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CORPORATE WORKSHOP

Microsampling techniques and LC-MS/MS: therapeutic drug monitoring research to help personalize medicine for our children

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What is Therapeutic Drug Monitoring research?

**Therapeutic drug monitoring (TDM)** is a field of clinical chemistry and clinical pharmacology specialized in the measurement of drug concentrations in blood or body fluids.

Its main focus is on drugs with a narrow therapeutic range, i.e. drugs that can easily be under- or overdosed.

TDM aims at improving patient care by allowing the **individualization of the therapy** for which clinical experience or clinical trials have shown it improved outcome in the general or special populations.
TDM in pediatrics: critical issues

- The critical issues are the same as in adults, but several additional factors must be considered.

Different clinical pharmacokinetic (ADME) and pharmacodynamic parameters must be considered in children compared to adults.

- Off-label use of drugs: approximately 10% of all drugs prescribed are for children.

- Co-medication: the average number of drugs administered in neonatal intensive care units to premature infants < 1Kg is usually in the range of 15-20; infants < 2.5 Kg usually receive 4-10 drugs during their hospital stay.

- Drug interactions needs to be considered.
Availability of a reliable analytical method

“General” requirements

- High specificity
- High sensitivity
- High accuracy
- High reproducibility
- High throughput
- Fast turn Around Times
- Low costs
“Special” requirements for pediatrics

- Higher sensitivity due to the low volume of available samples (especially for very low birth weight infants)
- A dynamic range that can accommodate a wide range of analyte concentrations in a heterogeneous patient population that ranges from 0 to 18 years
- Sufficient robustness to withstand the non-standard matrix effects encountered in hemolytic, lipemic, icteric, and hyperuricemic samples
- Possibility to use “non conventional matrices”: dried matrices, saliva, cerebrospinal fluid...
LIQUID CHROMATOGRAPHY COUPLED TO TANDEM MASS SPECTROMETRY (LC-MS/MS) IS THE GOLD STANDARD METHODOLOGY FOR THERAPEUTIC DRUG MONITORING.

For research use only. Not for use in diagnostic procedures.
Sample Injection

Chromatographic Separation

Molecule Ionization

Molecule Fragmentation

Mass Spectra Generation

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Q1: Precursor ion Selection

Q2: Fragmentation

Q3: Product Ion Selection

For research use only. Not for use in diagnostic procedures.
Bioanalytical Method Development

✓ Optimization of MS conditions (ionization source, ionization parameters, SRM parameters)

✓ Optimization of chromatographic conditions (HPLC columns, mobile phases, temperature, internal standard, etc...)

✓ Optimization of sample preparation
Amount of non-volatile material left behind by King et al., JASMS (2000) 11, 942-950

LLE = 0.20mg  SPE = 0.30mg  PPT = 3.35mg
Despite a lower degree of purification, protein precipitation is more rapid!
Small volume Plasma or DBS → Extraction buffer + Internal standard → 15 min → Injection → Upper phase collection

For research use only. Not for use in diagnostic procedures.
Bioanalytical Method Validation

According to updated reference guidelines (EMA, ICH, FDA)

Specificity and selectivity

Carry-over

Matrix effects/ion suppression

Accuracy

Precision

Recovery

Reproducibility

Lower limit of quantification (LLOQ)

Long term and short term stability

Lower limit of detection (LLOD)

Dilution integrity

Guidance for Industry

**Bioanalytical Method Validation**

**DRAFT GUIDANCE**

This guidance document is being drafted and for comment purposes only.

Comment and suggestions regarding this draft document should be submitted in triplicate to the above addresses within 28 days of publication in the Federal Register.

The draft guidance is subject to the availability of the final guidance. Federal Register announcement at http://www.federalregister.gov/

For further comments, please send to: Division of Drug Metabolism (C96), Food and Drug Administration, 3514-3545, Rockville, MD 20855. All comments should be identified with the subject matter listed in the above guidance. Any published feedback on the draft guidance will be maintained in the United States

Preparation, licensing and regulation of clinical research in the United States:

U.S. Department of Health and Human Services

Food and Drug Administration

Center for Drug Evaluation and Research (CDER)

Center for Veterinary Medicine (CVM)

September 2013

Revised:

Revision 1

Guideline on bioanalytical method validation

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Summary

This guidance defines key elements necessary for the validation of bioanalytical methods. The guidelines reflect the validation of the bioanalytical methods for the determination of drug concentrations in biological fluids. Guidance on the application of these validated methods in the routine analysis of study samples from animal and human studies.
Evaluation of matrix effect/ion suppression

Strategies for the Assessment of Matrix Effect in Quantitative Bioanalytical Methods Based on HPLC–MS/MS

B. K. Matuszewski, * M. L. Constanzer, and C. M. Chavez-Eng

Assessment of Matrix Effect. The assessment of matrix effect and assay reliability is critical when homologues rather than stable isotope-labeled analytes are utilized as internal standards. By comparing the peak areas of the analyte standards, standards spiked before and after extraction into different lots of plasma, and the peak area ratios of analytes to an IS, the recovery and ion suppression or enhancement associated with a given lot of plasma were assessed.
Proficiency tests (if available)!
Advantages of LC-MS/MS over immunoassay methods

- Higher specificity
- Higher sensitivity
- Higher accuracy
- Multiplexing
- Different matrices
- Low sample volumes
- Lower costs
- Less inter-lot variability
- Possibility to develop “in house” methods for clinical research use
From immuno-assay to LC-MS/MS...
Ultra high performance liquid chromatography-tandem mass spectrometry vs. commercial immunoassay for determination of vancomycin plasma concentration in children. Possible implications for everyday clinical practice.

Analysis of the clinical discordance between the two methods. Numbers represent samples in each interpretative category.

N=138 samples
Microsampling

- Low sample volume ($\leq 50$ µL plasma or serum)
- Dried Blood Spot (DBS)
- Volumetric microsampling
- Dried Plasma Spot
Examples of LC-MS/MS micromethods developed at G.Gaslini Institute for clinical research use

CINACALCET
LC-MS/MS method for the simultaneous quantification of five antibiotics from 50 µL plasma

G. Cangemi – MICRO SAMPLING TECHNIQUES AND LC-MS/MS: TDM RESEARCH TO HELP PERSONALIZE MEDICINE FOR OUR CHILDREN
LC-MS/MS method for the quantification of ciprofloxacin and daptomicin from 50 µL plasma
LC-MS/MS method for the quantification of an antimicotic agent (micafungin) in 50 µL plasma
Microsampling

✓ Low sample volume (≤ 50 µL plasma or serum)
✓ Dried Blood Spot (DBS)
✓ Volumetric microsampling
✓ Dried Plasma Spot
Dried blood spot

The DBS is obtained by piercing heel or finger with a retractable needle and the capillary blood that emerges is sampled on a suitable absorbent paper. The paper is dried and stored in an airtight container with desiccant.

“it is admitted that a \(3.2\) mm DBS punch from a \(100\ \mu\text{L}\) blood sample with a Hct of 55% corresponds to a blood volume of \(3.42\ \mu\text{L}\)”

Mei et al., 2010
Advantages

☑️ Easy and non-invasive sampling
☑️ Use of a small volume of sample
☑️ Stability: many analytes are stable in the DBS and can be stored at room T
☑️ Shipping: can be performed by ordinary mail without special restrictions

Disadvantages

☒ Small sample volumes require a very sensitive instruments
☒ The analyte concentration in capillary blood may differ from the concentration in venous blood
☒ Further validation of the method must take into account some characteristic effects of DBS that can generate analytical errors

European Bioanalysis Forum
Hematocrit effect

The hematocrit (Ht) is the percentage of the corpuscular part of the whole blood volume

Variations in Ht influence blood viscosity

Viscosity causes a different diffusion of blood on adsorbent paper

Ht can negatively influence the accuracy of analysis
Hematocrit effect: influence on accuracy

Hematocrit effect: influence on recovery

Chemical-physical differences of the analytes and/or paper quality can generate non-homogeneous distribution of analytes on the stain surface.

*Figure 3.* Chromatographic effects: radioactivity distribution in DBS obtained by spotting blood spiked with $^{14}C$-labeled compounds on five different DBS papers. Top row: pictures of DBS. Middle row: autoradiograms. Bottom row: Radioactivity concentrations, expressed as photon-stimulated luminescence/mmol, along the diameter of the autoradiogram. From Ren et al. (2010), with copyright permission.
Blood spot homogeneity

Assessment of the sample homogeneity by using different samplings of the entire surface of the paper

Effect of blood volume

The calibration curve and the quality controls are registered on paper in defined volumes, whereas unknown samples are picked up by direct contact with blood of unknown volume.

Even this effect can generate a different distribution of the analyte on the surface of the paper.

DBS: critical issues

- Chemical-physical characteristics of the analyte
- Quality of paper
- Blood volume adsorbed on paper
- Hematocrit

Main variable determining the robustness and reproducibility of the method

Before transferring the method from a conventional matrix to DBS
It is suggested to proceed with further validation experiments and then test the method with a set of real samples.
Hematocrit effect

Adults: Males 40-50% Females 35-45%

Neonates 42 – 65 % dropping during the first 28 days of life

Jopling et al. Pediatrics (2009) 123(2) e333-e337
DBS–LC–MS/MS assay for caffeine: validation and neonatal application

Aim: DBS might be an appropriate microsampling technique for therapeutic drug monitoring of caffeine in infants. Nevertheless, its application presents several issues that still limit its use. This paper describes a validated DBS–LC–MS/MS method for caffeine. Results: The results of the method validation showed an hematoctit dependence. In the analysis of 96 paired plasma and DBS clinical samples, caffeine levels measured in DBS were statistically significantly lower than in plasma but the observed differences were independent from hematocrit. Conclusion: These results clearly showed the need for extensive validation with real-life samples for DBS-based methods. DBS–LC–MS/MS can be considered to be a good alternative to traditional methods for therapeutic drug monitoring or PK studies in preterm infants.

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Published online: 17 August 2016

Keywords: caffeine • DBS • LC–MS/MS • newborn • PK • preterm • therapeutic drug monitoring

Caffeine is considered a standard treatment for apnea of prematurity, which is one of the infant's pathologies. Possible toxic effects of caffeine include tachycardia, tachypnea, tremors, opines.

Matteo Bruschi et al.
Sebastiano Baroni
Olga Romaniello
Francesco Risso
Italian German
Raniero Ambrosi
Luca A. Ramenghi
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Thermo Fisher Scientific

GASLINI
Measurement of caffeine in preterm neonates, correlation between plasma and DBS

n 44 (19 F – 25 M)

Ht 25 – 69 %

Gestational age 24-33 weeks

During the validation experiments Ht effects has been confirmed

We choose to use calibrators with Ht values close to the median value of the patients included in the study
Caffeine concentration in DBS resulted 10% lower than in plasma

\[ r^2 = 0.911 \]

The differences observed were independent from Ht
What can we do in order avoid or minimize Ht-effect?

- Standardize the Ht in the calibration standards close to the expected Ht of the samples
- Analyze the entire DBS
- Use of volumetric microsampling devices: PCDBS (pre-cut DBS), DMPD (dried matrix on paper disc), VAMS (volumetric absorptive microsampling)
- Use of dried plasma spot (DPS)

Accurate volumetric application becomes the most critical parameter and requires trained people.
VAMS: volumetric absorptive microsampling

Advantages

✓ No Ht effect!
✓ More standardized sampling
✓ Possibility to use more “agressive” solvents in sample prep
✓ Same advantages of DBS in terms of analyte stability and shipment (to be tested...)

Disadvantages

✗ Higher cost per sample
Microsampling

- Low sample volume ($\leq 50 \mu L$ plasma or serum)
- Dried Blood Spot (DBS)
- Volumetric microsampling
- Dried Plasma Spot
Dried Sample Spot Devices (DSSD)

Courtesy of Laboratori Biomicron, Turin, Italy
Advantages of DPS over DBS:

- No Ht effect
- Efficacy and toxicity target levels from clinical studies for several drugs refer to plasma levels

Disadvantages of DPS over DBS:

- Longer procedure for sample collection due to a centrifugation step
- Efficacy and toxicity target levels of immunosuppressants refer to whole blood
Development and validation of UHPLC–MS/MS methods for the quantification of colistin in plasma and dried plasma spots

Gianluca D’Avolio, Sebastian Bao, Elio Castagnola, Gino Tripodi, Fabio Favata, Antonio D’Avolio, BSc, MSc, SM

Abstract
Quantification of colistin in plasma samples may be very useful in optimizing therapy especially in special patient populations. Nevertheless, the specific drug monitoring of colistin is still limited probably for the low number of laboratories which perform this analysis and for high shipment costs. We developed, and validated new UHPLC–MS/MS methods to quantify colistin in plasma and in dried plasma spots (DPS) collected on dried sample spots devices (DSSD). Colistin A, Colistin B and polymyxin B, used as internal standard, were detected using multiple reaction monitoring (MRM) of the following specific transitions 488.6 –> 444.0, 576.8 –> 527.0, 588.9 and 600.6 –> 547.9, 609.8 respectively. Colistin A and B were extracted from plasma using proteins precipitation and from DSSD using an extraction basic solution. Both methods were validated, and the mean intra- and inter-day accuracy and precision were in accordance with FDA and EMA guidelines. Colistin in DSSD was found to be stable for at least one week at room temperature (20-25°C). A statistically significant linear correlation was found between colistin extracted from plasma and from DSSD (r² = 0.9964, p < 0.0001; 95% CI 0.9960-0.9967) for colistin A and (r² = 0.9986, p < 0.0001; 95% CI 0.9984-0.9989) for colistin B, respectively. DPS on EMD represent a safe and cheap strategy to store and ship at room temperature plasma samples. Thus, it is suited for pharmacokinetic studies and therapeutic drug monitoring of colistin.

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TDM in pediatrics: from research to clinics

What do we need?

- Lower sample volumes
- Highly sensitive LC-MS/MS instruments
- Analytical methods, instruments and microsampling devices validated for clinical use
THANKS TO...

For the kind invitation

My collaborators:
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Photos used in this presentation are courtesy of
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