

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

Chad D. Christianson, Casey J. Johnson, Chrystal N. Sheaff, Derek F. Laine, Jennifer S.D. Zimmer, Shane R. Needham; Alturas Analytics, Inc., Moscow, Idaho

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

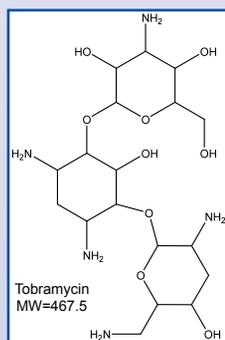
The analysis of dry blood spots (DBS) for the determination of drug concentrations in whole blood is well known.^{1,2} Recently, novel methods for visual indication of clear fluids (e.g. synovial fluid and CSF) on the collection paper using the DBS technique have been developed.³ This novel technique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

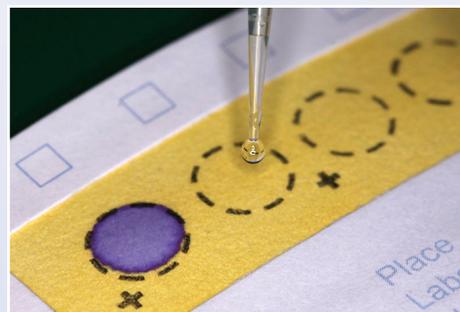
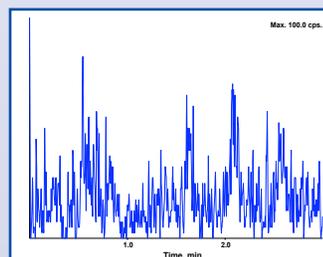
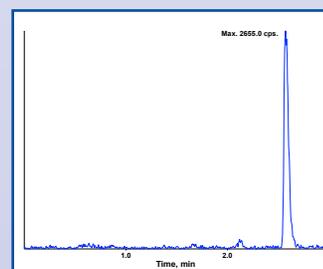


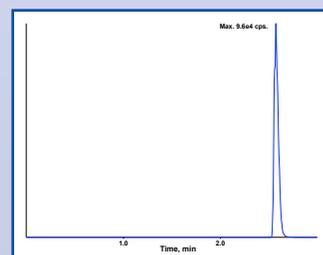
Figure 1. Picture of DMS Analysis of Tears on a FTA DMPK-C Card (GE Healthcare) with Addition of Proprietary Color-Indicating Technology Developed at Alturas Analytics.



HPLC/MS/MS Chromatogram from the Analysis of a Blank Tears Sample Using the DMS Technique.



HPLC/MS/MS Chromatogram from the Analysis of a Tears Sample Fortified with 0.5 µg/mL (LLOQ) Tobramycin Using the DMS Technique.



HPLC/MS/MS Chromatogram from the Analysis of a Tears Sample Fortified with 15 µg/mL (ULOQ) Tobramycin Using the DMS Technique.

Table 1. QC Results for the HPLC/MS/MS Analysis of Tobramycin from Human Tears Using a Novel DMS Technique.

QC Level (µg/mL)	Assay Accuracy and Precision (% ± %CV)	Matrix Factor
12	90.0 ± 4.5	NA
3.0	96.2 ± 0.07	NA
1.5	101 ± 5.7	0.97

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

1. Barfield, et. al. *J. Chromatogr B* 2008, 870: 32-37.
2. Spooner, et al. *Anal. Chem.* 2009, 81:1557-1563.
3. Needham, Christianson, et. al. *Bioanalysis*. November 2010
4. Dasgupta, *Clinica Chimica Acta*. 2007, 377: 1-13.