aaps American Association of Pharmaceutical Scientists
Division of Pharmacy Professional Development
UNIVERSITY OF WISCONSIN-MADISON

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

July 13th, 2020

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Session Description and Objectives

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, as well as being 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.

- 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

- 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

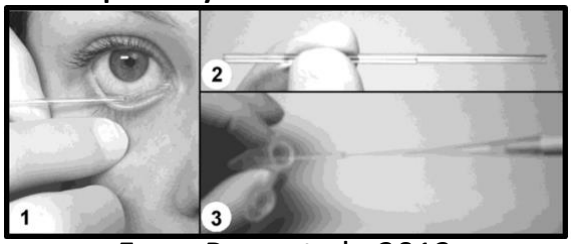
Practical considerations

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

Tears

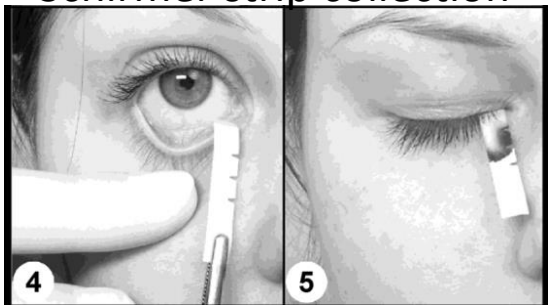
- Constantly produced at 1.2 µL/min
- Unstimulated volume ~ 7 - 10 µL
- 0.6 - 0.8% protein (0.4% albumin)
- pH 6.5 - 7.6
- Differences between stimulated and unstimulated collection

Capillary tube collection



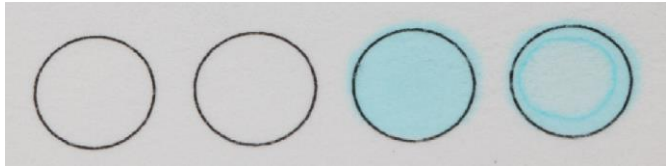
From Posa et al., 2012

Schirmer strip collection

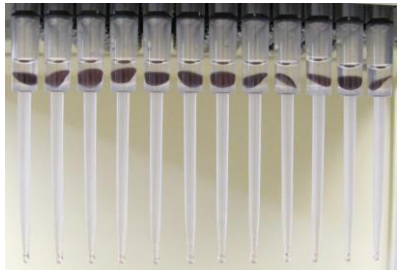


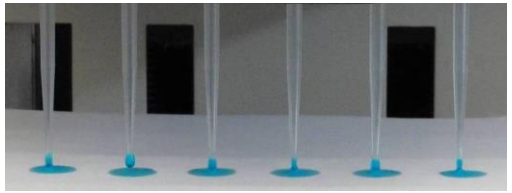
From Posa et al., 2012

Adapting blood microsampling techniques for non-invasive matrices



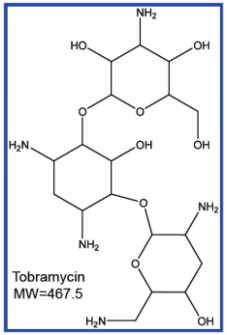
Improved precision and accuracy using color-indicating dyes

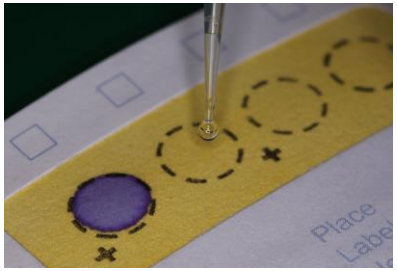




Improving extraction workflow with common Bioanalytical tools

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

Sweat

- Collected on sweat wipes or patches
- Low sample volume/need for normalization
- pH 4 - 6.8 when resting.
  - High sweat/plasma ratio for basic drugs
- Commonly used for monitoring drugs of abuse

Saliva

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

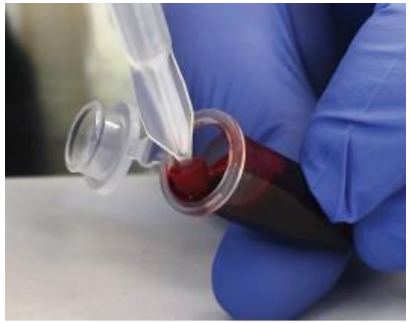
Fig. 4. A selection of different swab-based devices for sampling saliva: (A) Super Sal™ (Oasis Diagnostic Corporation); (B) Versi Sal™ (Oasis Diagnostic Corporation); (C) Quantisal® (Immunalysis Corporation); (D) Intercept® (Orasure Technologies Inc.); (E) Salivette® (blue cap, Sarstedt); (F) SOS (Salimetrics); (G) Toothette-Plus swabs (Sage Products Inc.); (H) OraQuick Advance HIV-1/2 (Orasure Technologies Inc.); (I) BBL CultureSwab orange and white cap.

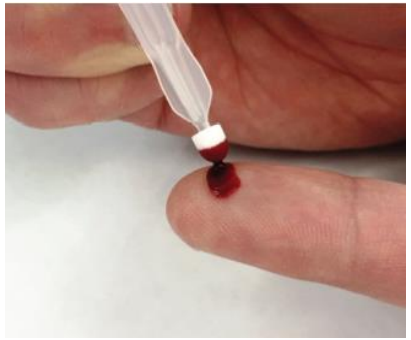
Bellagambi et al., 2020

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

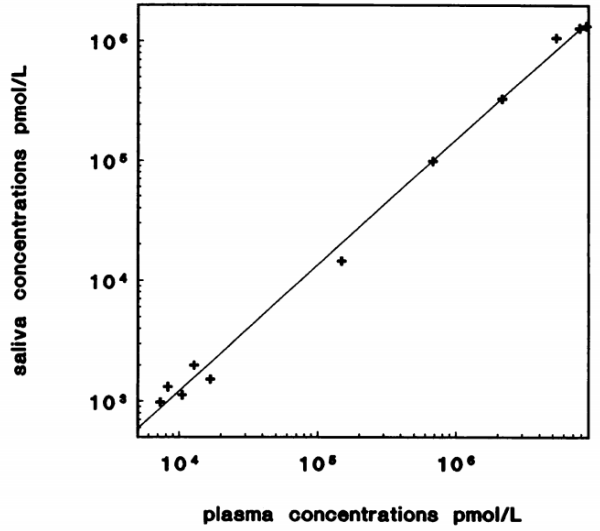
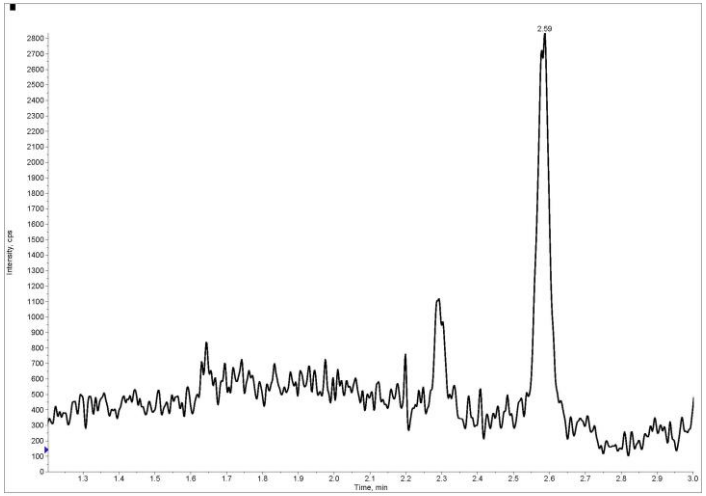


Fig. 2. Correlation between concentrations of dexamethasone in plasma and saliva taken simultaneously from healthy subjects and patients pretreated with dexamethasone.

Thijssen et al., 1996

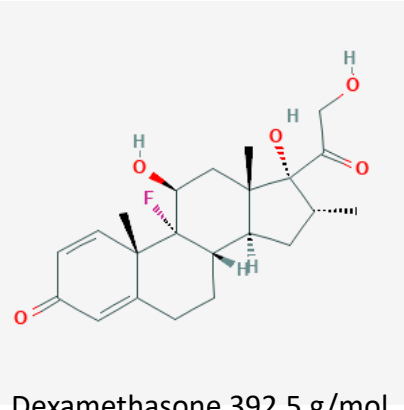
Simple extraction of dexamethasone from saliva using a Mitra® Microsampling device

1. Collect sample on 20 µL Mitra® tip
2. Dry for at least 2 hours at ambient temperature
3. Place dried tip in 96 well plate with curve and QCs
4. Add 25 µL 100 ng/mL IS (Dex-D4 in ACN:H20 1:1)
5. Add 300 µL ACN. Vortex and incubate 30 min
6. Transfer extract to fresh plate. Evaporate to dryness
7. 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

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

Dexamethasone structure from https://pubchem.ncbi.nlm.nih.gov/compound/Dexamethasone

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Acknowledgements

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