CONSIDERATIONS FOR THE USE OF VOLUMETRIC ABSORPTION MICRO SAMPLING (VAMS) DEVICES FOR A BIOANALYTICAL ASSAY

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Introduction

What are VAMS ™?

PRODUCT OVERVIEW

- Volumetric Absorptive Microsampling (VAMS™)
  - MITRA™ device
    - Produced by Neoteryx®
  - Hydrophilic polymer
  - Absorbs fixed liquid volume
    - Currently available in 10, 20 & 30 µL format
  - Potential for home sampling
  - Sample the dried for simple storage prior to analysis

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What are VAMS ™?

BENEFITS

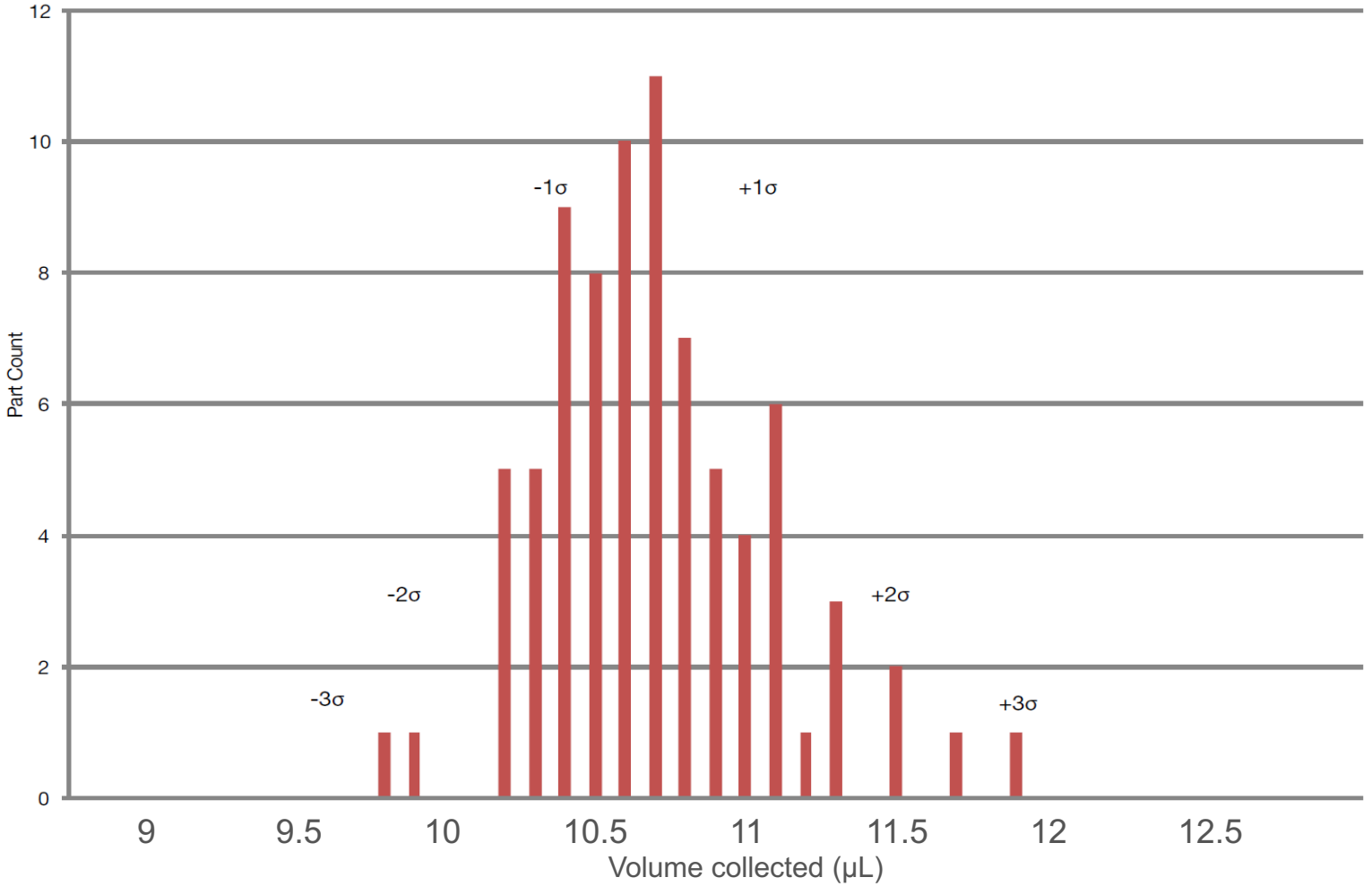
- Fixed volume wicked by device
- Overcomes traditional haematocrit issues faced by other dry sampling techniques
- Not impacted by blood partitioning
- Cheaper sample storage/ transportation
  - No requirements to be chilled

What are VAMS ™?

POTENTIAL ISSUES

- Haematocrit can still cause issues
  - Recovery based, requires further method development to improve
- Samples continue to ‘dry’ throughout lifetime
  - Difficult to assess during typical development time
  - Further reductions in analyte recovery resolved through method development
- Variability of wicking volume within and between lots
  - Unrelated to extraction recovery
  - Requires alternate solution

Wicking volumes across multiple tip lot numbers



Source: Batch to Batch Variability in MITRA ™ Tips, Kansal et al (2017)
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Approaches to solve VAMS volume variation and analyte recovery

Can we normalise tip volume and recovery?

- Addition of an internal standard onto the tip could
  - Normalise tip volume
  - Compensate for variable recovery
- Addition pre- or post- sampling
  - Can tips be loaded with internal standard after sampling?
- How does this compare to ISTD addition during sample extraction (standard practice)

Methodology

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Methodology

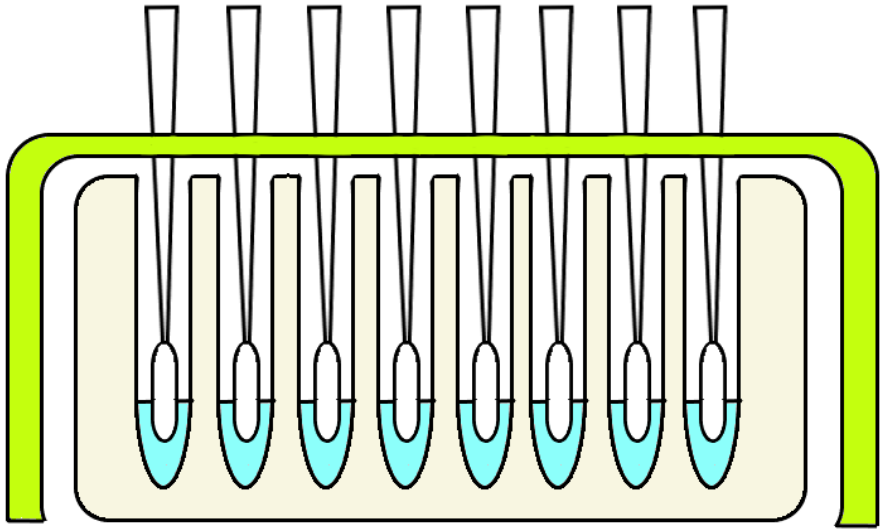
ANALYTE SELECTION

- Utilising Covance owned methodology
- Analytes with wide range of polarities selected for trial
  - Levetiracetam
  - Topiramate
  - N-Desmethylclobazam
  - Clobazam
  - Stiripentol
- All have an isotopically labelled internal standard
- Range of analogue internal standards also examined
  - 7 in total
  - Paired with analytes based on chromatographic similarity

Methodology

Tip preparation and spiking

- Internal standards spiked into solution of methanol: water: formic acid (50:50:0.05)
- MITRA™ tips loaded into 96-well rack (10 & 20 µL)
- 50 µL of internal standard solution per well to 96-well plate
  - Enough to make contact but not fully submerge
- Rack placed over 96-well plate
- Lowered into well for 1 minute
- Dried for minimum of 3 hours under airflow
- ISTD addition before and after blood sampling



Picture produced by Covance

Methodology

Tip preparation and spiking

- Blood of normal ‘healthy’ haematocrit (approx. 46%)
  - Assessed by packed cell volume method
- Low and high haematocrit assessed by adding red cells or plasma to whole blood
  - ±15% haematocrit assessed
    - Plasma added to dilute
    - Red cells added to concentrate
- Lipidaemic blood assessed
  - Blood centrifuged and healthy plasma exchanged with lipidaemic plasma
- Blood then spiked with analyte and introduced to MITRA™ tip by hand at single concentration

Methodology

Tip Extraction

- Initial sonication in methanol: water: formic acid (50:50:0.05) (Containing ISTD for samples not pre-loaded)
  - Tips removed and solvent crashed with acetonitrile
- Tips then placed into second plate containing MTBE and further sonicated
  - Tips removed
- Crash supernatant drawn off and added to MTBE extract
- Samples evaporated then reconstituted for analysis

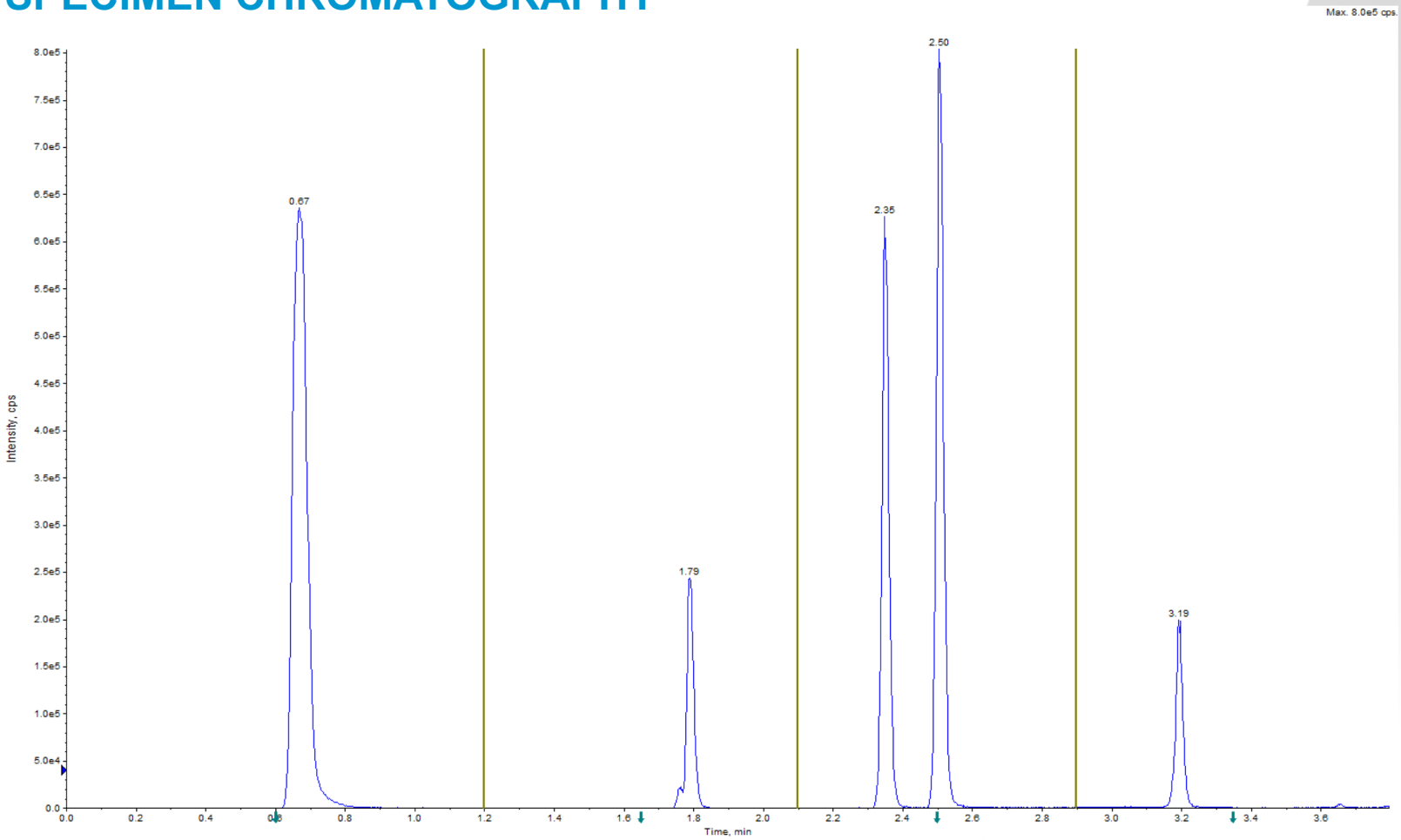
Methodology

SAMPLE ANALYSIS

- Waters™ Acquity™ LC system coupled with AB Sciex™ 5500 mass spectrometer
- Data captured and processed using Analyst® software
- As only one concentration used %BIAS is based on normal ‘healthy’ blood spikes

Methodology

SPECIMEN CHROMATOGRAPHY



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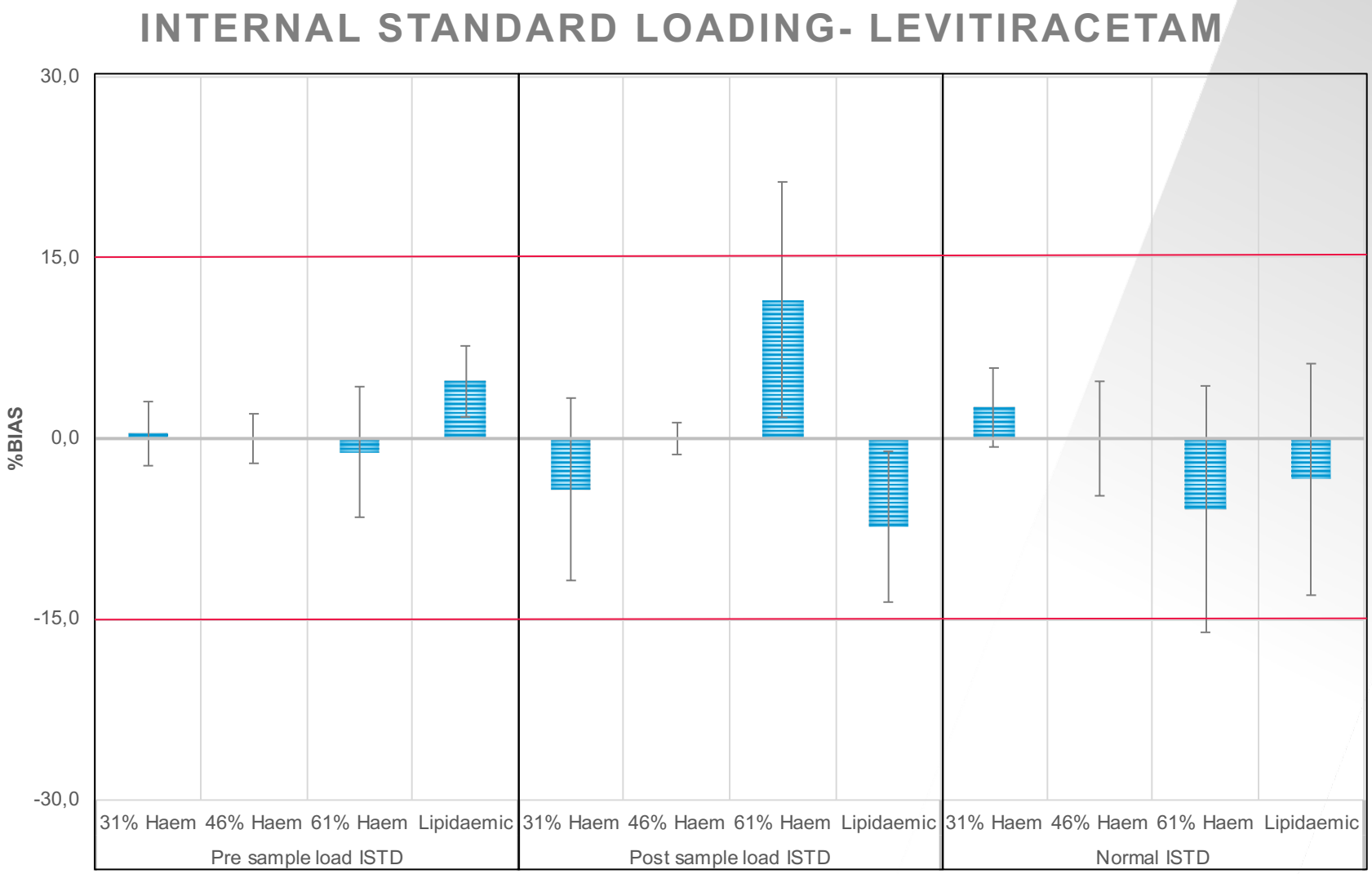
Results / Conclusions

Results



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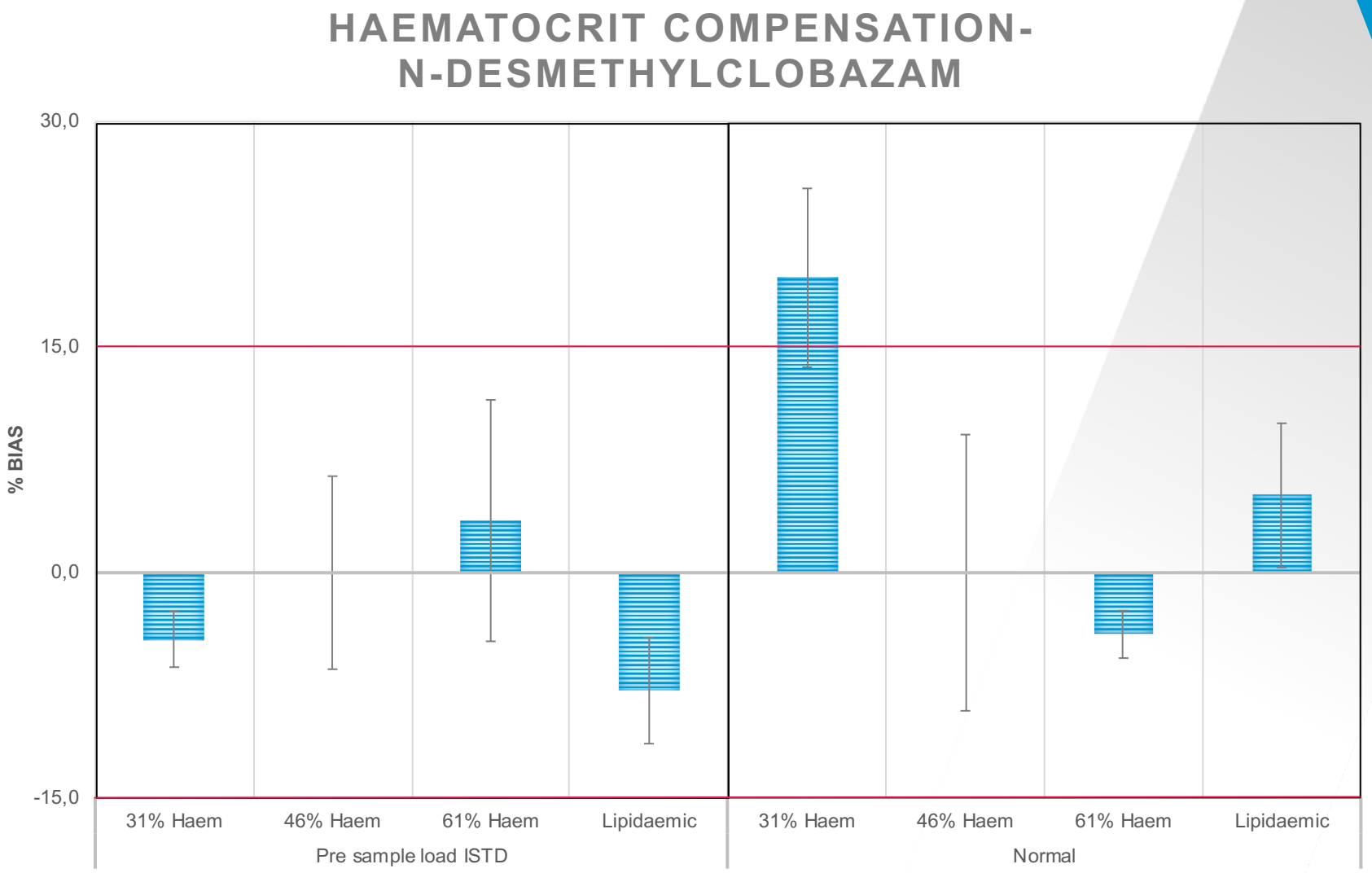
Conclusions

INTERNAL STANDARD LOADING

- Loading prior to tip sampling shows good accuracy and precision
  - Similar accuracy and better precision than addition during extraction
- Loading post sampling shows poorer accuracy and precision
  - Possibility that presence of red cells hinders wicking of further solvent

Results



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Conclusions

HAEMATOCRIT COMPENSATION

- Normal SILIS addition shows haematocrit affect
  - Analyte recovery lowers as haematocrit increases
  - SILIS recovery not varying with haematocrit
- Pre-spiked tips not showing this effect
  - SILIS recovery tracking analyte recovery and normalising

	Recovery		
Sample Name	N-Desmethylclobazam (%)	SILIS (%)	Difference
Pre-ISTD 31%	82.4	84.4	2.0
Pre-ISTD 46%	80.0	78.5	1.5
Pre-ISTD 61%	75.5	71.8	3.7
Pre-ISTD Lipid	75.9	80.7	4.7
Norm-ISTD 31%	100.1	98.6	1.5
Norm-ISTD 46%	82.2	96.8	14.6
Norm-ISTD 61%	77.4	95.1	17.7
Norm-ISTD Lipid	88.8	99.5	10.6

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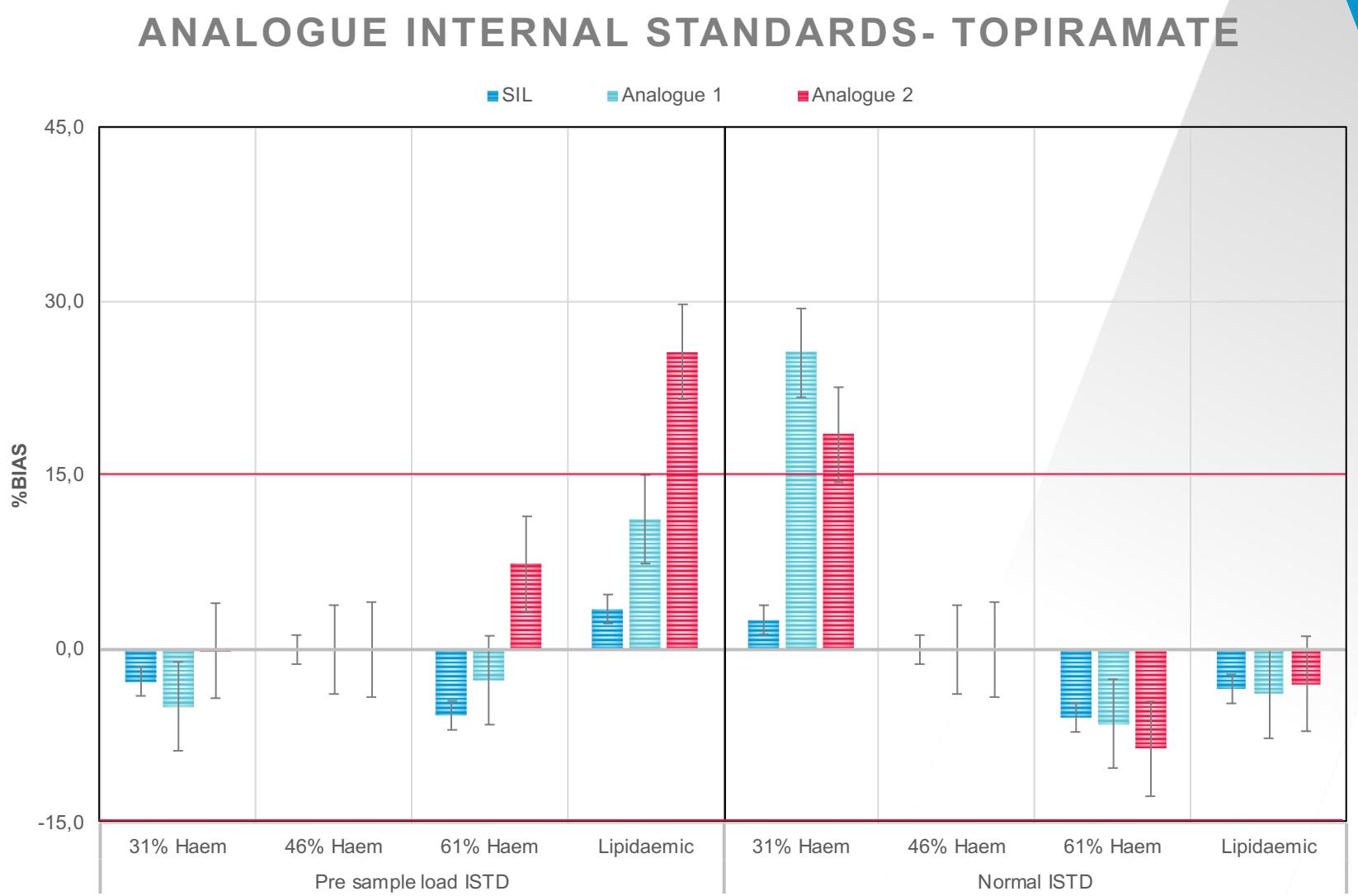
Results



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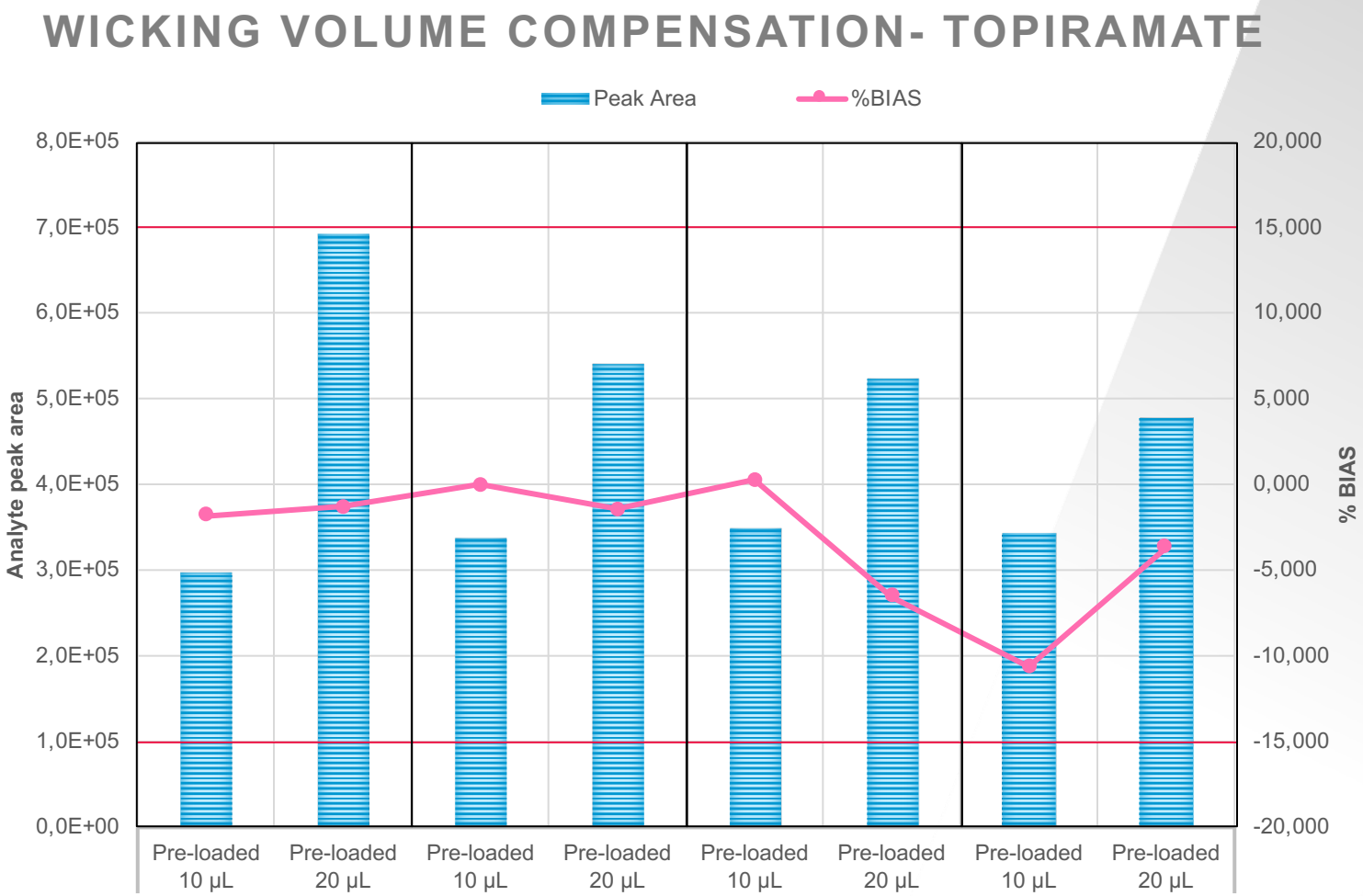
Conclusions

Using an Analogue Internal Standard

- Analogue internal standards more variable than SILIS
- Pre-loaded has overcome some issues seen in normal extraction (haematocrit)
- LC/MS method developed around use of SILIS
  - May see improvements if further optimised for analogue internal standard usage

Results



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Conclusions

Wicking volume compensation

- Larger wicking volumes lead to larger analyte response
- Pre-loading with internal standard compensates effectively for this
- Can allow multiple tip lots to be used across study without concern about impact to data

Thank You

Thanks to Johannes Stanta and Matt Ewles of Covance, and James Rudge of Neoteryx®.

Any Questions?

About Covance

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