Alturas Analytics
The LC-MS Experts

TECH NOTE

OVERVIEW

Purpose

Non-invasive matrices such as tears, sweat, saliva, and milk offer an enticing alternative to traditional sampling from blood, serum, and plasma. Collection of these matrices is simpler and more affordable than venipuncture. It is also much less unpleasant for patients and study subjects allowing for more frequent sampling, higher compliance, and removal of fear of needles as a barrier to clinical trial recruitment. However, bioanalysis of these matrices is not without challenges. Sample volumes can be very low, the more complex matrices such as milk may require extensive sample preparation, and the use of the matrix must be biologically relevant. Using the example of a method we developed at Alturas Analytics Inc. to measure Tobramycin from human tears, we will discuss practical applications of microsampling of non-invasive matrices for bioanalysis, and improvements offered by emerging technologies.

Objectives

- Understand the benefits and challenges of using non-invasive matrices in bioanalysis
- Explore currently available microsampling technologies and advances in analytical techniques
- Discuss strategies for collecting, extracting, and analyzing non-invasive matrices

Microsampling of Non-Invasive Matrices: Practical Examples Using Tears and a Perspective of Past and Emerging Technologies

Presented by Jason Watts, Ph.D.
Alturas Analytics, Inc., Moscow, ID, United States

INTRODUCTION

Microsampling

» Typically uses ≤50 µL
» Simple collection and storage
» Possibility for at-home sampling
» Site-Centric → Patient-Centric

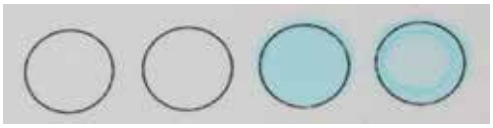
Non-invasive Matrices

» Tears, sweat, saliva, milk
» High correlation with non-protein bound plasma concentrations for many drugs

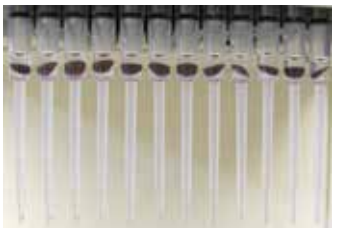
Practical Considerations

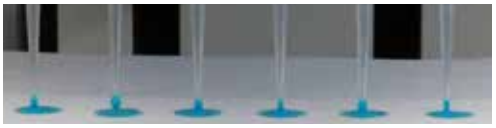
» Can sufficient sensitivity be achieved?
» Analyte stability
» Does the matrix concentration correlate with plasma concentrations?
» What is gained over traditional sampling?

Adapting blood microsampling techniques for non-invasive matrices



» Improved precision and accuracy using color-indicating dyes



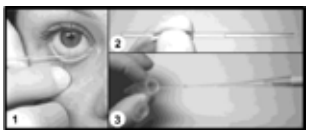


» Improving extraction workflow with common Bioanalytical tools

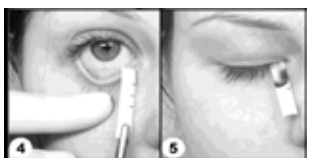
MATRICES

Tears

» Constantly produced at 1.2 µL/min
» Unstimulated volume ~ 7 - 10 µL
» 0.6 - 0.8% protein (0.4% albumin)
» pH 6.5 - 7.6
» Differences between stimulated and unstimulated collection

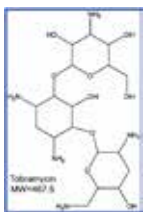


Capillary tube collection
From Posa et al., 2012



Schirmer strip collection
From Posa et al., 2012

Tobramycin from Human Tears



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

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Alturas Analytics is a GLP compliant bioanalytical CRO specializing in MS/MS solutions supporting early discovery through late phase clinical trials.

In addition to providing PK support services to pharmaceutical companies worldwide, Alturas maintains an intensive research effort of applying new technologies leading to scientific advancement.

CONTINUED FROM FRONT

Sweat

» Collected on sweat wipes or patches

» Volume variability: normalized to sodium and potassium levels

» pH 4 - 6.8 when resting
- High sweat/plasma ratio for basic drugs

» Commonly used for monitoring drugs of abuse

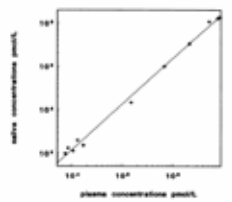
Saliva

» Production and composition
- 0.5 mL/min
- pH ~ 6 - 7
- Excretion of drugs dependent on permeability and protein binding

» Production and composition
- Unstimulated
- Stimulated

Using blood microsampling device for saliva sampling

» Mitra® (Neoteryx) microsampling device
- Precise sample collection
- Ideal for pediatric or at home sampling
- 10, 20 or 30 µL sample volume

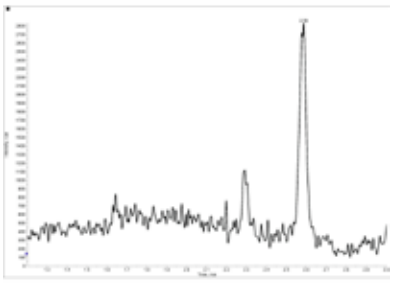


Correlation between plasma and saliva dexamethasone concentrations
Thijssen et al., 1996

SIMPLE EXTRACTION OF DEXAMETHASONE FROM SALIVA USING MITRA® MICROSAMPLING DEVICE

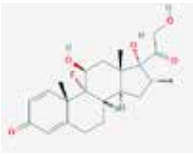
Method

» Collect sample on 20 µL Mitra® tip
» Dry for at least 2 hours at ambient temperature
» Place dried tip in 96 well plate with curve and QCs
» Add 25 µL 100 ng/mL IS (Dex-D4 in ACN:H20 1:1)
» Add 300 µL ACN. Vortex and incubate 30 min
» Transfer extract to fresh plate. Evaporate to dryness
» Reconstitute in 100 µL of ACN:H20 with 0.1% formic acid



0.4 ng/mL Dexamethasone extracted from Saliva

Dexamethasone extracted from saliva



Dexamethasone 392.5 g/mol
Structure from https://pubchem.ncbi.nlm.nih.gov/compound/Dexamethasone

Concentration (ng/mL)	Assay Accuracy and Precision (% ± % CV)	% Recovery
0.8	98.3 ± 0.94%	94
50	103.5 ± 0.68%	117
400	100.1 ± 7.0%	121

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