

Application News

Liquid Chromatograph Mass Spectrometer LCMS-8060

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

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User Benefits

- ◆ Full solution provided by Shimadzu and RECIPE®
- ◆ Fully automated sample preparation
- ◆ Verified method for RECIPE® ClinMass® TDM Kit System for Antibiotics in Serum / Plasma

Introduction

Dosage recommendations for antibiotics approved for use in humans are based on the manufacturers instructions.

Despite existing guidelines from medical societies, the rational use of antibiotics in the outpatient setting still leads to problematic patient compliance, with consequences for the success of individual therapy and the development of resistance.

In the clinical setting, there is the possibility of targeted antibiotic therapy, defined after the determination of bacterial resistance (antibiogram). In the it, the sensitivity of the pathogen for various type of antibiotics is tested. Depending on the results, therapy is given based on the Minimal Inhibitory Concentration (MIC). Recommendations are based on the fourfold MIC value. Under clinically controlled conditions, monitoring of blood levels by Therapeutic Drug Monitoring (TDM) can be used to adjust an optimized blood concentration to ensure efficacy and avoid toxicity.

TDM of antibiotics is therefore an essential prerequisite for the successful as well as individualized treatment.

RECIPE's fully validated analytical method provides the quantification of 15 Antibiotics (Table 2) for TDM using LC-MS/MS.^[1] By addition of the Shimadzu CLAM (Clinical Laboratory Automated sample preparation Module) in front of the LC-MS/MS system (Figure 1) the required sample preparation could be fully automated which achieves results on a fast and high-precision analytical workflow.

To prove that the automated sample preparation leads to reliable results a method verification procedure was evaluated according to the CLSI Guidelines EP06-A, EP15-A3, EP17-A2.

15 antibiotics in serum were verified using the ClinMass® TDM Platform (order no. MS9000) in combination with the ClinMass® Add-on Set for Antibiotics in Serum / Plasma (order no. MS9700) (RECIPE®, Germany).

Lyophilized, matrix-based calibrator and control samples were reconstituted, aliquoted and stored until use.

Then the samples were loaded directly into the CLAM-2040. It was programmed to perform protein precipitation using Precipitant P including internal standards from the ClinMass® TDM Kit System for Antibiotics followed by filtration and sample collection. The sample is then transported using an arm from the CLAM-2040 to the LC without human intervention for LC-MS/MS analysis. Due to overlapped sample preparation (Figure 2) and analysis the throughput was one complete analysis each 13 min. Analytical conditions are listed in Table 1. The optimized MRM transitions are summarized in Table 2.

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.



Fig. 1 CLAM LCMS TQ

Materials and Methods

Fast, sensitive and robust LC-MS/MS systems provide the basis for routine analysis in clinical laboratories. For the described verification, a Shimadzu CLAM-2040 coupled with a Nexera X3 UHPLC system and a LCMS-8060 triple-quadrupole mass spectrometer was used.

Fig. 2 Scheme fully automated sample preparation and analysis

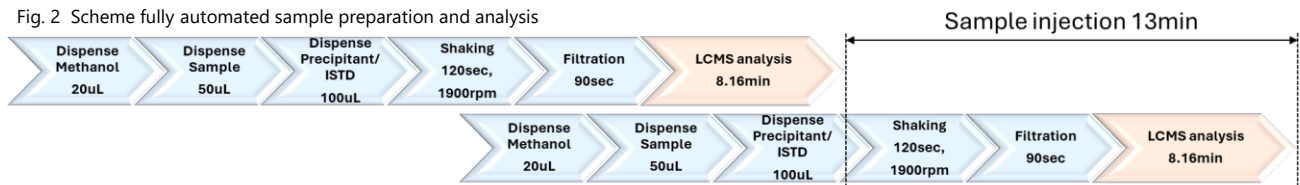


Table 2 MRM transitions and parameters of the analytes and isotope-labelled substances

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

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

■ Results

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 Table 3. The acceptance criteria of CV<15% (<20% near LLOQ) and Bias \pm 20% were fulfilled.

For determination of the linearity and the lower limit of quantification (LLOQ) several dilutions of the CliniCal® Serum Calibrator Set lyophil. for Antibiotics (order no. MS9713, RECIPE®, Germany) were prepared in 3-fold and analyzed in a single analysis sequence. The results for linearity evaluation and for the LLOQ are summarized in Table 4.

The acceptance criteria used to define LLOQ were the precision with a CV<20% and the Bias \pm 20%. The criteria for linearity were the precision with a CV<15% and the Bias \pm 15%.

To determine the precision intraday two different levels of QC samples were prepared in 8-fold and analysed in a single analysis sequence. And for the intraday precision, the 2 QC samples were prepared in 5-fold and analysed in a single analysis sequence on 3 days. The intra and interassay precision for each level is summarized in Table 5 and 6. The acceptance criteria of CV<15% (<20% near LLOQ) was fulfilled.

Table 3 Trueness of measurement

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

Table 4 Linearity evaluation, including LLOQ / LOD

Analyte	Linear Range (mg/L)	R ²	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

Table 5 Intraassay results [CV%]

Analyte	Sample	Target value (mg/L)	Measured value (mg/L); Mean (n=8)	CV (%)
Ampicillin	MS9782 lot 2063, Level I	2.19	2.36	1.5
	MS9782 lot 2063, Level II	22.1	23.4	4.3
Cefazolin	MS9782 lot 2063, Level I	6.40	7.25	2.7
	MS9782 lot 2063, Level II	67.8	64.8	6.3
Cefepime	MS9782 lot 2063, Level I	7.03	7.40	3.7
	MS9782 lot 2063, Level II	69.9	73.0	4.6
Cefotaxime	MS9782 lot 2063, Level I	2.47	2.53	2.8
	MS9782 lot 2063, Level II	22.9	24.7	2.5
Cefuroxime	MS9782 lot 2063, Level I	6.68	6.81	3.0
	MS9782 lot 2063, Level II	69.9	60.5	5.0
Chloramphenicol	MS9782 lot 2063, Level I	1.31	1.36	5.3
	MS9782 lot 2063, Level II	12.3	11.6	3.3
Clindamycin	MS9782 lot 2063, Level I	0.52	0.53	1.1
	MS9782 lot 2063, Level II	4.87	4.93	1.3
Daptomycin	MS9782 lot 2063, Level I	10.5	11.9	4.1
	MS9782 lot 2063, Level II	112	103	4.7
Flucloxacillin	MS9782 lot 2063, Level I	4.81	4.47	2.4
	MS9782 lot 2063, Level II	53.2	49.4	4.0
Linezolid	MS9782 lot 2063, Level I	1.45	1.38	11.4
	MS9782 lot 2063, Level II	14.1	14.0	3.5
Meropenem	MS9782 lot 2063, Level I	5.28	5.15	1.4
	MS9782 lot 2063, Level II	54.8	52.5	3.1
Piperacillin	MS9782 lot 2063, Level I	4.95	5.28	3.7
	MS9782 lot 2063, Level II	49.8	49.1	5.4
Sulbactam	MS9782 lot 2063, Level I	3.23	3.33	3.9
	MS9782 lot 2063, Level II	31.6	32.4	2.5
Tazobactam	MS9782 lot 2063, Level I	1.43	1.49	2.0
	MS9782 lot 2063, Level II	14.2	14.2	1.7
Vancomycin	MS9782 lot 2063, Level I	2.41	2.19	4.4
	MS9782 lot 2063, Level II	25.7	25.0	4.3
	MS9782 lot 2063, Level II	67.8	49.4	4.0

Table 6 Interassay results [CV%]

Analyte	Sample	Target value (mg/L)	Measured value (mg/L); Mean (n=5)	CV (%)
Ampicillin	MS9782 lot 2063, Level I	2.19	2.28	3.8
	MS9782 lot 2063, Level II	22.1	23.0	4.5
Cefazolin	MS9782 lot 2063, Level I	6.40	7.32	3.9
	MS9782 lot 2063, Level II	67.8	70.3	7.3
Cefepime	MS9782 lot 2063, Level I	7.03	7.22	4.3
	MS9782 lot 2063, Level II	69.9	72.0	4.5
Cefotaxime	MS9782 lot 2063, Level I	2.47	2.54	2.7
	MS9782 lot 2063, Level II	22.9	24.7	3.0
Cefuroxime	MS9782 lot 2063, Level I	6.68	6.70	3.3
	MS9782 lot 2063, Level II	69.9	62.3	3.2
Chloramphenicol	MS9782 lot 2063, Level I	1.31	1.33	6.5
	MS9782 lot 2063, Level II	12.3	11.7	3.4
Clindamycin	MS9782 lot 2063, Level I	0.52	0.538	1.6
	MS9782 lot 2063, Level II	4.87	4.95	2.2
Daptomycin	MS9782 lot 2063, Level I	10.5	11.8	5.5
	MS9782 lot 2063, Level II	112	105	3.9
Flucloxacillin	MS9782 lot 2063, Level I	4.81	4.79	5.7
	MS9782 lot 2063, Level II	53.2	49.1	3.8
Linezolid	MS9782 lot 2063, Level I	1.45	1.38	9.2
	MS9782 lot 2063, Level II	14.1	14.0	2.5
Meropenem	MS9782 lot 2063, Level I	5.28	5.08	2.4
	MS9782 lot 2063, Level II	54.8	52.0	2.7
Piperacillin	MS9782 lot 2063, Level I	4.95	5.21	3.9
	MS9782 lot 2063, Level II	49.8	49.6	3.4
Sulbactam	MS9782 lot 2063, Level I	3.23	3.32	3.9
	MS9782 lot 2063, Level II	31.6	32.2	3.6
Tazobactam	MS9782 lot 2063, Level I	1.43	1.47	3.9
	MS9782 lot 2063, Level II	14.2	13.9	2.5
Vancomycin	MS9782 lot 2063, Level I	2.41	2.33	8.7
	MS9782 lot 2063, Level II	25.7	26.2	5.5

■ Conclusion

The ClinMass® TDM Kit System for Antibiotics in Serum / Plasma (order no. MS9000 and MS9700) was successfully verified on the CLAM-2040 with the analytical system LCMS-8060 from Shimadzu.

All 15 analytes passed the acceptance criteria for accuracy (trueness, precision) and linearity.

The lower limit of quantification (LLOQ) was below published clinical reference ranges.

■ References

1. Instruction Manual, ClinMass® TDM Kit System, Antibiotics in Serum / Plasma, RECIPE® Chemicals + Instruments GmbH



Shimadzu Corporation
www.shimadzu.com/an/

SHIMADZU Europa GmbH,
www.shimadzu.eu

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The analysis method described is intended solely to illustrate the potential application opportunities.

In the case of a potential clinical application, follow the instructions on the ClinMass® TDM Kit System.

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