DMS) analysis.

The desire of researchers to obtain distribution data from multiple compartments continues to expand the application of DMS to other fluids such as tears and dialysate. Additionally, research has suggested that using other compartments such as tears can be an alternative for therapeutic drug monitoring.4 However, the collection of tears can be problematic due to the limited volume of the fluid and difficulty with sampling into a tube or vessel. Dried matrix spot analysis offers an attractive alternative to the collection and analysis of tears and other clear fluids. As an example for analysis of clear fluids, here we report on a novel DMS preparation method coupled with LC/MS/MS to provide an accurate and precise assay for the determination of Tobramycin from human tears.

Methods
**Extraction**
- Tobramycin extracted from human tears using DMS procedure
- Card type: FTA DMPK-C (GE Healthcare)
- Used proprietary color-indicating technology developed at Alturas Analytics, Inc. to visualize spot (See Figure 1.)
- 15 µL sample volume
- Punch diameter: 6 mm
- Solvent 10% TCA in water

**HPLC**
- Gradient HPLC using acetonitrile and water with 0.05% HFBA
- Flow rate = 0.5 mL/minute
- HSC18 2.1x50 mm (Supelco)

**Mass Spectrometry**
- Sciex API4000 operating in MRM mode
- ESI
- Positive ion mode
- MRM analysis for Tobramycin

**Results**
- Range from 0.5 to 15 µg/mL with accuracies and precision better than ± 15%

Conclusions
- As an example of the applications of DMS, we developed a novel DMS analysis procedure coupled with HPLC/MS/MS to quantify Tobramycin in human tears
- To date this DMS technique has been used to quantify several compounds in many different fluids
- More research continues with applications of DMS to highly aqueous fluids where non-specific binding occurs such as urine and in-vitro fluids.
- Additional applications include therapeutic drug monitoring where simple yet standard collection techniques are required

References