



# High throughput LC-MS/MS of Pyrimethamine and Sulfadoxine in limited volume plasma samples: combining selectivity from chromatography and fast ion separation by MS

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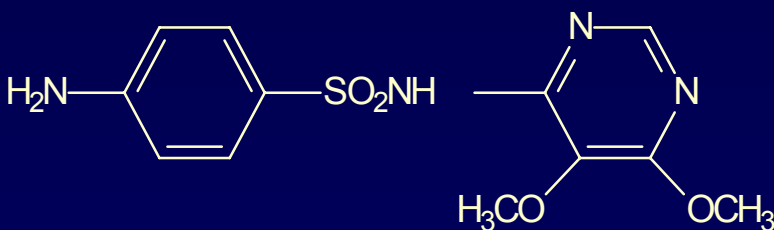


# Introduction

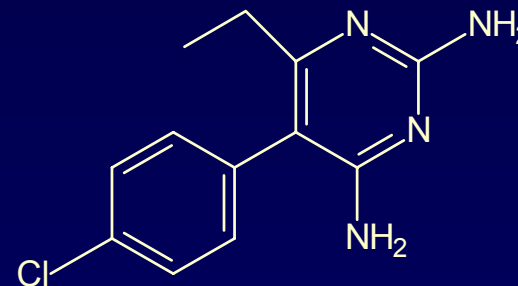
## ■ Pyrimethamine + Sulfadoxine:



- ✓ Prevention and treatment of *Malaria P. falciparum*
- ✓ ~~Chloroquine~~ in Eastern Africa
- ✓ Antifolate-sulfonamide combination: new first-line drug



PYR



SUL



## ■ Drug resistance:

- ✓ Due to exposure to **sublethal concentrations**
  - Suboptimal dosage
  - Use of substandard drug formulations
- ✓ **Therefore:**

### **Tablet formulations on African market:**

Acceptable quality ?

Constant quality (shelf time under tropical conditions) ?

*In vitro* availability:

- Dissolution tests
- ≠ between formulations

*In vivo* bio-availability:

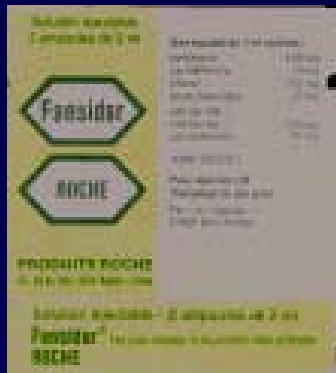
- Clinical trial
- **Same ≠ ?**



# Aim

From an **analytical viewpoint**:

Sound **simultaneous quantitation** of both drugs in human plasma samples



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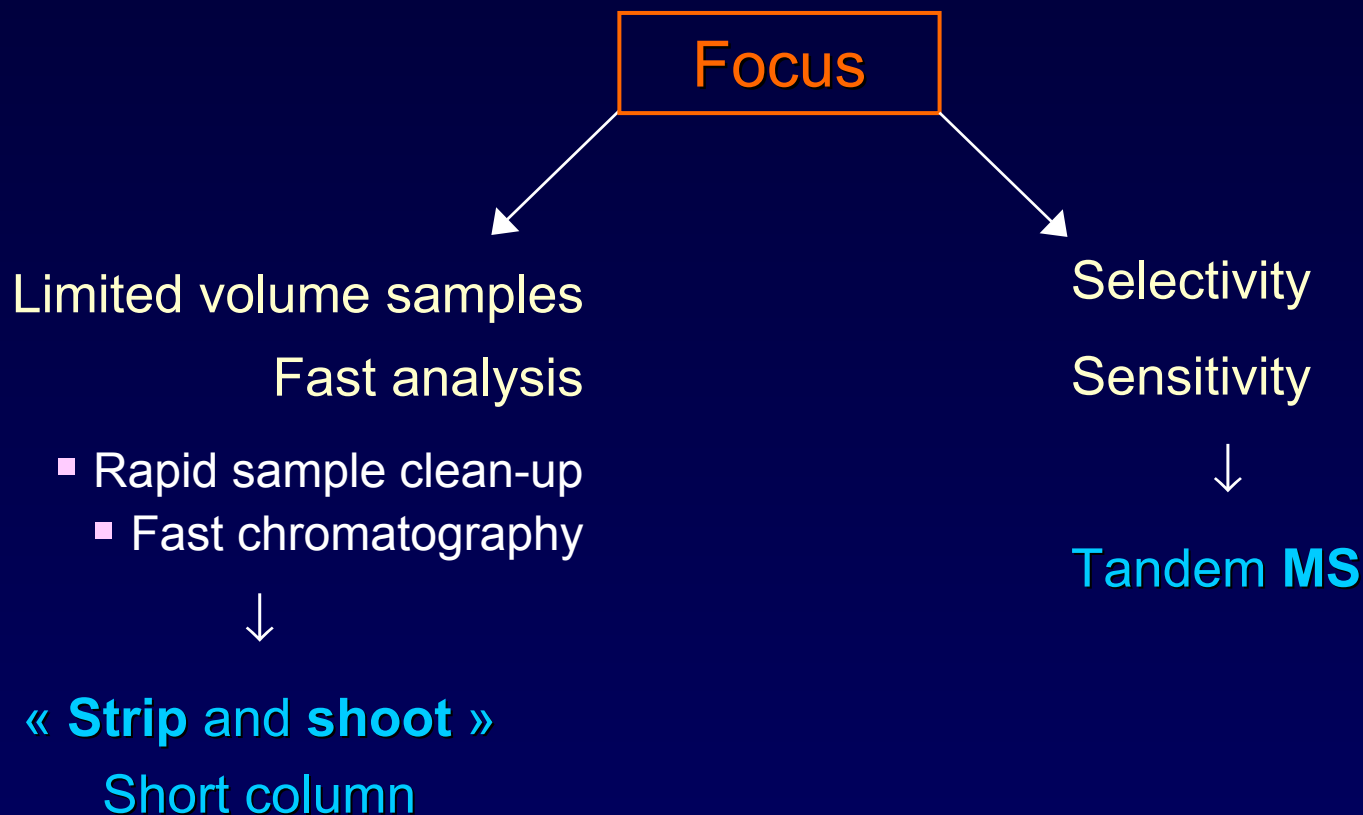
Tablet: 25 mg PYR + 500 mg SUL

**Challenge:**

- ✓ **Huge sample load**
- ✓ **Repetitive samples / volunteer**
- ✓ **Largely different concentrations**



# LC-MS/MS method: development



## ■ Sample pretreatment:

« Single step  
- one tube »

- ✓ 250  $\mu\text{L}$  crude plasma
- ✓ Deproteinization: 100  $\mu\text{L}$   $\text{ZnSO}_4$  0.1 M  
+ 100  $\mu\text{L}$  AcCN
- ✓ Removal of lipids: 300  $\mu\text{L}$   $\text{CHCl}_3$

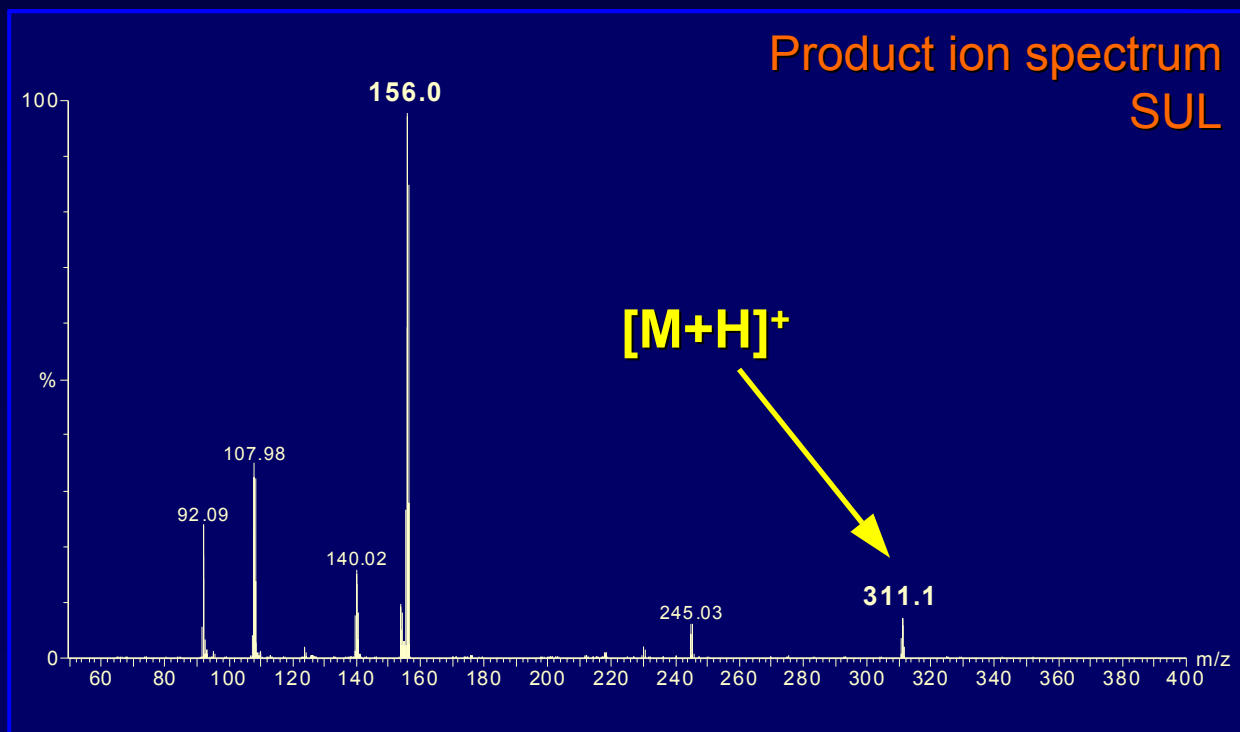
## ■ Chromatography:

- ✓ 10  $\mu\text{L}$  supernatant injected
- ✓ XTerra MS  $\text{C}_{18}$  column (3.5  $\mu\text{m}$  p. s., 50\*1 mm)
- ✓ Elution gradient:  $\text{H}_2\text{O}/\text{AcCN}$  + 0.5% formic acid  
ammoniumformate (20 mM)
- ✓ IS: Sulfamerazine



## ■ MS/MS conditions:

- ✓ Triple quadrupole (Q. Ultima, Micromass)
- ✓ ESI +
- ✓ MRM



- MS/MS conditions:

Compound	Precursor ion		Product ion	Cone voltage (V)	Collision energy (eV)
	Ion	<i>m/z</i>	<i>m/z</i>		
PYR	[M+H] <sup>+</sup>	249.1	233.1	30	30
SUL	[M+H] <sup>+</sup>	311.1	156.0	70*	15
IS	[M+H] <sup>+</sup>	265.2	110.0	35	14

Detuned to extend linear dynamic range

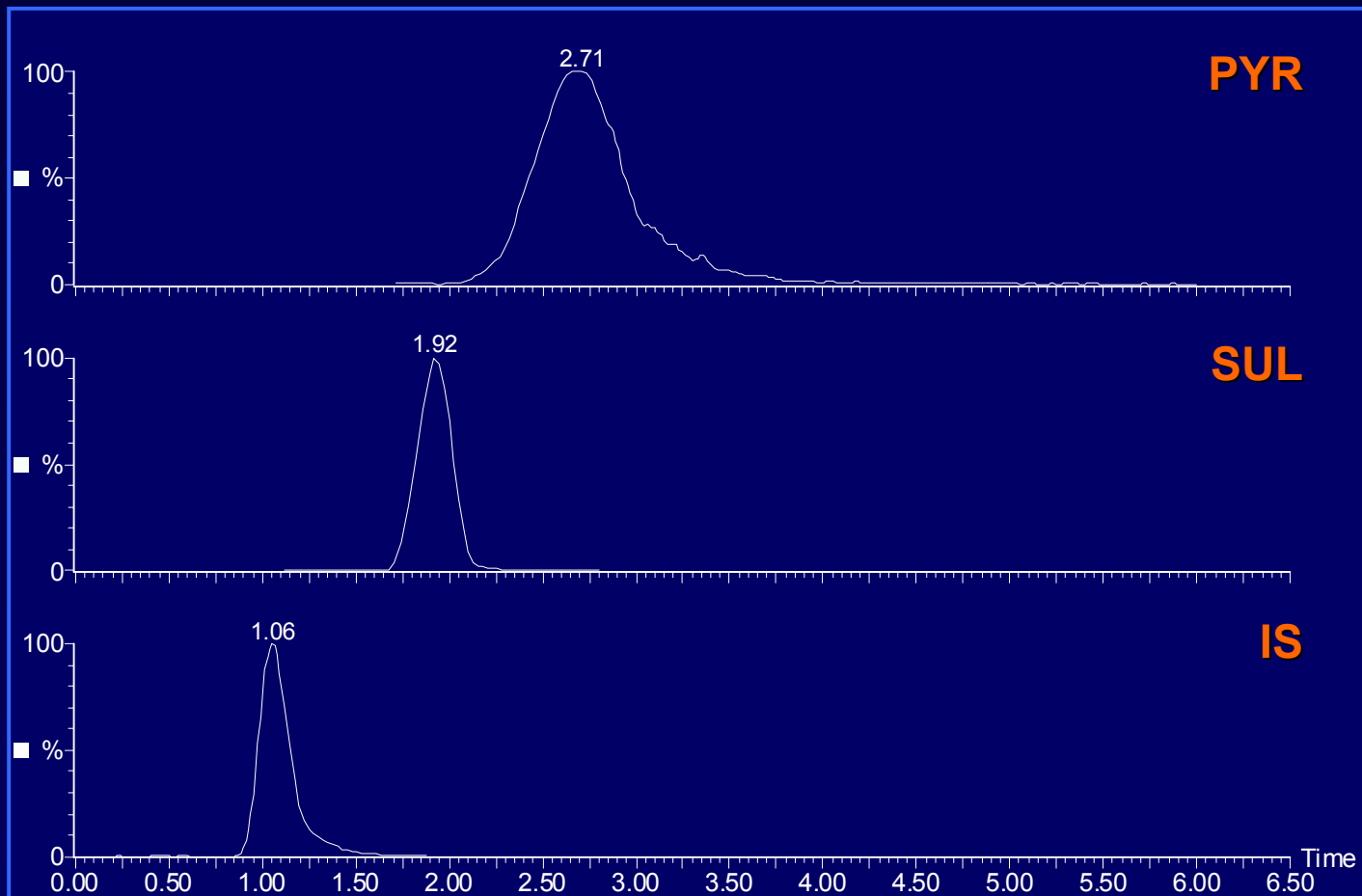




# LC-MS/MS method: results & discussion

Fortified  
plasma  
sample

40 ng/mL PYR  
4 µg/mL SUL



## Sample clean-up

« Single step - one tube »

- ✓ Fast, efficient, simple
- ✓ No unduly dilution



Clear supernatant

Sensitivity  
in bio-analysis ?

## Chromatography

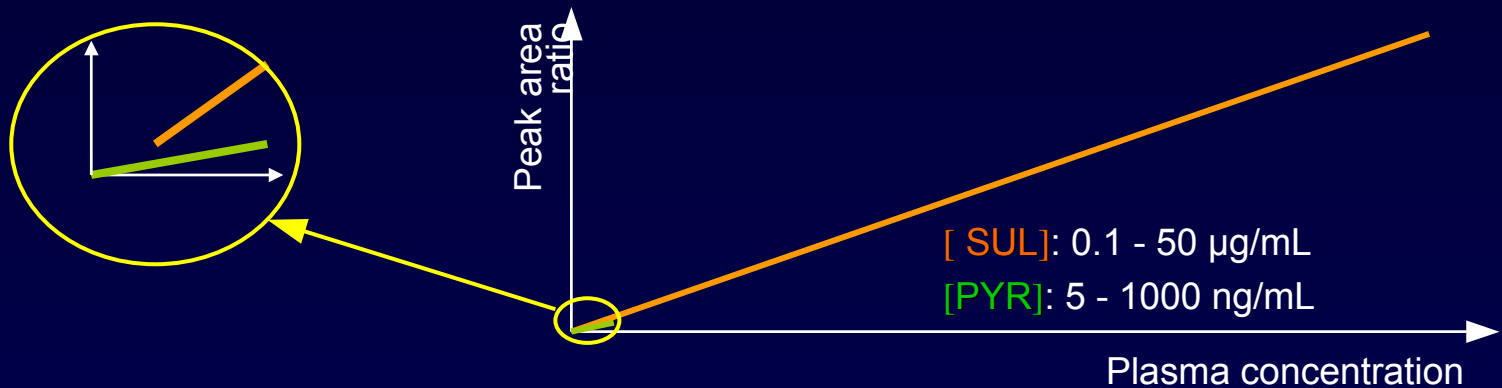
- ✓ Fast
- ✓ Partial separation
- ✓ 1 mm-column → sensitivity ↑

## MS/MS

- ✓ Selectivity → MRM
- ✓ Sensitivity



- **Low** [PYR]  $\leftrightarrow$  **High** [SUL]



*Problems:* carry-over  
saturation (ionisation and detector level)

⇒ Reconciliation in one-acquisition method ?

1. Quantitative interval
2. Detuning for SUL: cone voltage



# LC-MS/MS method: validation

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## ■ Linearity:

- ✓ Range: [SUL] 0.1 - 50 µg/mL  
[PYR] 5 - 1000 ng/mL
- ✓ Weighed linear regression: 1/x
- ✓ SUL R= 0.9978  
PYR R= 0.9984

■ **LOD:** SUL 0.01 µg/mL  
PYR 1 ng/mL

■ **LOQ:** Lowest calibration point

■ **Selectivity:** common antimalarial drugs + sulfonamides

MRM



- Other parameters:

Compound	SUL			PYR		
	Conc. level					
	µg/mL					
	1	10	50	10	100	1000
<b>Total reproducibility</b> (CV, %) (n=5)	9.8	9.7	6.5	14.9	8.5	8.2
<b>Within-day reproducibility</b> (CV, %) (n=7)	7.0	3.8	3.6	9.9	5.1	4.8
<b>Accuracy</b> (Recovery ± SD, %) (n=5)	103.7 ± 8.7	103.9 ± 6.9	100.2 ± 4.4	100.9 ± 6.2	100.7 ± 9.7	101.8 ± 4.7



# Conclusion

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- Sensitivity:
  - ✓ « No longer an issue » for majority of applications
  - ✓ Shift towards reproducibility and carry-over
- Trend: « fast analysis »
  - ✓ Tempting respons to ever increasing demands
  - ✓ However:

Rudimentary sample clean-up (+ fast chromatography)

≠

« Quick & dirty »

