Dried Blood Spot Analysis: It's Not Just for Whole Blood Anymore

Chad Christianson, Shane Needham, and Derek Laine • Alturas Analytics, Inc.
Moscow, Idaho 83843

Overview

- **Purpose** - Develop a Dried Blood Spot (DBS) extraction method and an HPLC/MS/MS method to determine concentrations of Indomethacin in human urine, human saliva, 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 DBS for the determination of drug concentrations in whole blood is well known.[1,2] However, very little work has been done to apply this technique on fluids other than whole blood. Traditional analyses on other fluids, such as synovial fluid, have been cumbersome due to the limitation on sample volume. Other fluids, such as urine, often require freezing a large volume sample after collection.

DBS techniques have provided an alternative method to traditional forms of analysis of low volume assays, methods that require a large volume of sample collection/storage, or complex storage conditions not readily available at some sample collection sites.

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, and mini-pig synovial fluid.

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

QC Level (ng/mL)	Intra-assay Accuracy and Precision (% ± %CV)	Recovery (%)	Matrix Factor
Human Urine			
400	99.3 ± 6.3	118	NA
25.0	102 ± 2.0	85.6	NA
3.00	105 ± 8.7	77.0	0.80
Human Saliva			
400.0	104 ± 6.4	96.7	NA
25.0	107 ± 6.6	80.1	NA
3.00	96.9 ± 9.3	74.2	0.71
Mini-Pig Synovial Fluid			
400.0	90.5 ± 4.7	90.2	NA
25.0	105 ± 4.4	120	NA
3.00	94.1 ± 4.5	91.5	0.84

Methods

Extraction

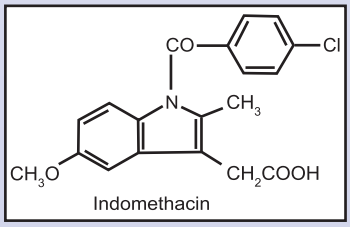
- Indomethacin extracted from human urine, human saliva, and mini-pig synovial fluid using a DBS extraction procedure
- Card type: FTA DMPK-C (GE Healthcare)
- Punch diameter: 3 mm
- Solvent: Methanol

HPLC

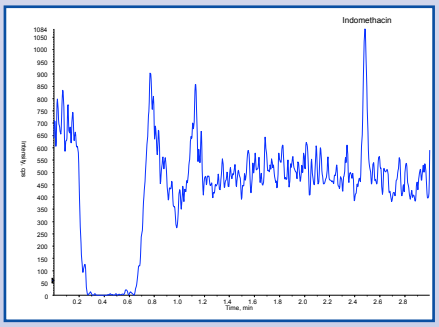
- Gradient HPLC using acetonitrile and water
- Flow rate = 0.7 mL/minute
- HSC18 2.1x50 mm (Supelco)
- Column heated to 50°C

Mass Spectrometry

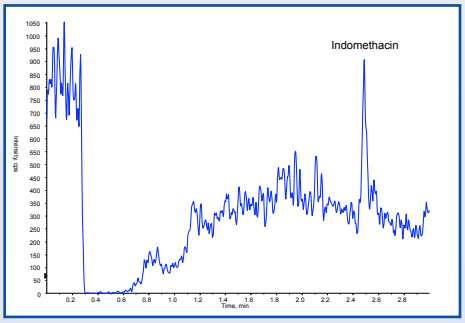
- Sciex API5000 operating in MRM mode
- ESI
- Negative ion mode
- MRM transitions for Indomethacin and Indomethacin d_4
  356.1 → 311.9
  360.2 → 315.9



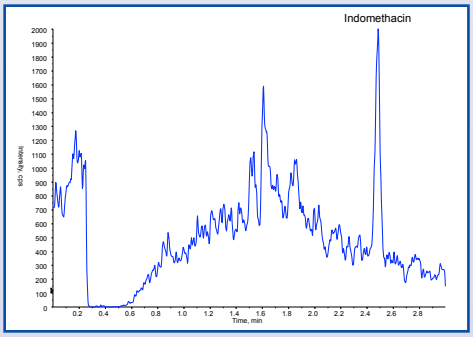
Indomethacin



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



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



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

Conclusions

- Developed a DBS extraction procedure and an HPLC/MS/MS method to quantify Indomethacin in human urine, human saliva, and mini-pig synovial fluid
- To date the DBS technique has been used to quantify several compounds in many different fluids
- LLOQ could be improved >3 times by using a 6 mm punch
- A full validation using a DBS technique in an alternative fluid is being conducted

References

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

Acknowledgements

Alturas Analytics, Inc. gratefully acknowledges GE Healthcare for their generous support and products.