Dried Blood Spot Analysis – Utilizing the Technique to Develop Assays in Rare or Limited Matrices

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Overview

- **Purpose** – Develop a DBS extraction method and an HPLC/MS/MS method to determine concentrations of Indomethacin in human urine, human saliva, human CSF, human tears, and mini-pig synovial fluid
- **Methods** – DBS methanol extraction and HPLC/MS/MS (API5000)
- **Results** – Range from 1-500 ng/mL with accuracies and precision better than ± 15% using HPLC/MS/MS

Introduction

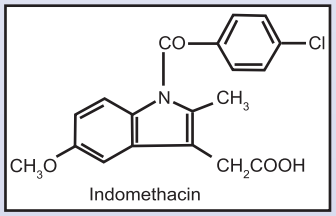
The analysis of dried blood spots (DBS) for the determination of drug concentrations in whole blood is well known.1,2 However, minimal work has been done to apply this technique for fluids other than whole blood. Traditional analyses on other fluids, such as synovial fluid, tears, or cerebral spinal fluid (CSF) have been cumbersome due to the limitation on sample volume. DBS techniques have provided an alternative collection and extraction method to traditional forms of analysis of low volume assays due to the much smaller volume requirement. Because collected samples are stored at ambient temperatures, this technique can be applied when studies are conducted in areas that do not have access to the low temperature freezers required to store traditional matrices.

Here we report on a simple DBS preparation method coupled with HPLC/MS/MS to provide an accurate and precise assay for the determination of Indomethacin from human urine, human saliva, human CSF, human tears, and mini-pig synovial fluid.

Methods

Extraction

- Indomethacin extracted from human urine, human saliva, human CSF, human tears, and mini-pig synovial fluid using a DBS extraction procedure
- Card type: FTA DMPK-C
- 15 µL sample volume
- Punch diameter: 3 mm
- Solvent: Methanol



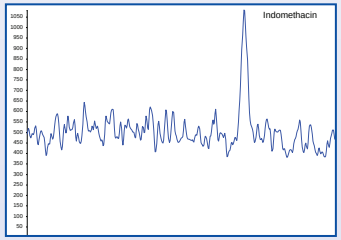
Indomethacin

HPLC

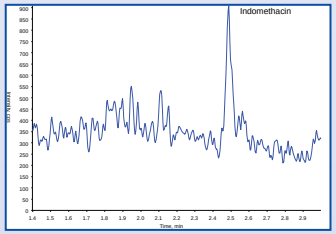
- Gradient HPLC using acetonitrile and water
- Flow rate = 0.7 mL/minute
- HSC18 2.1x50 mm (Supelco)
- Column heated to 50°C

Mass Spectrometry

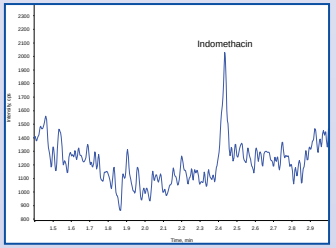
- Sciex API5000 operating in MRM mode
- ESI
- Negative ion mode
- MRM transitions for Indomethacin and Indomethacin 356.1 → 311.9



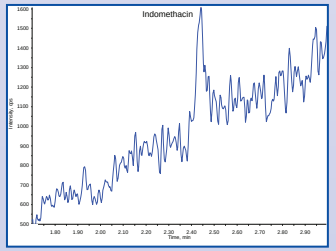
HPLC/MS/MS Chromatogram from the Analysis of Human Urine Fortified with 1.0 ng/mL of Indomethacin



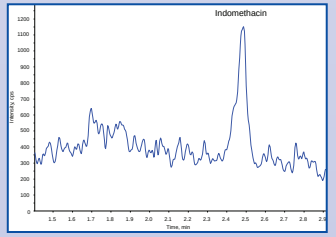
HPLC/MS/MS Chromatogram from the Analysis of Human Saliva Fortified with 1.0 ng/mL of Indomethacin



HPLC/MS/MS Chromatogram from the Analysis of Human CSF Fortified with 1.0 ng/mL of Indomethacin



HPLC/MS/MS Chromatogram from the Analysis of Human Tears Fortified with 1.0 ng/mL of Indomethacin



HPLC/MS/MS Chromatogram from the Analysis of Mini-Pig Synovial Fluid Fortified with 1.0 ng/mL of Indomethacin

Table 1. QC Results for the HPLC/MS/MS Analysis of Indomethacin in Human Urine, Human Saliva, Human CSF, Human Tears, and Mini-Pig Synovial Fluid.

QC Level (ng/mL)	Intra-assay Accuracy and Precision (% ± %CV)	Recovery (%)	Matrix Factor	IS Normalized Matrix Factor
Human Urine				
400	99.3 ± 6.3	118	NA	NA
25	102 ± 2.0	85.6	NA	NA
3	105 ± 8.7	77.0	0.80	1.0
Human Saliva				
400	104 ± 6.4	96.7	NA	NA
25	107 ± 6.6	80.1	NA	NA
3	96.9 ± 9.3	74.2	0.71	1.0
Human CSF				
400	104 ± 1.9	78.3	NA	NA
25	102 ± 3.6	74.8	NA	NA
3	98.7 ± 5.9	72.1	0.78	0.81
Human Tears				
400	114 ± 5.9	108	NA	NA
25	110 ± 2.5	84.8	NA	NA
3	109 ± 7.5	78.2	0.91	1.0
Mini-Pig Synovial Fluid				
400	90.5 ± 4.7	90.2	NA	NA
25	105 ± 4.4	120	NA	NA
3	94.1 ± 4.5	91.5	0.84	0.97

Conclusions

- Developed a DBS extraction procedure and an HPLC/MS/MS method to quantify Indomethacin in human urine, human saliva, human CSF, human tears, and mini-pig synovial fluid
- To date, the DBS technique has been used to quantify several compounds in many different fluids

References

1. Barfield, et. al. *J. Chromatogr B* 2008, 870:32-37.
2. Spooner, et al. *Anal. Chem*. 2009, 81:1557-1563.

Presented at ASMS Conference, Salt Lake City UT, May 2010