

# Quantification of antifungal TDM using LC-MS/MS: Problem solving and case discussion and the way forward

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# Combine drug assays



# Advantages of combining drug assays

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Reduce analytical run time

2

Reduce turnaround time

3

Improve frequency of TDM reporting

4

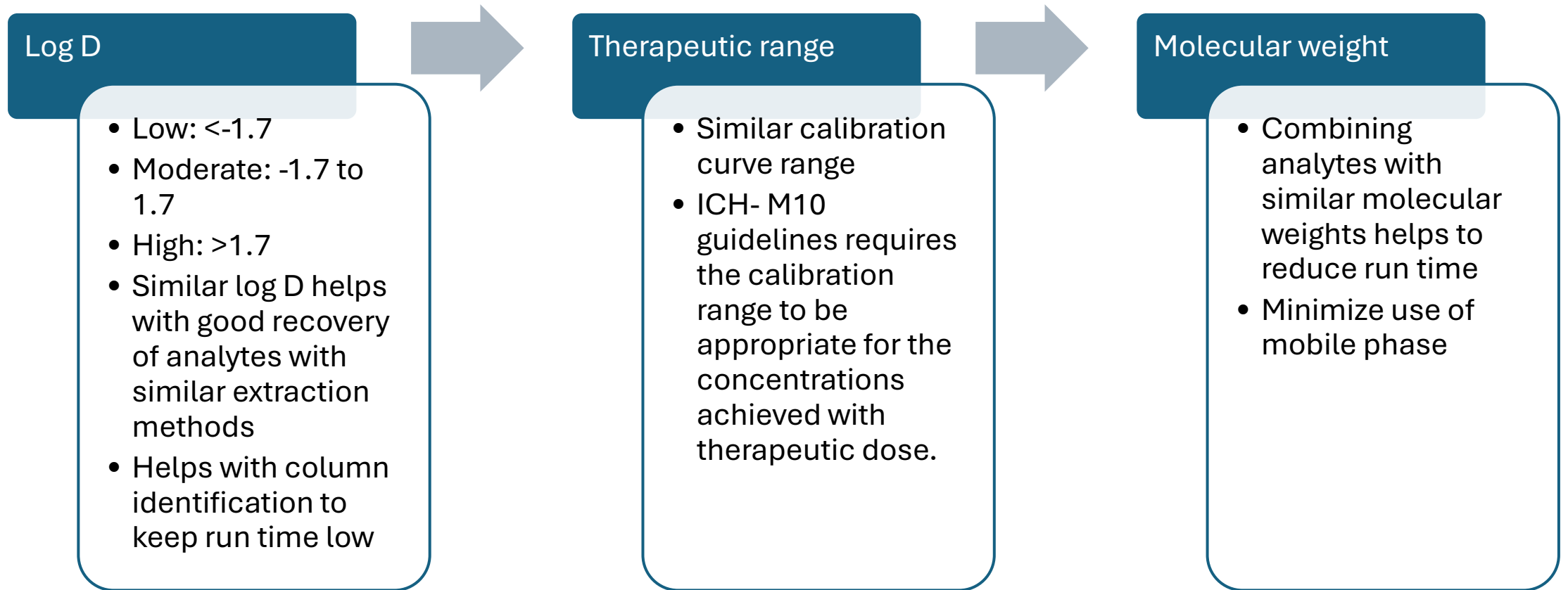
Single extraction method

5

Cost effective:

- Minimal use of mobile phase
- Reduced run time
- Minimal use of stable isotope labelled internal standards

# Flowchart



# List of drugs: Anti-infectives (Log D at pH 3)

## High Log D (> 1.7)

- Voriconazole
- Posaconazole
- Itraconazole
- Hydroxyitraconazole
- Isavuconazole

## Moderate Log D (- 1.7 to 1.7)

- Isoniazid
- Pyrazinamide
- Rifampicin
- Linezolid
- Fluconazole
- Piperacillin
- Cefoperazone

## Low Log D (< -1.7)

- Teicoplanin
- Vancomycin
- Ampicillin
- Amoxicillin
- Ceftazidime
- Meropenem
- Tigecycline
- Gentamicin
- Amikacin
- Polymyxin B
- Colistin

# Extraction methods according to Log D

## High Log D



☐ Precipitation: MTBE + ACN/ Methanol

☐ Reconstitution:  
[Organic: Water containing FA] at [4:1]

## Moderate Log D



☐ Precipitation: Methanol/ ACN

☐ Final solvent:  
[Water containing FA: Organic] at [5:1]

## Low Log D



☐ Precipitation: Methanol/ ACN

☐ Final solvent: Water containing FA:  
organic at 5:1

## High Log D



☐ LLE: MTBE (70%) + DCM (30%)

☐ Reconstitution:  
[Organic: Water containing FA] at [4:1]

## Moderate Log D



☐ LLE: EA

☐ Final solvent:  
[Water containing FA: Organic] at [5:1]

## Low Log D



☐ LLE: EA. (may not work for analytes with  
extremely low log D).

☐ DCM to remove phospholipids

☐ Final solvent: Water containing FA

# Matrix effect

Need for high organic ratio in mobile phase and possibility of ion suppression

# Matrix effect: Antifungal drugs

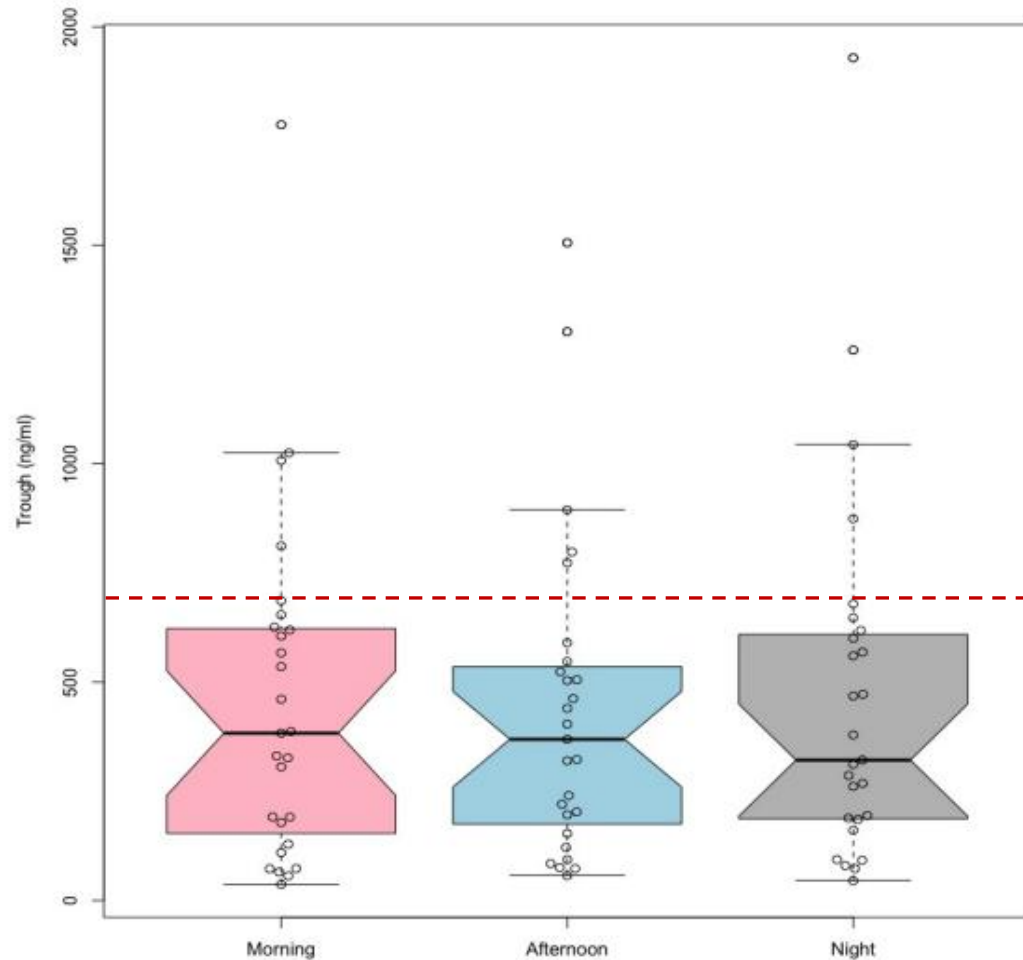
- High organic ratio in mobile phase
- To elute highly lipophilic analytes
- C8 column
- Isotopes as internal standards
- Isotopes improve accuracy and precision
- In the absence of an isotope, use other internal standards or a combination of internal standards to quantify analyte. Do extensive validation



# Curious case of posaconazole

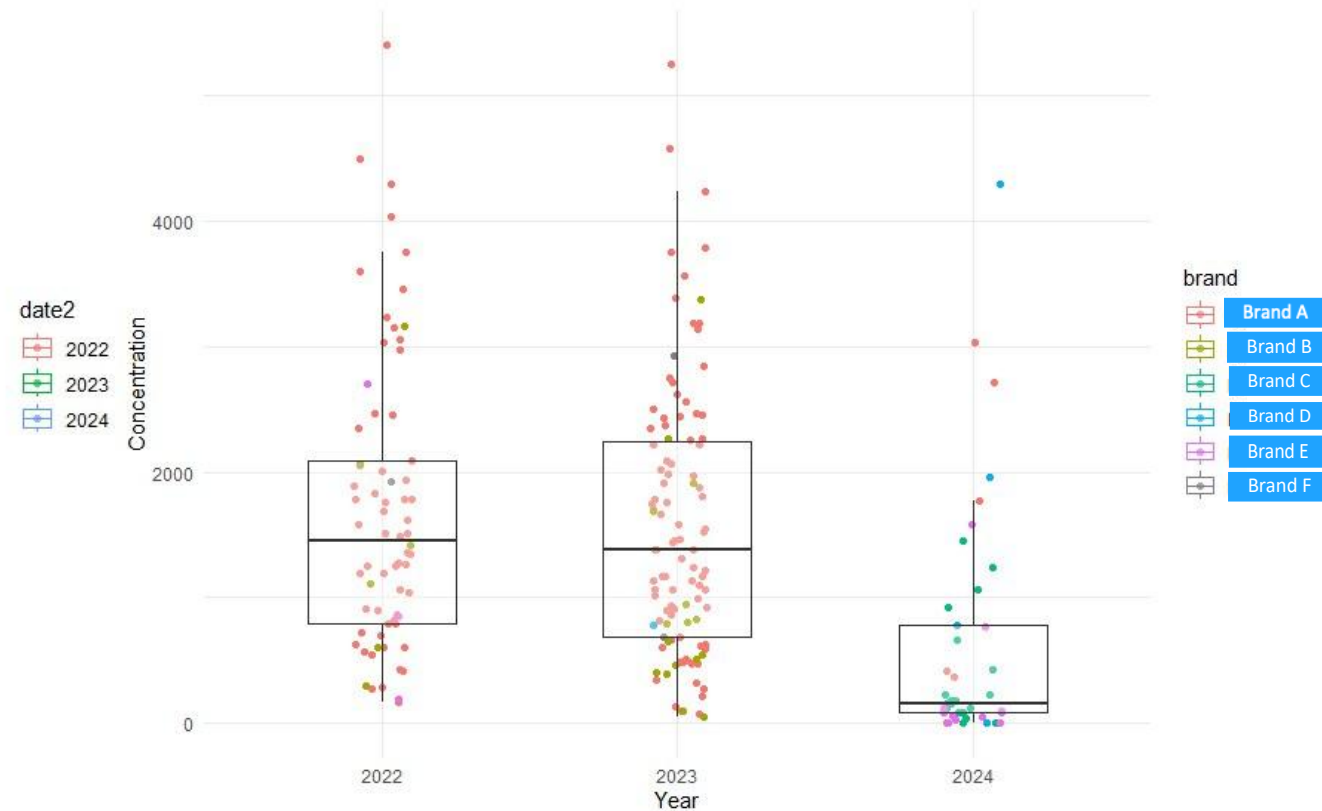
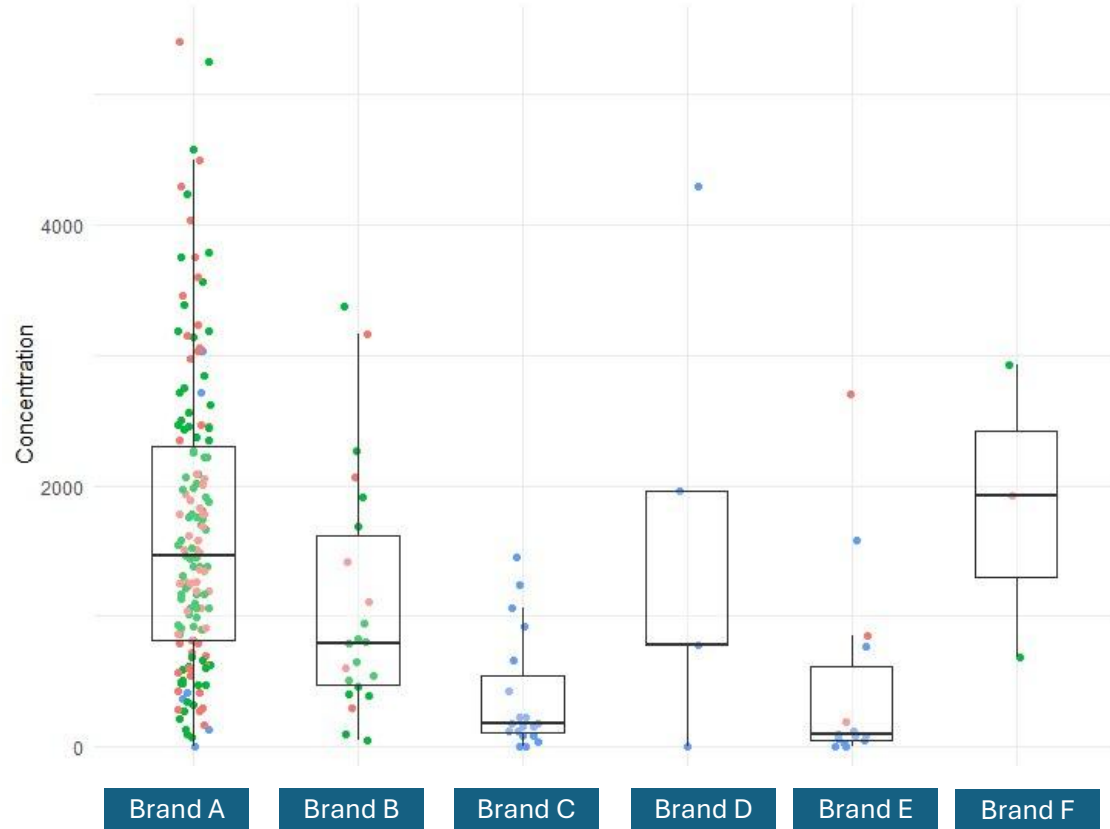
Brand issues

# Posaconazole exposure in AML and BMT



- Overall inter-patient variability (AUC): CV = 79.9%
- Posaconazole syrup
- CMC Vellore, not yet published data (n = 27)
- Study performed by Serin et.al
- 83% had low exposure
- 9 (33%) developed IFD within 2 months.

# Posaconazole Tablet



Courtesy of: Clinical Pharmacology Unit, Christian Medical College, Vellore

# Alternate matrices for quantification

According to ICH M10 guidelines

# Other matrices

- Synovial fluid
- Ascitic fluid
- CSF
- Alternate markers for tissue penetration



*Medical Mycology*, 2019, 57, S161–S167

doi: 10.1093/mmy/myy067

Review Article



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## Review Article

### **Beyond tissue concentrations: antifungal penetration at the site of infection**

**Yanan Zhao<sup>1,\*</sup>, Brendan Prideaux<sup>1</sup>, Shane Baistrocchi<sup>2</sup>, Donald C. Sheppard<sup>2</sup> and David S. Perlin<sup>1</sup>**

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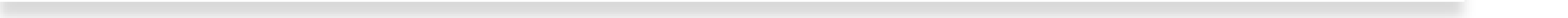
# Dilution integrity

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- For rare matrices, surrogate matrix could be used for dilution
- Precision and accuracy after dilution of matrix should be evaluated

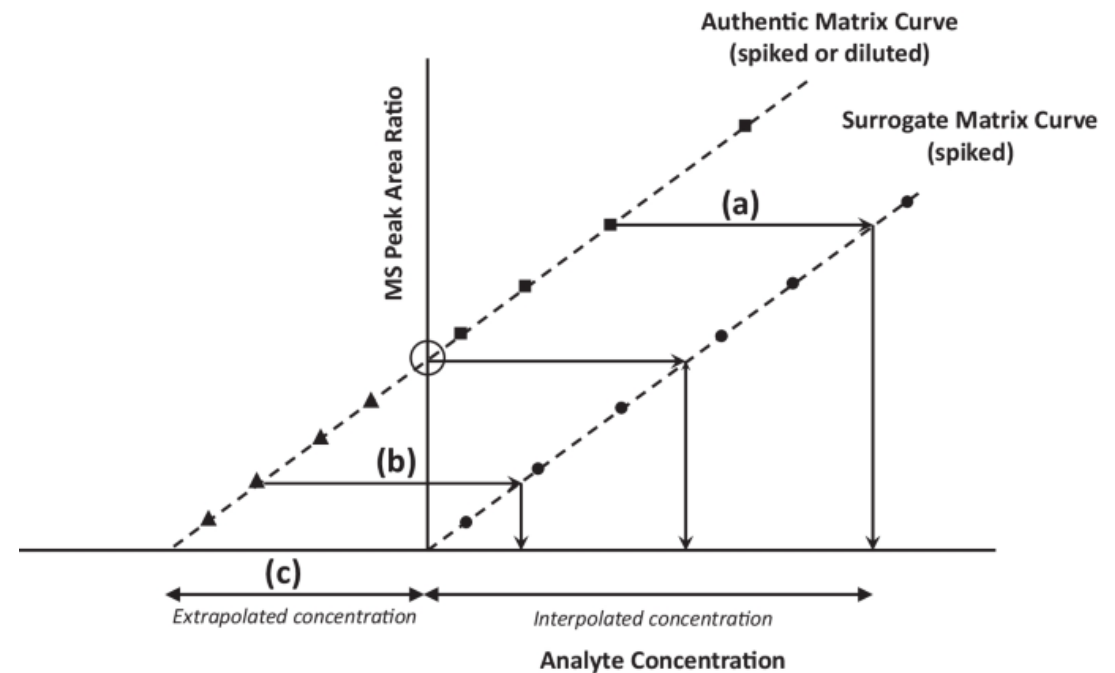
# Accuracy and Precision



- Accuracy and Precision should be evaluated at the LLOQ, Low QC and High QC level for alternate matrix
  - The QCs should be read against the surrogate calibration curve.
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# Parallelism

- Parallelism assures that observed changes in response per given changes in analyte concentrations are equivalent for the surrogate and the authentic biological matrix across the range of the method.
- Using isotope labelled internal standards ensure parallelism







# Stability

Stability of the analyte should be demonstrated in the surrogate matrix.