

# Applications & Challenges in LCMS/MS for Anti-Infective drugs analysis

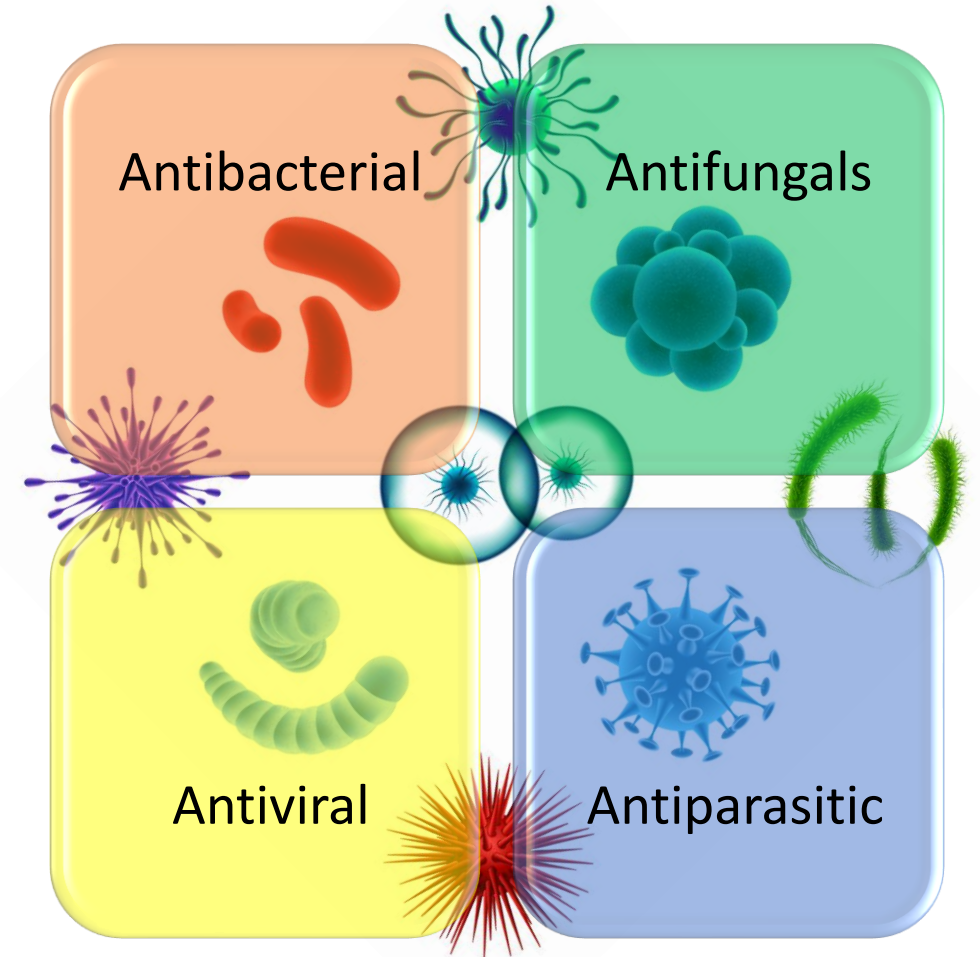
Ang May Yen | Application Manager, CSC.

9<sup>th</sup> April 2025



# What is Anti-infective agents?

- ⊕ Anti-infectives are medicines that work to prevent or treat infections.
- ⊕ Anti-infectives are crucial in modern healthcare, enabling the treatment and prevention of a wide range of infections, including serious ones like pneumonia or tuberculosis.
- ⊕ Optimal, timely and appropriate anti-infective therapy is an important public health issue to minimize the development of antimicrobial resistance in available agents.



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# Therapeutic Drug Monitoring (TDM)

A branch of clinical chemistry and pharmacology that specializes in the **measurement of medication concentrations** in blood, serum or plasma.

Improving patient care by **adjusting the dose of drugs** which have been shown to **improve outcome** in the general or special populations.

Therapeutic drugs monitoring aims to promote **optimum drugs treatment** by **maintaining serum drug concentration** within a “**Therapeutic Range**”

**Precision Medicine!!**

# Therapeutic Drug Monitoring (TDM) workflows

## Immunoassay workflows

- Advantages:
  - Fast, no extraction & simple handling.
- Challenges:
  - Limited number of drugs can be monitored.
  - Possible cross-reactivity between the monitored drug with its metabolites.
  - No multiplexing - 1 kit per compound.
  - Lacks Sensitivity.

## HPLC –UV workflows

- Advantages:
  - Multiplexing possible - as long as same group of drugs.
  - More types of drugs can be monitored.
- Challenges:
  - Require extensive sample preparation - Clean extract before injection.
  - Long analysis time per sample - all drugs compound analyze must be separated well.
  - Lacks Sensitivity

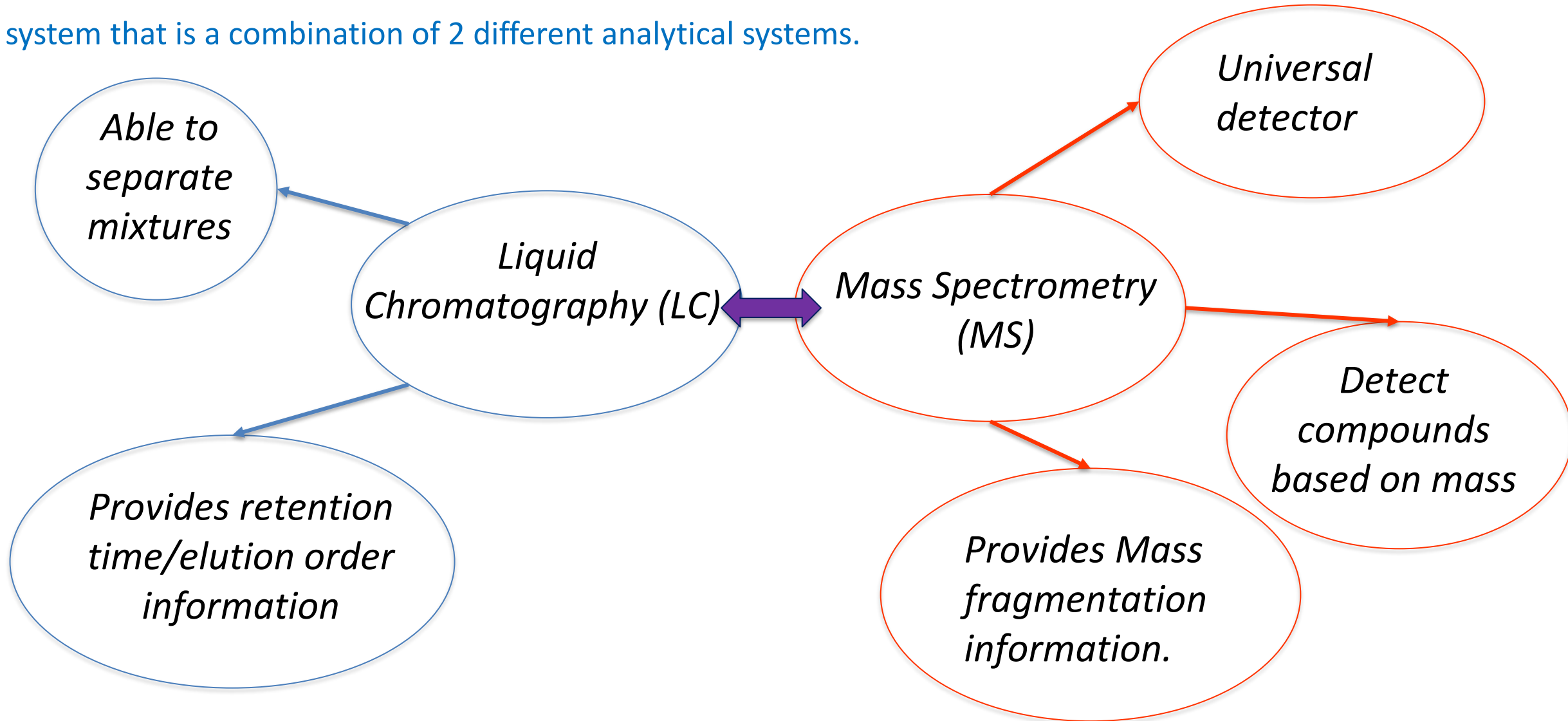
## LCMS/MS workflows

- Advantages
  - Multiplexing possible.
  - Short runtime
  - Less extensive sample preparation.
  - Highly Sensitive
- Challenges
  - Labor extensive - Possibility of Automation?
  - Requires specific internal standards to compensate matrix effects
  - Complex understanding required.

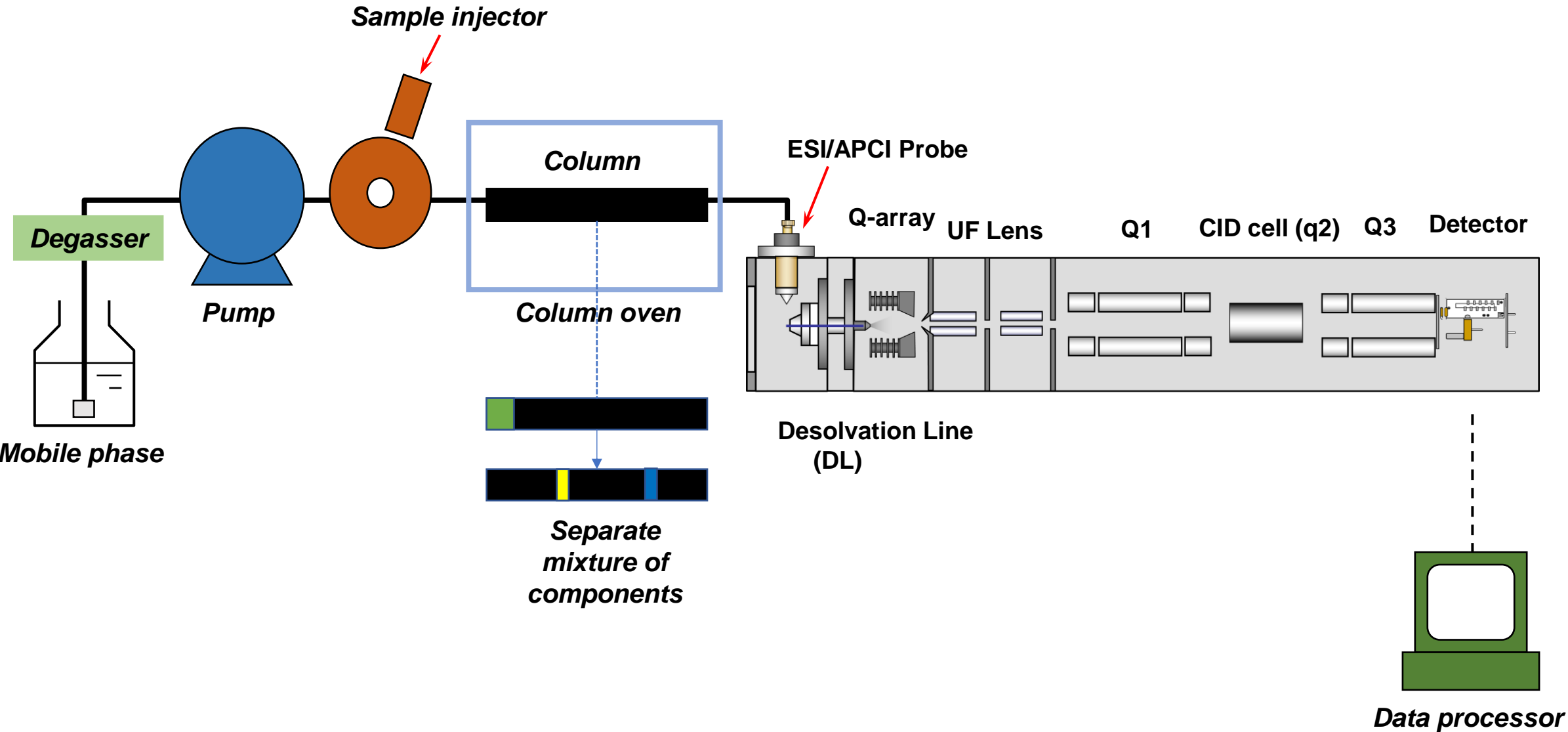
Highly  
Selective

# What is a Liquid Chromatography-Mass Spectrometry instrument?

A system that is a combination of 2 different analytical systems.



# LCMS/MS Schematic Diagram

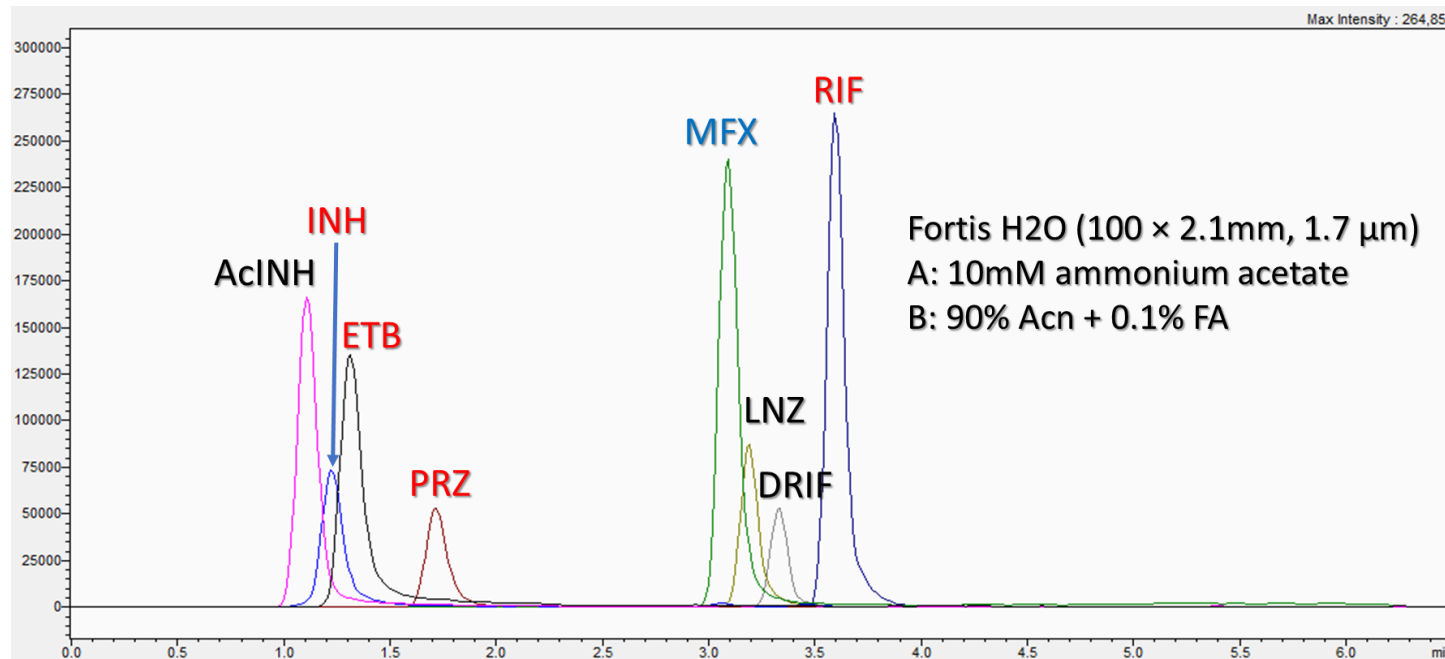


## **Advantages of using LCMS/MS in TDM of anti-infective drugs**



## A) Multiple analyte with fast analysis time

- ⊕ Able to analyze a multitude of drugs in one run.
- ⊕ Relatively short-run time as total separation of each drug during analysis is not required as identification, quantitation & confirmation are based on mass.

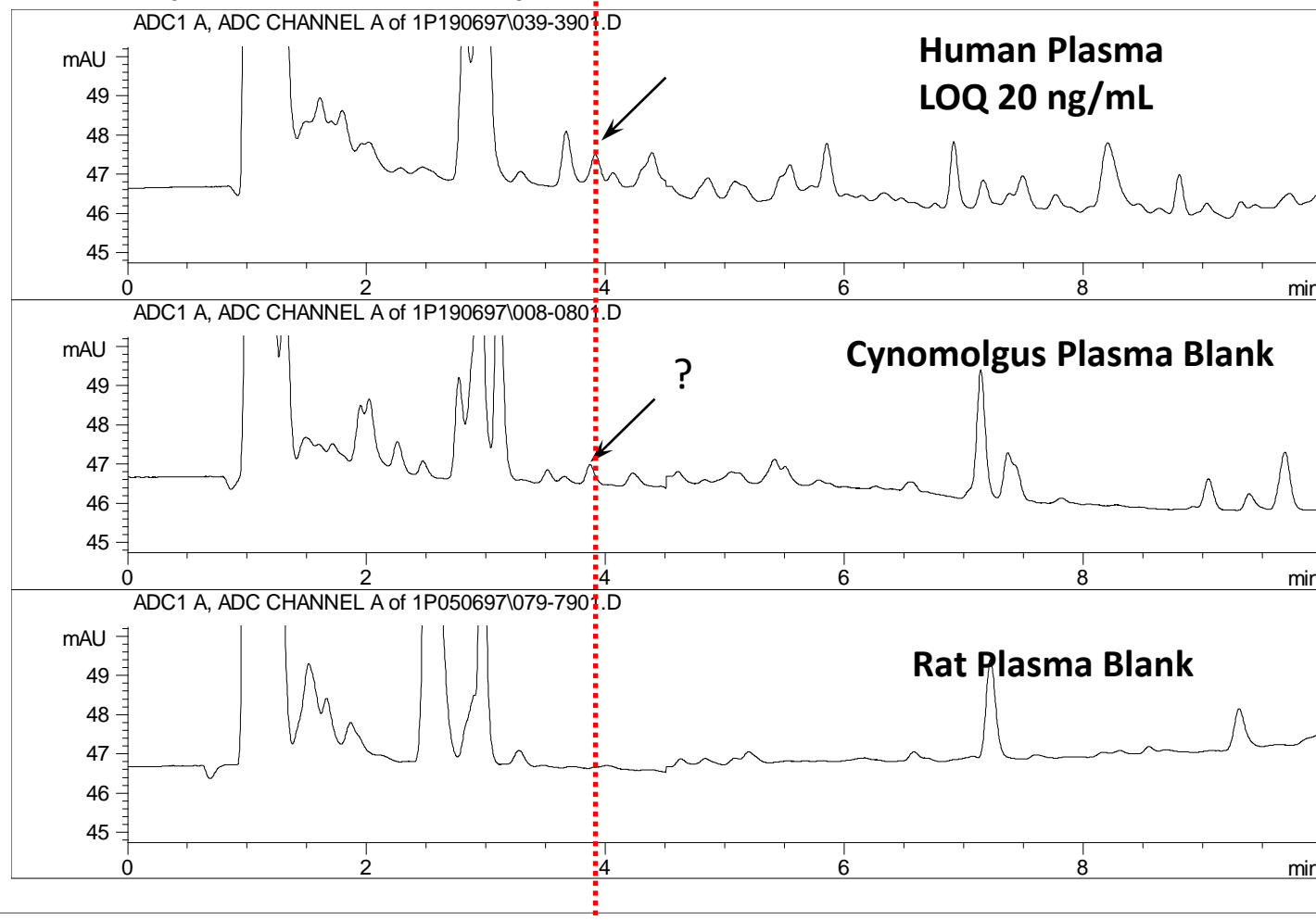


First- and second-line anti-TB drugs and metabolites. All in 1 method! All eluted by 4.0mins.



## B) Highly Selective & Sensitive analysis

- ⊕ Identification & quantitation in LCMS/MS analysis is carried out in MRM mode that ensures high selectivity and sensitivity.

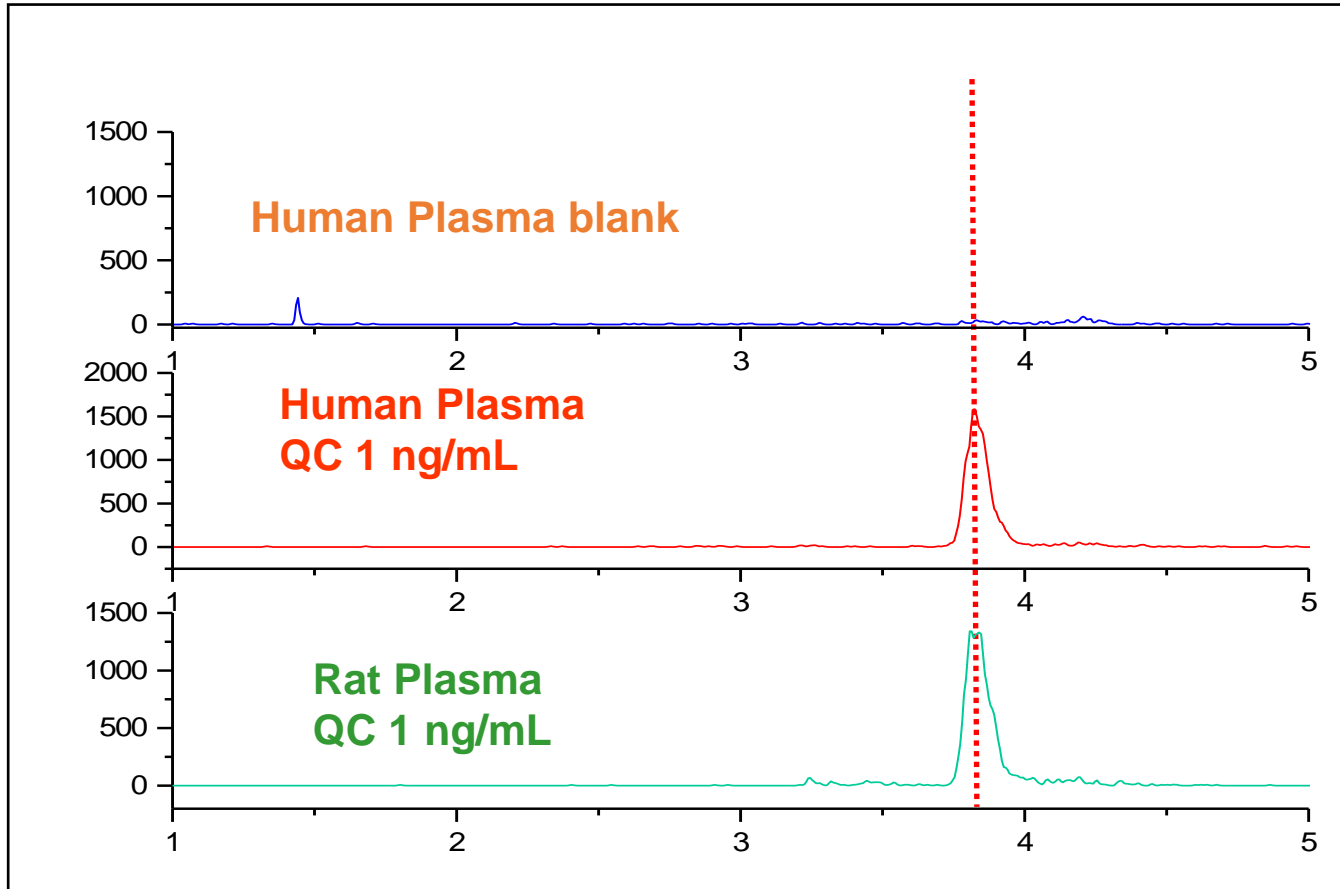


LC-UV Analysis of Methotrexate  
(Anti-metabolite drug)

*Slide from Prof G. Hopfgartner*

## B) Highly Selective & Sensitive analysis

- ☒ Identification & quantitation in LCMS/MS analysis is carried out in MRM mode that ensures high selectivity and sensitivity.

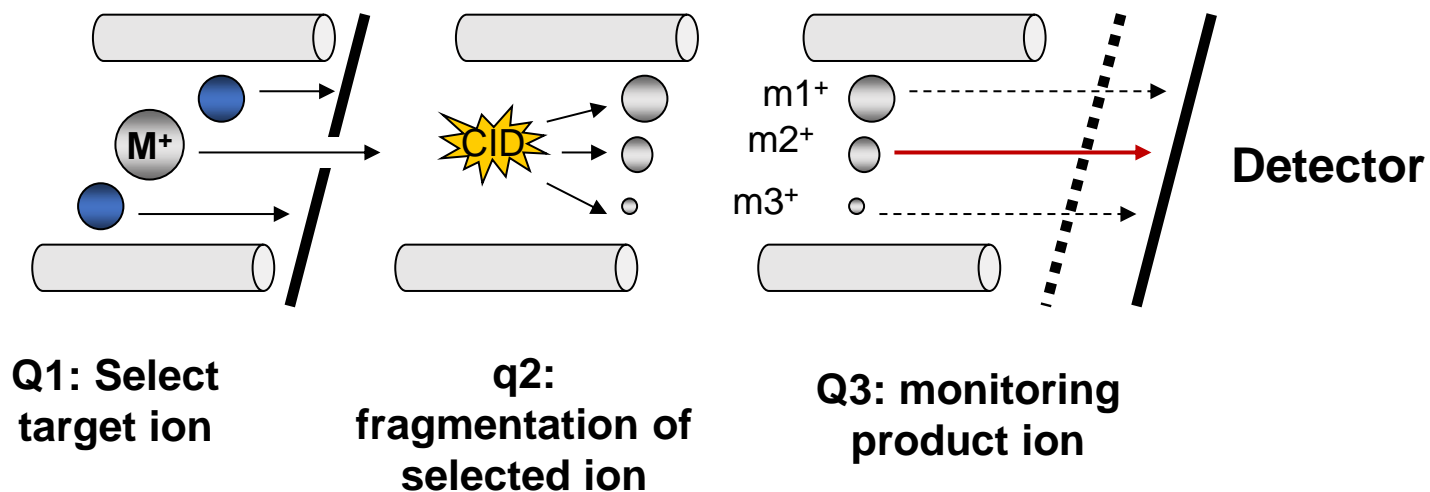


LCMS/MS Analysis of Methotrexate  
(Anti-metabolite drug)

*Slide from Prof G. Hopfgartner*

# What is MRM Mode?

- MRM (**Multiple Reaction Monitoring**) is the standard method of quantification on LC/MS/MS
- One MRM corresponds to a fragmentation reaction (elimination or neutral loss) of the precursor ion to a product ion.

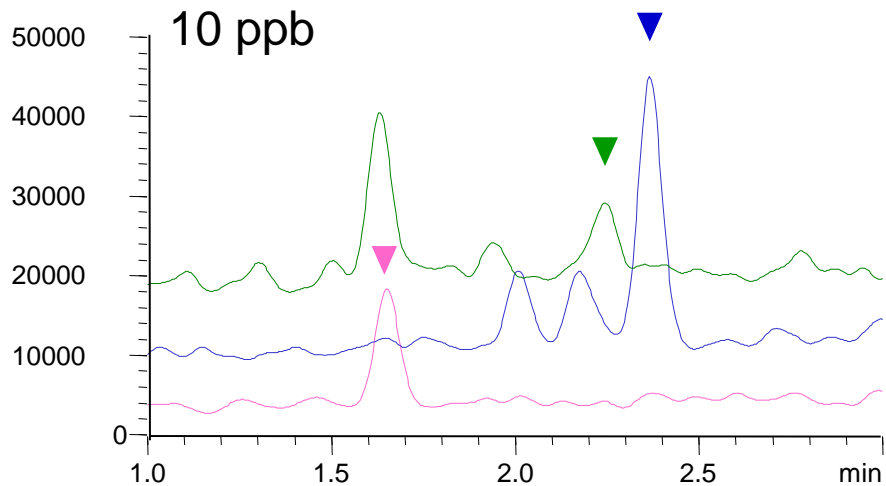


## Key Terminology:

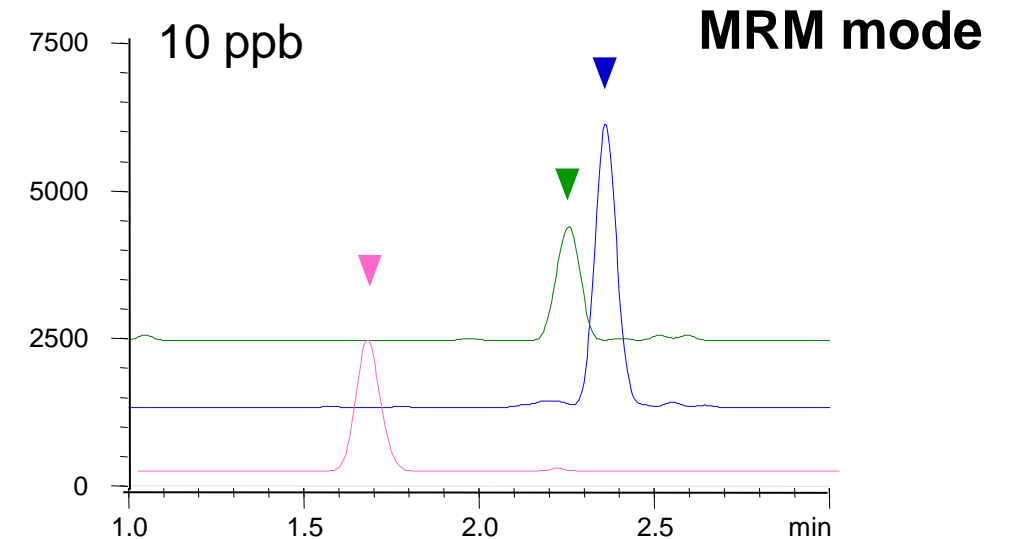
- Parent ion = precursor ion e.g.,  $[M+H]^+$
- Daughter ion = product ion
- Neutral loss = lost molecule in MRM like  $H_2O$ ,  $NH_3$ ,  $CH_3OH$  etc.
- CE optimization = obtain the best CE for a particular MRM

# Why is MRM mode adopted?

- MRM for quantitation has two outstanding advantages:
  - Extremely high mass selectivity (due to extreme low noise)
  - Highest sensitivity due to optimized CE value for every compound
- MRM offers a best method for trace-level quantitation of targets in complex matrix (food, biological samples etc.)



**High peak intensity and relatively high baseline and noise too, S/N is low.**



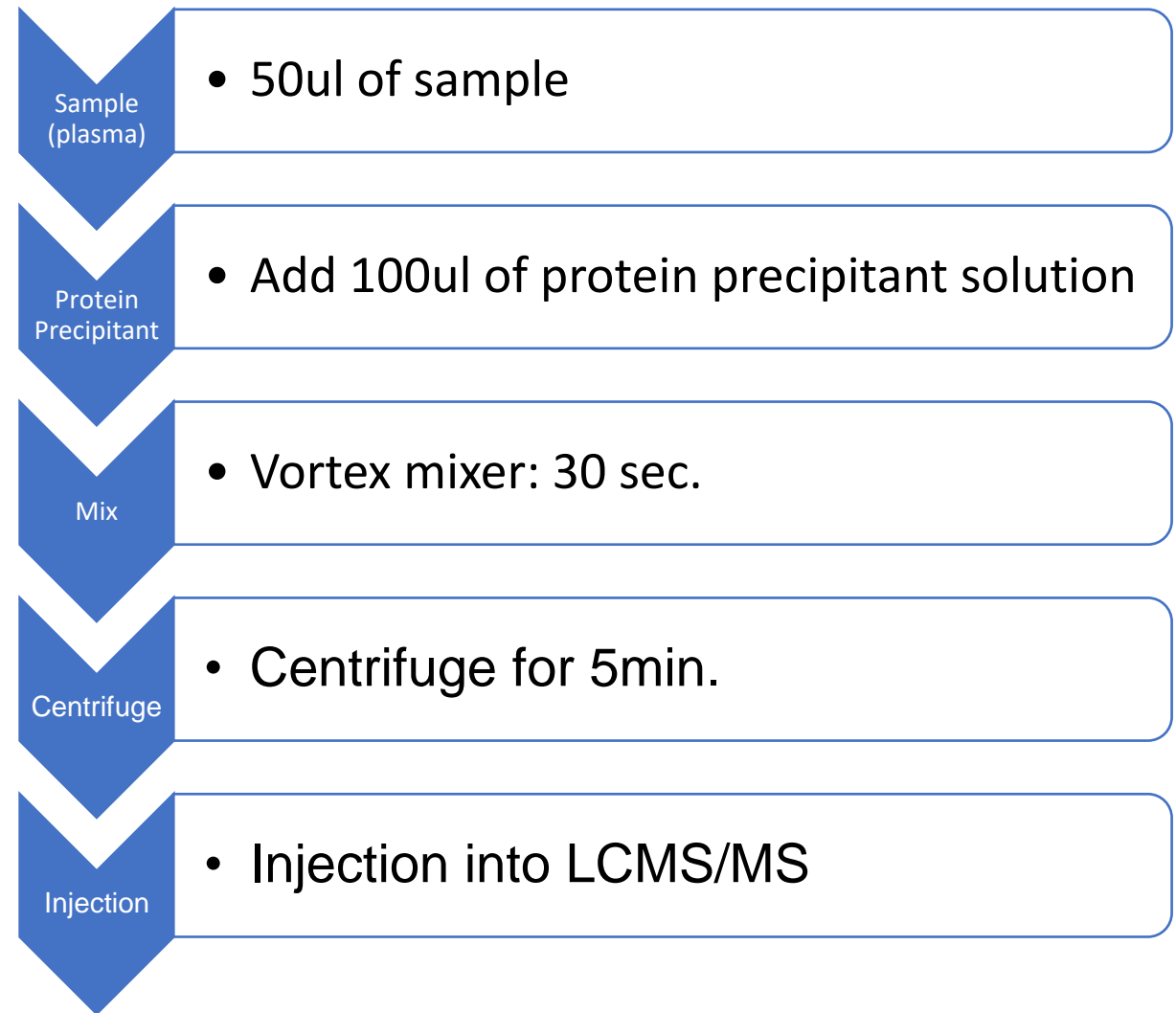
**Extremely low baseline and noise, S/N is significantly high.**

# **Challenges faced in LCMS/MS analysis for TDM of anti-infective drugs.**



## A) Labor Extensive

- For LCMS/MS analysis, it is still require for the biological sample to under go some simple extraction procedures like protein precipitation before it can be analyzed by the LCMS.
- The steps are relatively simple but can be time consuming when a large volume of samples needed to be processed.



## A) Labor Extensive

- Solution: Shimadzu has a setup that enables the automation of the sample pre-treatment (protein precipitation step). CLAM (Clinical Laboratory Automation Module)

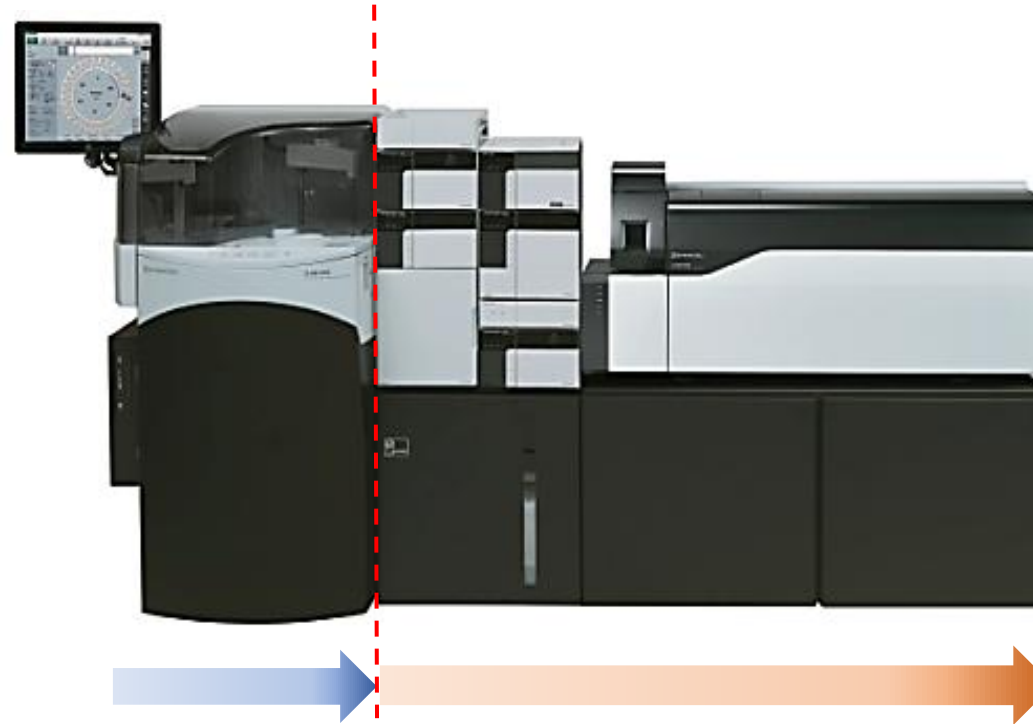
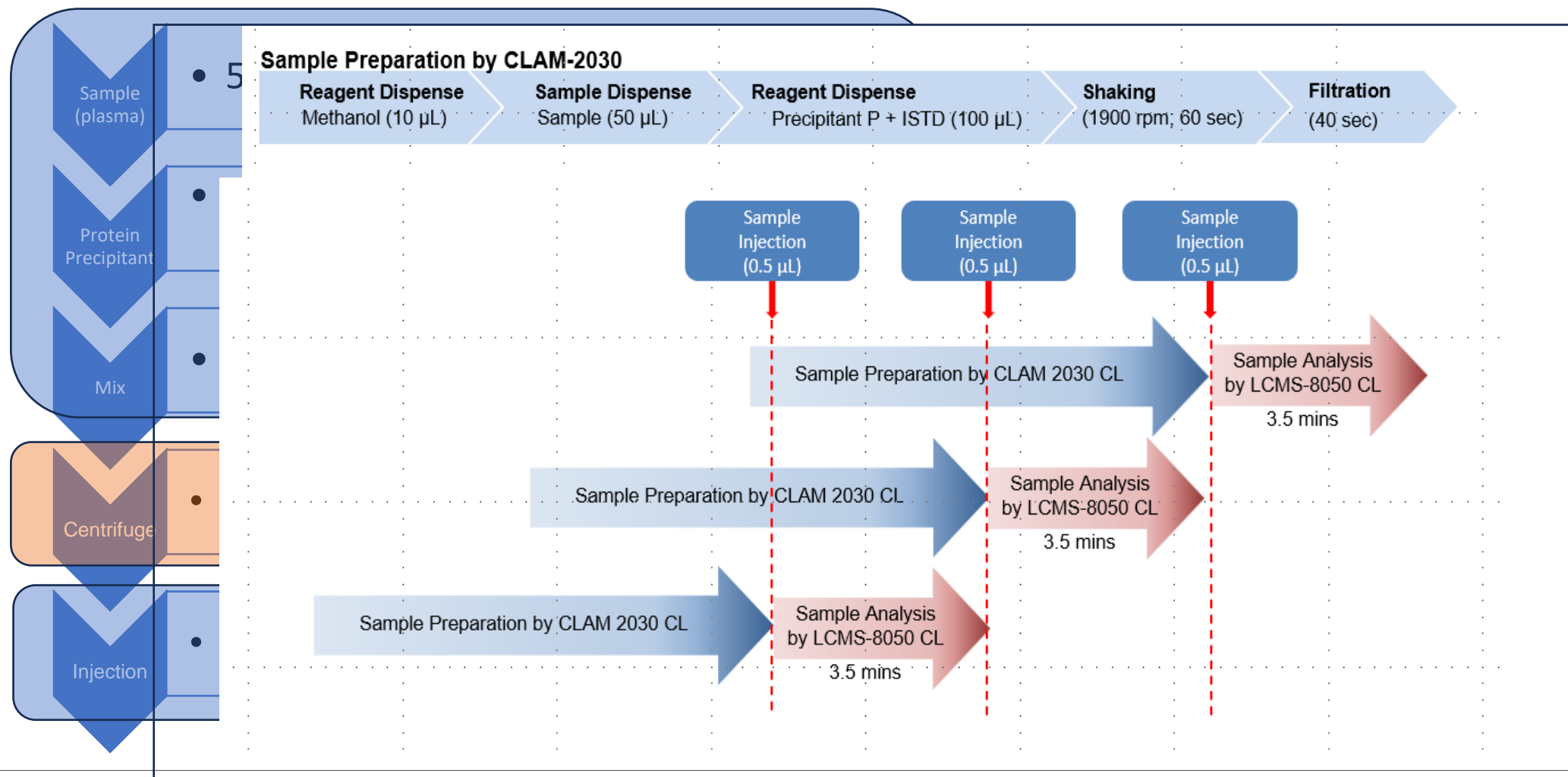


Figure 1. CLAM-2030 CL with LCMS-8050 CL.

- It is an on-line automated setup where the end-user just needs to put the plasma samples into the CLAM system. After the sample pre-treatment is completed, the extract is directly sent to the LCMS/MS for analysis.

# A) Labor Extensive

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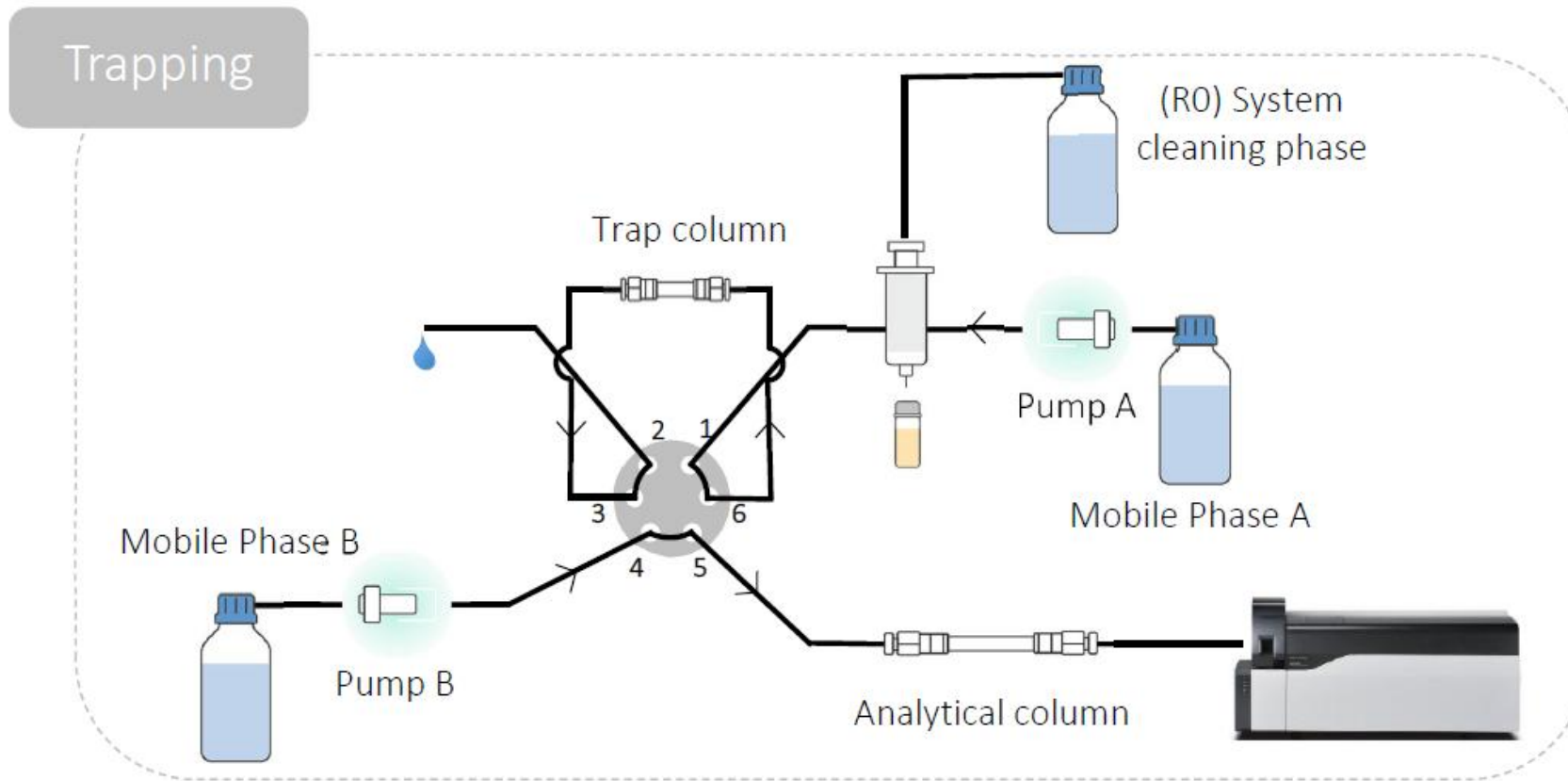


## B) Matrix Effect

- As the sample pretreatment is relatively simple, endogenous compounds including lipids, phospholipids, and fatty acids are not sufficiently removed from the blood/serum/plasma sample with protein precipitation.
- These compounds can interfere with the ionization process resulting in ionization suppression during LCMS/MS detection. This is commonly known as “Matrix Effect”.
- In ionization suppression, the signal produced in a biological matrix is lower than that in a neat solution, leading to an underestimation of the target value.“
- There are several common countermeasures:
  - Matrix calibration curve.
    - Standards & Internal Standards are spiked into the blank biological samples.
  - Isotope type Internal standards:
    - compounds where some atoms in the analyte are replaced with their stable isotopes (like  $2\text{H}/\text{D}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$ ) to aid in accurate quantification, particularly in mass spectrometry.

## B) Matrix Effect

- There are a few counter-measure that are normally taken:
  - Further sample pretreatment clean up - SPE or on-line SPE:
  - Example of on-line SPE:



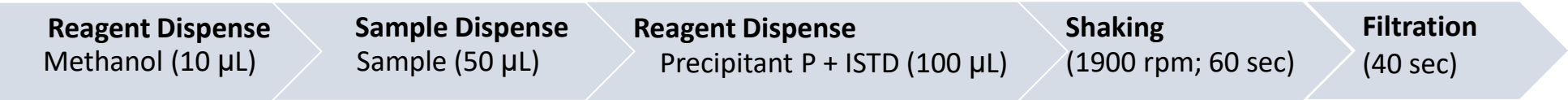
# Applications on TDM of Anti-infective drugs



# 1) Analysis of Antimycotics in Serum Using an Integrated Automated System: CLAM 2030 and LCMS-8050 with the RECIPE ClinMass® TDM Kit

- Eight types of antimycotics were monitored simultaneously in one method with a LCMS/MS run time of 3.5mins.

## Sample Pretreatment by CLAM-2030



## LCMS/MS Analytical Condition

UHPLC conditions : Nexera	
Injection Volume	: 0.5 µL
Oven Temperature	: 40 °C
LC Time Prog. (%B Conc.)	: 0.00 min (0 %) > 0.10 min (30 %) >
	2.10 min (60 %) > 2.20 min (98 %) >
	2.40 min (98 %) > 2.41 min (0 %) >
	3.50 min (0 %)
MS conditions : LCMS 8050	
Nebulizing Gas	: 3 L/min
Heating Gas	: 10 L/min
Interface	: 300 °C
Drying Gas	: 5 L/min
DL	: 250 °C
Heat Block	: 250 °C

## MRM Transitions

Analyte	MRM	Internal Standards (IS)	MRM
5-Fluorocytosine	130.10 > 57.95 130.10 > 113.00	13C,15N2-5-Fluorocytosine	133.10 > 115.00
Fluconazole	306.90 > 238.10 306.90 > 220.00	d4-Fluconazole	310.90 > 242.10
Isavuconazole	438.1 > 224.00 438.10 > 369.00	13C,d4-Isavuconazole	443.10 > 224.00
Itraconazole	705.20 > 392.20 705.20 > 432.20	d5-Itraconazole	710.20 > 397.20
Ketoconazole	531.10 > 489.10 531.10 > 244.00	d8-Ketoconazole	539.10 > 497.10
OH-Itraconazole	721.20 > 408.20 721.20 > 392.20	d5-Hydroxy-Itraconazole	726.20 > 413.20
Posaconazole	701.30 > 614.30 701.30 > 344.10	d4-Posaconazole	705.30 > 618.30
Voriconazole	350.00 > 281.00 350.00 > 127.00	d3-Voriconazole	353.00 > 284.00

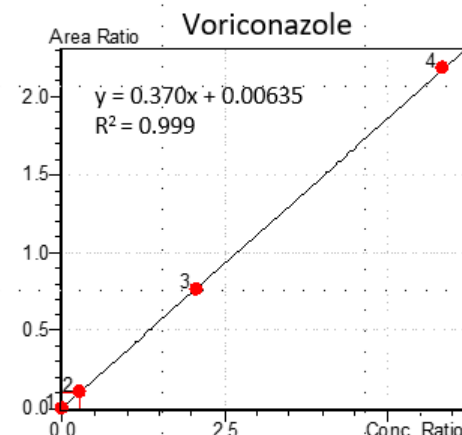
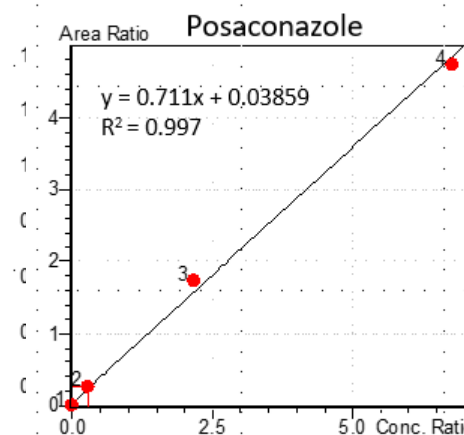
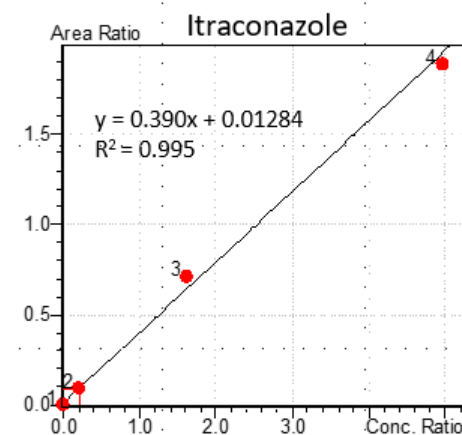
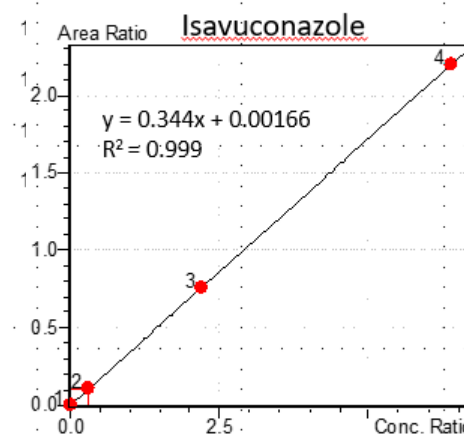
# 1) Analysis of Antimycotics in Serum Using an Integrated Automated System: CLAM 2030 and LCMS-8050 with the RECIPE ClinMass® TDM Kit

Concentrations of each calibration point. (Recipe kit)

Analyte	Concentration (mg/L)			
	Level 0	Level 1	Level 2	Level 3
5-Fluorocytosine	0.000	5.230	37.800	111.000
Fluconazole	0.000	0.669	4.710	14.100
Isavuconazole	0.000	0.458	3.500	10.200
Itraconazole	0.000	0.130	0.968	2.980
Ketoconazole	0.000	0.401	2.990	8.840
OH-Itraconazole	0.000	0.182	1.310	3.910
Posaconazole	0.000	0.231	1.750	5.420
Voriconazole	0.000	0.259	2.080	5.850

LOD & LOQ for each analytes.

Analyte	Cal 1 (mg/L)	S/N	LOD (mg/L)	LOQ (mg/L)
5-Fluorocytosine	5.230	124.32	0.1394	0.4224
Fluconazole	0.669	688.74	0.0031	0.0095
Isavuconazole	0.458	1532.12	0.0010	0.0030
Itraconazole	0.130	39.88	0.0103	0.0312
Ketoconazole	0.401	521.77	0.0025	0.0075
OH-Itraconazole	0.182	2579.98	0.0002	0.0007
Posaconazole	0.231	1937.71	0.0004	0.0012
Voriconazole	0.259	928.56	0.0009	0.0029



# 1) Analysis of Antimycotics in Serum Using an Integrated Automated System: CLAM 2030 and LCMS-8050 with the RECIPE ClinMass® TDM Kit

- Two levels of control samples provided in the RECIPE ClinCheck® kit—Level I (QC L) and Level II (QC H)—were injected four times daily for five days (n = 20).
- The accuracy and precision of each point were evaluated based on the calibration curve plotted daily. The accuracy for both QC standards for all compounds was within the range of 88 % to 119 % which complies with the RECIPE® guideline of 100 ± 20.
- The %CV for all compounds at both control levels remained below 6.97 % demonstrating good precision throughout the analysis period.

%Accuracy of Quality Control samples (n = 20).

Analyte	%Accuracy (n = 20)	
	QC L	QC H
5-Fluorocytosine	96-114	92-111
Fluconazole	97-116	91-115
Isavuconazole	93-109	85-112
Itraconazole	98-118	92-115
Ketoconazole	103-117	90-117
OH-Itraconazole	99-117	94-118
Posaconazole	108-119	90-119
Voriconazole	98-118	88-113

%CV of Quality Control samples (n = 20).

Analyte	% CV (n = 20)	
	QC L	QC H
5-Fluorocytosine	4.99	4.37
Fluconazole	4.24	6.09
Isavuconazole	3.68	6.56
Itraconazole	4.22	5.85
Ketoconazole	3.63	6.19
OH-Itraconazole	4.50	6.97
Posaconazole	2.83	6.73
Voriconazole	4.72	6.02

## 2) Analysis of Antibiotics in Serum / Plasma Using RECIPE® ClinMass® TDM Kit System with Fully Automated Sample Preparation LC/MS/MS System

- Eight types of antimycotics were monitored simultaneously in one method with a LCMS/MS run time of 3.5mins.

Fig. 2 Scheme fully automated sample preparation and analysis

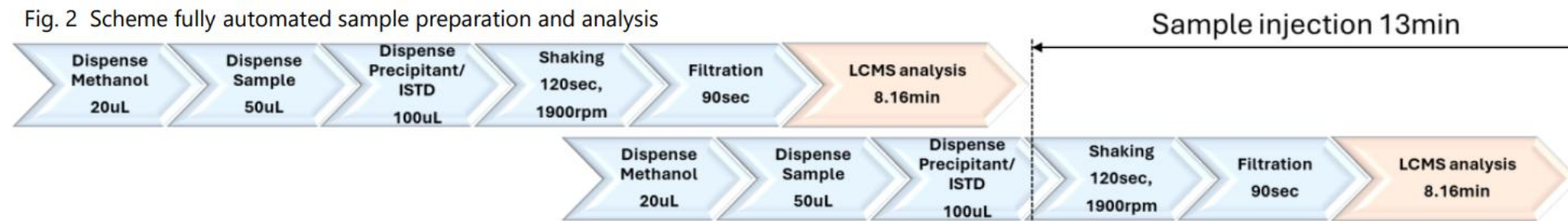


Table 1 Analytical conditions

Mass Spectrometer	: LCMS-8060
Ionization	: Electrospray Ionization (ESI), positive
Interface Voltage	: 1 kV
Heating Gas	: 10 L/min
DL Temp.	: 250 °C
Interface Temp.	: 300 °C
Nebulizing Gas	: 3 L/min
Drying Gas	: 10 L/min
Heat Block	: 400 °C
CID	: 270 kPa
UHPLC	: Nexera X3
Column Oven	: 40 °C
Injection Volume	: 7.0 µL, and automatic sample pretreatment for dilution
Flow rate	: 0.6 mL/min
Time Programme	: Binary gradient

Time (min)	Flow (mL/min)	Mobile Phase A (%)	Mobile Phase B (%)
Initial	0.6	100.0	0.0
0.01	0.6	100.0	0.0
0.50	0.6	100.0	0.0
3.00	0.6	97.5	2.5
6.50	0.6	50.0	50.0
7.00	0.6	50.0	50.0
7.10	1.0	20.0	80.0
8.10	1.0	20.0	80.0
8.15	1.0	100.0	0.0
8.16*	Stop		

\*The equilibration time at the end of the gradient has been shortened.

## 2) Analysis of Antibiotics in Serum / Plasma Using RECIPE® ClinMass® TDM Kit System with Fully Automated Sample Preparation LC/MS/MS System

Table 2 MRM transitions and parameters of the analytes and isotope-labelled substances

Analyte / IS	Quantifier MRM		Dwell Time	CE
	Precursor (m/z)	Product (m/z)		
<b>Ampicillin</b>	349.9	114.0	10	-28
<b>Cefazolin</b>	454.9	323.0	10	-20
<b>Cefepime</b>	481.0	86.9	10	-5
<b>Cefotaxime</b>	455.9	125.0	10	-20
<b>Clindamycin</b>	427.0	126.1	10	-15
<b>Daptomycin</b>	810.6	159.1	20	-43
<b>Flucloxacillin</b>	453.9	196.0	50	-34
<b>Linezolid</b>	338.0	235.1	10	-10
<b>Meropenem</b>	384.0	114.0	10	-11
<b>Piperacillin</b>	518.0	115.0	10	-33
<b>Vancomycin</b>	724.9	100.0	50	-37
<b>Cefuroxime</b>	423.1	317.8	50	15
<b>Chloramphenicol</b>	323.0	152.1	10	11
<b>Sulbactam</b>	232.2	140.1	10	17
<b>Tazobactam</b>	299.2	207.1	50	10
<b>d5-Ampicillin (1)</b>	355.0	114.0	10	-31
<b>d3-Cefepime (2)</b>	484.0	89.9	10	-15
<b>d3-Clindamycin (3)</b>	430.0	129.1	10	-18
<b>d3-Linezolid (4)</b>	341.0	235.1	10	-20
<b>d6-Meropenem (5)</b>	390.0	114.0	10	-25
<b>d5-Piperacillin (6)</b>	523.0	116.1	10	-55

Analyte / IS	Qualifier MRM		Dwell Time	CE
	Precursor [m/z]	Product [m/z]		
<b>Ampicillin</b>	350.1	159.9	10	-14
<b>Cefazolin</b>	454.9	156.0	10	-5
<b>Cefepime</b>	481.0	396.0	10	-25
<b>Cefotaxime</b>	455.9	395.9	10	-22
<b>Clindamycin</b>	427.0	377.1	10	-10
<b>Daptomycin</b>	810.6	341.0	20	-27
<b>Flucloxacillin</b>	453.9	238.1	50	-23
<b>Linezolid</b>	338.0	296.1	10	-35
<b>Meropenem</b>	384.0	141.1	10	-40
<b>Piperacillin</b>	518.0	143.1	10	-33
<b>Vancomycin</b>	724.9	144.5	50	-14
<b>Cefuroxime</b>	422.9	207.0	50	15
<b>Chloramphenicol</b>	323.1	256.9	10	11
<b>Sulbactam</b>	232.2	188.0	10	17
<b>Tazobactam</b>	299.0	255.1	50	10



## 2) Analysis of Antibiotics in Serum / Plasma Using RECIPE® ClinMass® TDM Kit System with Fully Automated Sample Preparation LC/MS/MS System

Linearity evaluation, including LLOQ / LOD

Analyte	Linear Range (mg/L)	R <sup>2</sup>	LLOQ (mg/L)	LOD (mg/L)	CV (%)	Bias (%)
Ampicillin	0.19 – 45.6	0.998	0.19	0.06	4.9	10.0
Cefazolin	2.69 - 144	0.992	2.69	0.90	2.8	3.9
Cefepime	1.24 - 148	0.997	1.24	0.41	3.3	1.9
Cefotaxime	0.78 - 44.3	0.995	0.78	0.23	0.6	-13.7
Cefuroxime	2.48 - 153	0.992	2.48	0.83	4.8	3.0
Chloramphenicol	0.43 – 24.8	0.999	0.43	0.14	6.1	5.2
Clindamycin	0.09 – 9.42	0.999	0.09	0.03	1.4	-12.9
Daptomycin	1.68 - 196	0.994	1.68	0.56	13.0	-9.9
Flucloxacillin	1.51 – 94.3	0.998	1.51	0.50	3.4	-7.7
Linezolid	0.12 – 28.3	1.000	0.12	0.04	10.7	6.8
Meropenem	1.72 - 107	0.998	1.72	0.57	3.7	0.9
Piperacillin	0.76 - 96.4	0.999	0.76	0.25	8.6	-14.2
Sulbactam	0.55 - 62.7	0.998	0.55	0.18	2.5	10.1
Tazobactam	0.12 – 28.3	1.000	0.12	0.04	3.8	12.3
Vancomycin	0.37 – 47.8	0.995	0.37	0.12	8.1	13.3

## 2) Analysis of Antibiotics in Serum / Plasma Using RECIPE® ClinMass® TDM Kit System with Fully Automated Sample Preparation LC/MS/MS System

- The trueness was determined by 4-fold analysis of two different quality control (QC) samples in a single analysis sequence.
- The results (precision in CV% and deviation from the target in % Bias) are summarized in the table.
- The acceptance criteria of CV<15% (<20% near LLOQ) and Bias  $\pm$ 20% were fulfilled.

Analyte	Sample	Target value (mg/L)	Measured value (mg/L); Mean (n=4)	CV (%)	Bias (%)
Ampicillin	MS9782 lot 2063, Level I	2.19	2.34	1.7	6.9
	MS9782 lot 2063, Level II	22.1	23.9	2.1	8.2
Cefazolin	MS9782 lot 2063, Level I	6.40	7.27	3.8	13.6
	MS9782 lot 2063, Level II	67.8	66.9	6.6	-1.3
Cefepime	MS9782 lot 2063, Level I	7.03	7.50	4.9	6.0
	MS9782 lot 2063, Level II	69.9	74.9	5.2	7.1
Cefotaxime	MS9782 lot 2063, Level I	2.47	2.51	3.3	1.7
	MS9782 lot 2063, Level II	22.9	24.3	1.9	6.3
Cefuroxime	MS9782 lot 2063, Level I	6.68	6.87	2.9	2.9
	MS9782 lot 2063, Level II	69.9	62.7	3.8	-10.3
Chloramphenicol	MS9782 lot 2063, Level I	1.31	1.40	5.3	7.1
	MS9782 lot 2063, Level II	12.3	11.9	3.4	-3.6
Clindamycin	MS9782 lot 2063, Level I	0.52	0.54	1.0	2.9
	MS9782 lot 2063, Level II	4.87	4.91	1.1	0.8
Daptomycin	MS9782 lot 2063, Level I	10.5	12.1	3.3	14.8
	MS9782 lot 2063, Level II	112	106	4.6	-4.9
Flucloxacillin	MS9782 lot 2063, Level I	4.81	4.54	2.5	-5.5
	MS9782 lot 2063, Level II	53.2	50.1	2.2	-5.9
Linezolid	MS9782 lot 2063, Level I	1.45	1.25	4.0	-13.7
	MS9782 lot 2063, Level II	14.1	14.1	1.3	0.1
Meropenem	MS9782 lot 2063, Level I	5.28	5.13	1.7	-2.9
	MS9782 lot 2063, Level II	54.8	52.7	2.0	-3.9
Piperacillin	MS9782 lot 2063, Level I	4.95	5.40	2.5	9.1
	MS9782 lot 2063, Level II	49.8	51.2	4.0	2.8
Sulbactam	MS9782 lot 2063, Level I	3.23	3.42	2.6	5.9
	MS9782 lot 2063, Level II	31.6	32.7	3.0	3.4
Tazobactam	MS9782 lot 2063, Level I	1.43	1.48	2.5	3.7
	MS9782 lot 2063, Level II	14.2	14.3	1.1	0.9
Vancomycin	MS9782 lot 2063, Level I	2.41	2.18	4.7	-9.7
	MS9782 lot 2063, Level II	25.7	24.6	4.2	-4.4

Thank You  
Q & A

