

A Rapid and Simple HPLC-MS/MS Method for the Quantitative Determination of Colistin for Therapeutic Drug Monitoring in Clinical Practice

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Abstract: Colistin is the last-line option for the treatment of multidrug-resistant gram-negative bacterial infections with narrow therapeutic window. It is essential to ensure its efficacy and safety by therapeutic drug monitoring (TDM). Quantitative determination of colistin is difficult due to its complex ingredients. Previous determination methods demand intricate sample pre-treatment which are not only time-consuming but also costly, and is difficult to apply in clinical practice. Therefore, in order to carry out quantitative determination of colistin accurately and quickly, we establish a rapid high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) with simple sample pre-treatment process. The sample was purified by acetonitrile to remove the plasma protein. Then purified colistin was effectively separated from terfenadine, an internal standard (IS) using Phenomenex Kinetex C18 column (50.0×2.1mm, 5µm) with acetonitrile and water mobile phase at a flow rate of 0.5 mL/min and 40°C column temperature. Colistin and IS were monitored in positive ion mode. Our method expressed good linearity in 50.0–6000 ng/mL of colistin B and 28.31–3397.51 ng/mL of colistin A in plasma. Methodology validations, including selectivity, precision, accuracy, recovery, stability, matrix effect, and dilution integrity met acceptance criteria of *Bioanalytical Method Validation (MI0)* of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH).

Keywords: colistin, HPLC-MS/MS, human plasma, protein precipitation, rapid and simple detection

Introduction

Polymyxins, isolated from the culture medium *Paenibacillus polymyxa*, are well-known lipopeptide antibiotics which are used to treat Gram-negative pathogenic infections. Polymyxins mainly contain five subtypes (A, B, C, D, and E). In 1947, polymyxin A and polymyxin D were firstly discovered in United States and England.^{1,2} Then other groups of polymyxins, polymyxin B, C and E, were subsequently developed.³ Among them, polymyxin B and polymyxin E (colistin) were further developed for clinical practice due to their least nephrotoxic in vivo models compared to other types of polymyxins. Polymyxin B is used as polymyxin B sulfate for intravenous administration in clinical practice.⁴ As the inactive prodrug of colistin, colistimethate (CMS) was reported less toxic an irritant at the injection site compared with the parent antibiotic and had similar in vivo antibacterial activity.⁵ Then CMS was authorized by the US FDA in 1959^{6,7} and have been widely used in daily clinical practice during 1960s and 1970s,^{8,9} whereas colistin sulfate has been available for intravenous administration only in China.⁵ However, since the 1970s, with the advent of novel, more active and less toxic antibiotics, such as β-lactams, quinolones and aminoglycosides, the clinical use of polymyxins limited due to the highly frequent nephrotoxicity and neurotoxicity. Nowadays, along with gram-negative pathogens gradually displaying multidrug-resistant and extensively drug-resistant,¹⁰ polymyxins were reused as the last-line options to treat infections caused by multidrug-resistant Gram-negative bacteria. However, polymyxins have a narrow therapeutic

window because of their concentration-dependent nephrotoxicity¹¹ and neurotoxicity,¹² so therapeutic drug monitoring (TDM) for dose adjustment is necessary in clinical use.¹³

There are several techniques that have been reported to determine colistin in plasma, such as microbiological assays¹⁴ and chromatographic methods including capillary electrophoresis chromatographic detection,¹⁵ liquid chromatography (LC) combined with ultraviolet detection^{16,17} or fluorescence detection,^{18,19} liquid chromatography-tandem mass spectrometry (LC-MS/MS).²⁰ Microbiological assays and capillary electrophoresis chromatographic detection were limited due to lack selectivity and time consuming.²¹ LC-based methods are the preferred methods to determine the concentration of colistin due to their accuracy, precision, sensitivity and selectivity.

However, various LC-based methods demand intricate sample pre-treatment, such as SPE cartridges for solid-phase extraction,^{22–25} derivatization¹⁸ or nitrogen blow-drying and reconstitution²⁰. For example, colistin cannot be quantified with high sensitivity by LC combined with ultraviolet detection or fluorescence detection without derivatization with UV-absorbing or fluorescent reagents, because it has very weak ultraviolet absorption and no native fluorescence. The detection LC-MS/MS is based on the ratio of mass to charge (m/z) which does not require special structure of the sample. Therefore, LC-MS/MS is used more widely in clinical practice. However, the mass spectrometer requires high purity of the sample, hence, the sample purification method becomes the rate limiting step of the method. Currently existing sample purification method for colistin quantification by LC-MS/MS were SPE cartridges for solid-phase extraction or nitrogen blow-drying and reconstitution which is not only time-consuming but also costly. Hence, to optimize the detection steps, we are committed to develop and validate a quantitative determination method of colistin with the characteristic of simplicity of operator, time-saving and cheap and then apply the method to TDM in clinical practice and finally lay a foundation for pharmacokinetic/pharmacodynamic (PK/PD) investigations of colistin to ensure the correct individualized dosing.

Materials and Methods

Standards and Reagent

Colistin sulfate (polymyxin E) was purchased from CFW Laboratories Inc. (Walnut, USA). Terfenadine was provided by China National Institute for the Control of Pharmaceutical and Biological Products. Colistin sulfate contained 2 major components with a composition percentage of 30.46% for colistin A and 53.8% for colistin B, respectively. Terfenadine was used as an internal standard (IS). LC-grade methanol (MeOH) and water were from Honeywell Research Chemicals (North Carolina, USA). LC-grade acetonitrile (ACN) and LC-grade formic acid (FA) was purchased from ThermoFisher Scientific (Massachusetts, USA). Blank human plasma, human hemolytic plasma and human hyperlipidemia plasma were from hematology department in Chinese PLA General Hospital. The study was approved by the Ethics Committee of Chinese People's Liberation Army General Hospital (C2021-069-01). Therapeutic Drug Monitoring (TDM) represents a standard element of clinical practice; consequently, a waiver for informed consent has been granted following ethical review board approval. Throughout the study, all procedures adhere strictly to guidelines outlined in the Declaration of Helsinki.

LC-MS/MS System

The chromatographic system was the Agilent 1260 HPLC system (Agilent Technologies Inc., Santa Clara, CA, USA), which consist of a vacuum degasser, binary pumps, and an auto-sampler. Phenomenex Kinetex C18 column (50.0×2.1mm, 5 μ m) was used to separate the analyte from the IS with gradient elution. The mobile phase A was made up of distilled water with 0.2% formic acid and the mobile phase B was acetonitrile with 0.2% formic acid. The gradient elution procedure was shown in Table 1. The injection volume was 5.0 μ L. The temperature of auto-sampler was set at 4°C and the column was 40°C.

The Agilent 6460 triple-quadrupole mass spectrometer (Agilent Technologies, USA) was used to monitor the analyte and IS on multiple reaction monitoring (MRM) mode with positive ion condition of the electrospray ionization (ESI) source. Before analysis, the mass spectrometric parameters were optimized as follows: the ion spray voltage of 4000V, the temperature of sheath gas was set 350°C and the drying gas (N₂) 300 °C, the nebulizer pressure was set at 30 psi. The fragment voltage of colistin was 110 eV and 95 eV, respectively and 90 for IS. The collision energy of colistin and IS was

Table I HPLC Gradient Elution Procedures for Colistin in Human Plasma

Time (min)	% Mobile Phase A	% Mobile Phase B	Flow Rate (mL/min)
0.00	82	3	0.5
1.00	82	60	0.5
2.00	5	90	0.5
2.50	5	90	0.5
2.60	82	3	0.5
3.50	82	3	0.5

15 and 21 respectively. The mass transition pairs as follows: m/z 391.1 \rightarrow m/z 101 for colistin A, m/z 385.9 \rightarrow m/z 101 for colistin and m/z 472.4 \rightarrow m/z 454.3 for IS.

Preparation of the Stock Solutions, Calibration Standards and Quality Control (QC) Samples

Colistin stock solutions (1 mg/mL) were prepared by dissolving in 0.2% FA water including 0.5663 mg/mL colistin A and 1.00 mg/mL colistin B. In addition, terfenadine stock solutions (1 mg/mL) were prepared by dissolving in DMSO. Working solutions of colistin were prepared from stock solution by further diluting in 0.2% FA water. Similarly, 15.0 ng/mL terfenadine working solution was prepared from the stock solution by further diluting in ACN. Then working solutions were further diluted 20 times with blank human plasma to prepare calibration standards and quality control (QC) samples. The concentrations of calibration standards for colistin were set eight levels of concentration including 28.31, 56.63, 113.25, 283.13, 566.25, 1132.50, 2718.00, 3397.51 ng/mL for colistin A and 50, 100, 200, 500, 1000, 2000, 4800 and 6000 ng/mL for colistin B. The concentrations of QC samples for colistin were produced four levels including 28.31 ng/mL (lower limit of quantification, LLOQ), 84.94 ng/mL (low quality control, LQC), 849.38 ng/mL (middle quality control, MQC), 2265 ng/mL (high quality control, HQC) for colistin A and 50 ng/mL LLOQ, 150 ng/mL LQC, 1500 ng/mL MQC, and 4000 ng/mL HQC for colistin B. The terfenadine working solution was set as IS solution.

Sample Preparation

The organic solvent, ACN, was used for sample purification. Firstly, 200 μ L of IS solution and 50 μ L 2% FA water were placed into a 1.5 mL Eppendorf tube, then 50 μ L of plasma samples were added into the tubes. Then the mixture of sample and IS was vortexed for 1 min and the plasma proteins in the sample were fully precipitated by ACN, which is in the IS solution. Subsequently, purified sample was centrifuged at 14,000 g and 4 °C for 10 min. The 200 μ L of the supernatant was placed into a new Eppendorf tube, diluted with 200 μ L 2% FA water and vortexed. Finally, the 200 μ L supernatants were loaded into sample bottles, and 5 μ L of the supernatants were used for HPLC-MS /MS analysis.

Validation of the Assay Method

The method validation was carried out according to the *Bioanalytical Method Validation (M10)* of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). And the method parameters including selectivity, calibration curves and lower limit of qualification (LLOQ), precision and accuracy, matrix effect (ME) and extraction recovery, stability, and dilution integrity.

Selectivity

Selectivity is the ability of an analytical method to distinguish the analytes from potential interfering substances. The selectivity of our method was investigated by comparing the chromatograms of the blank human plasma, blank human plasma after the addition of IS, blank human plasma after the addition of only colistin sulfate standards and blank human plasma after the addition of colistin sulfate standards and IS. The acceptance criteria for selectivity was that responses of interfering components should be no more than 20% of the analyte response at the LLOQ and no more than 5% of the IS response in LLOQ sample.

Calibration Curve and LLOQ

The calibration curve was defined as colistin concentration versus a weighted ($1/x \cdot x$) linear regression of the peak area ratio of colistin to IS and the peak area of colistin is the sum of colistin A and colistin B peak areas.^{26,27} Eight concentrations of calibration standard were prepared to determine the linearity of the calibration curve including 28.31, 56.63, 113.25, 283.13, 566.25, 1132.50, 2718 and 3397.51 for colistin A and 50, 100, 200, 500, 1000, 2000, 4800 and 6000 ng/mL for colistin B.

LLOQ was defined as the lowest concentration of analytes that could be quantitatively determined with acceptable precision and accuracy. The analytical signal of colistin A and colistin B at LLOQ (28.31 ng/mL and 50 ng/mL) demand more than 10 times the noise of the blank sample at the same retention time.

Precision and Accuracy

The accuracy is defined as the closeness of the measured value to the nominal concentration, which is expressed by the ratio of measured value to nominal concentration. Precision, expressed by coefficient of variation, is described as the relative standard deviation of measured value. Inter-day accuracy and precision was evaluated with six replicates of LLOQ, LQC, MQC, and HQC including 28.31, 84.94, 849.38, 2265 ng/mL for colistin A and 50, 150, 1500, 4000 ng/mL for colistin B in three consecutive days and intra-day accuracy and precision was evaluated with the six replicates on the same day. The accuracy and precision should be within $\pm 15\%$ ($\pm 20\%$ for LLOQ) and below 15% (20% for LLOQ).

Matrix Effect and Extraction Recovery

Matrix effect (ME) is known as the changes in the response of the analyte under test method due to interfering substances in the biological matrix. In our method, matrix effect was evaluated by comparing the peak areas of analytes added to six batches of human plasma at three level QC concentrations (LQC, MQC, HQC) with the peak areas of analytes added to ultrapure water at the corresponding concentration for three assays in parallel, respectively. The test was accepted if the accuracy should be within $\pm 15\%$ of the nominal concentration in all matrix.

The extraction recovery (ER) was evaluated by comparing the peak areas of the analytes at three level QC samples (LQC, MQC, HQC) added to blank human plasma with the peak areas of the analytes added to the blank human plasma after extraction processing at the corresponding concentration levels for six assays in parallel, respectively. The test was accepted if the accuracy should be within $\pm 15\%$ of the nominal concentration.

Stability

Two levels of QC samples with 3 replicates were prepared for the evaluation of the stability of colistin in human plasma. The stability tests included freeze-thaw stability test (samples stored -80°C and -20°C underwent three freeze-thaw cycles), short-term stability tests (samples were stored at room temperature for 20 h, in an auto-sampler for 46 h and in a refrigerator at 4°C for 30 h).

Dilution Integrity

Dilution integrity was determined to ensure that the quantitative results were not affected by diluting samples. Blank human plasma samples, added with colistin, were diluted at a ratio of 1:2 to get the final sample concentration of 1698.76 ng/mL colistin A and 3000 ng/mL colistin B. The test was accepted if the accuracy and precision should be within $\pm 15\%$.

Results and Discussion

Method Development

In the study, we validated a HPLC-MS/MS method with more simpler sample pre-treatment for quantitative determination of colistin in human plasma on the basis of the previous study. It will be more suitable for TDM in the clinical practice because its simple operation, time and cost savings. Colistin is a cationic polypeptide with a cyclic heptapeptide ring and a tripeptide side-chain.¹⁵ It exhibits both hydrophobicity due to the fatty acid moiety, and basic properties due to the five unmasked γ -amino groups. Therefore, it is able to distribute well in both polar and non-polar environments. Based on the chemical property of colistin and previous research experience, we choose the distilled water with 0.2% formic acid as the aqueous phase and acetonitrile with 0.2% formic acid as organic phase in term of mobile phase

selection. In addition, analyte was purified from human plasma by direct protein precipitation with acetonitrile. This simplified the sample pre-treatment procedure to save time and cost without affecting the LLOQ. In the selection of IS, as we all know, an ideal IS usually had high similarity with the chemical structure of the analyte, does not overlap with other peaks and is stable for a reasonable time. In previous studies, polymyxin B was frequently used as an IS.^{6,18,20} In addition, netilmicin sulfate was also chosen as IS in a number of research.²⁸ In our method, we firstly chose polymyxin B1 (c=1000ng/mL, m/z=402.3–101.1) as IS according to previous studies. However, selectivity and internal standard interference tests were found that interference peaks were detected at the retention time of colistin A's ion channel (391.1–101) for 1.409 min during method validation. The peak area was greater than 20% of the LLOQ, and interference existed in all tests. We analyzed that the reason for the interference may be due to the low content of colistin A in the standard substance. So, we reduced the concentration of B1 internal standard to 200 ng/mL and found the interference peak was lower than 20% of the peak area of the lower quantitation limit, which meet the criterion. But in the interference test of the internal standard, interference peak appeared at the retention time of the internal standard 1.424 min, and the peak area of the interference peak was greater than 5% of the average peak area of the standard and the internal standard of quality control. So, we concluded that in our method, if polymyxin B1 was used as the IS, the selectivity parameter of colistin A did not meet the standard, and a more suitable internal standard compound should be selected. Then terfenadine was chosen as an IS because of its high similarity in physico-chemical properties with colistin and without overlapping with the peaks of all the analytes.

Method Validation

According to the international standards, colistin concentration range of 50–6000 ng/mL of colistin B and 28.31–3397.51 ng/mL of colistin A was validated in our method.²⁹ All the validation parameters required by international standard were investigated, including selectivity, calibration curve and LLOQ, accuracy and precision, matrix effect and extraction recovery, stability and dilution integrity.

Selectivity

The blank plasma used for selectivity validation was from six different people. The results (Figure 1) demonstrated that the response of the interfering substances was less than 20% of the LLOQ of analyte and less than 5% of the IS response. So, it indicated that endogenous substances and IS did not affect the determination of colistin in our developed method.

Calibration Curve and LLOQ

The regression equation (Figure 2) of colistin A was $Y = 3.438X + 8.769$ ($r^2 = 0.999$) and colistin B was $Y = 5.412X + 0.0001$ ($r^2 = 0.999$). Wherein X indicates the nominal concentration level of the analyte, and Y indicates the peak area ratio of analyte to the IS. The linear relations r^2 of colistin content in human plasma was no less than 0.99 in the range of 28.31 ng/mL and 4000 ng/mL. And the detected concentration was within $\pm 20\%$ of the nominal concentration at the LLOQ.

Accuracy and Precision

The results showed that the intra-day accuracy and precision of colistin A were 87.61%–106.34% and 0.80%–7.59% (Table 2), while the colistin B were 90.46%–104.78% and 0.54–7.18% (Table 3). The inter-day accuracy and precision of colistin A were 93.81%–102.10% and 0.15%–1.61% (Table 2), while colistin B were 95.68%–101.34% and 0.31%–2.61% (Table 3). The accuracy and precision results met the requirements of ICH principles, which should be within $\pm 15\%$ ($\pm 20\%$ for LLOQ) and below 15% (20% for LLOQ), respectively.

Matrix Effect and Extraction Recovery

Matrix effect factor is the ratio of the peak areas of analytes or IS added to human plasma to the peak areas of analytes added to ultrapure water at the corresponding concentration. The internal standard normalized matrix effect factor was the ratio of the matrix effect factor of analytes to the matrix effect factor of IS. The results showed that the accuracy of internal standard normalized matrix effect factor in all matrix was within $\pm 15\%$. It indicated that the developed method can distinguish the analyte from various endogenous substances in human plasma.

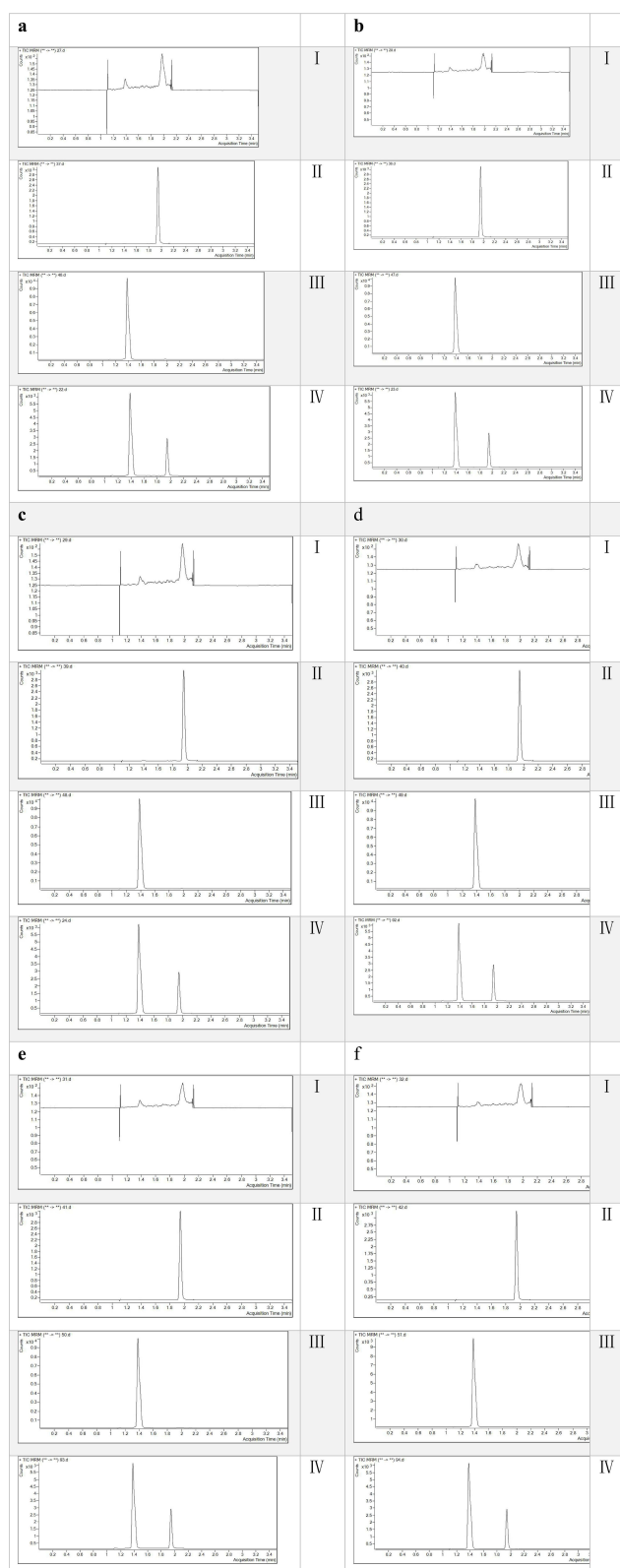


Figure I Blank human plasma was used to assess the selectivity of the method. From top to bottom are double blank (I), IS only (II), analytes only (III) and QC sample (IV) (in 6 different lots (a-f)).

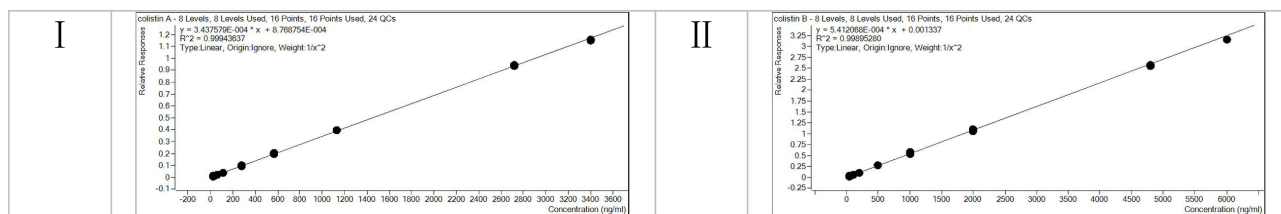


Figure 2 The regression equations for colistin A(I) and colistin B(II).

The ER was evaluated by comparing the peak areas of the analytes added to blank human plasma with the peak areas of the analytes added to the blank human plasma after extraction processing. In our study we evaluated the ER in three level QC samples (LQC, MQC, HQC) with six assays in parallel at each concentration level, respectively. Then the precision of the six assays in parallel was used as the evaluation parameters. The results showed that the precision of extraction recoveries of colistin A was 8.3534% in LQC, 4.2521% in MQC, and 1.8411% in HQC and the accuracy of extraction recoveries of colistin B was 10.0360% in LQC, 2.3178% in MQC, and 2.0717% in HQC. The precision of all the concentration levels were within 15%.

Table 2 Accuracy and Precision of the Method for Determination of Colistin A (n=6)

Concentration (ng/mL)	Batch	Intra-Batch (Mean±SD)	Accuracy (%)	CV (%)	Inter-Batch (Mean±SD)	Accuracy (%)	CV (%)
28.31	1	26.071±1.978	92.09	7.59	28.390±0.456	100.28	1.61
	2	28.996±1.067	102.42	3.68			
	3	30.104±1.567	106.34	5.21			
84.9	1	87.353±4.547	102.84	5.21	79.679±0.173	93.81	0.22
	2	77.266±4.225	90.96	5.47			
	3	74.417±4.495	87.61	6.04			
849	1	831.153±13.983	97.85	1.68	867.248±2.335	102.10	0.27
	2	896.420±9.630	105.54	1.07			
	3	874.170±13.269	102.92	1.52			
2265	1	2311.13±18.58	102.04	0.80	2250.45±3.46	99.36	0.15
	2	2233.33±25.47	98.60	1.14			
	3	2206.90±22.54	97.43	1.02			

Table 3 Accuracy and Precision of the Method for Determination of Colistin B (n=6)

Concentration (ng/mL)	Batch	Intra-Batch (Mean±SD)	Accuracy (%)	CV (%)	Inter-Batch (Mean±SD)	Accuracy (%)	CV (%)
50.0	1	50.109±1.978	100.22	3.04	49.747±1.300	99.49	2.61
	2	50.052±1.088	100.10	2.17			
	3	49.081±3.524	98.16	7.18			
150	1	157.177±1.411	104.78	0.90	143.523±1.355	95.68	0.94
	2	137.700±3.864	91.80	2.81			
	3	135.693±3.634	90.46	2.68			
1500	1	1440.860±13.983	96.06	1.43	1520.132±4.745	101.34	0.31
	2	1568.245±11.420	104.55	0.73			
	3	1551.291±18.034	103.42	1.16			
4000	1	3946.144±43.227	98.65	1.10	3882.529±13.037	97.06	0.34
	2	3886.126±20.963	97.15	0.54			
	3	3815.318±43.847	95.38	1.15			

Therefore, the matrix effects and recovery results of IS met the requirements of ICH principles. All the data was shown in Table 4.

Stability

As the data shown in Table 5, colistin was stable in human plasma at least 20 h at room temperature, 46 h in autosampler, 30 h in 4 °C refrigerator, one to three freeze-thaw cycles under freezing at -20°C and -80 °C.

Dilution Integrity

The results in Table 6 showed that the accuracy of colistin A and colistin B in plasma after 2-fold dilution were -0.93% and -2.80%, respectively, and the precision were 1.85% and 1.21%, respectively. The results met the ICH principles for accuracy and precision of $\pm 15\%$. This indicated that the dilution of human plasma samples did not affect the analysis results.

Table 4 ME and ER of Colistin A and Colistin B Between Different Batches of Matrix (n=6)

Batch	LQC		MQC		HQC	
	ME	ER	ME	ER	ME	ER
Colistin A						
1	1.4604	0.9941	1.2930	1.0831	1.2973	1.0264
2	1.3646	0.9586	1.3129	1.0791	1.2922	1.0274
3	1.4186	0.9467	1.2954	1.0777	1.2867	1.0334
4	1.3595	0.7337	1.2844	0.9774	1.3199	0.9963
5	1.4266	0.8402	1.2899	1.0981	1.3048	1.0516
6	1.3858	0.8166	1.2944	1.0343	1.2662	1.0427
Mean	1.4026	0.9290	1.2950	1.0583	1.2945	1.0297
SD	0.0394	7.7603	0.0096	4.4999	0.0180	0.0190
%CV	2.8072	8.3534	0.7424	4.2521	1.3918	1.8411
Colistin B						
1	1.2657	1.0308	1.1798	1.0818	1.2540	1.0475
2	1.2256	0.9761	1.2073	1.0707	1.2561	1.0406
3	1.2461	0.9168	1.2325	1.0641	1.2465	1.0516
4	1.3337	0.8119	1.2382	1.0261	1.2543	1.0212
5	1.2935	0.8712	1.1932	1.0925	1.2577	1.0667
6	1.3657	0.8119	1.2196	1.0920	1.2417	1.0846
Mean	1.2884	0.9339	1.2118	1.0712	1.2517	1.0521
SD	0.0535	9.3723	0.0227	2.4828	0.0062	2.1795
%CV	4.1506	10.0360	1.8738	2.3178	0.4978	2.0717

Abbreviations: ME, Matrix effect; ER, extraction recovery.

Table 5 Sample stability Investigation (n = 3)

Nominal Concentration (ng/mL)	Colistin A				Colistin B			
	LQC		HQC		LQC		HQC	
	mean \pm SD	CV (%)	mean \pm SD	CV (%)	mean \pm SD	CV (%)	mean \pm SD	CV (%)
0 h	81.76 \pm 7.77	9.51	2851.53 \pm 45.97	1.61	152.59 \pm 6.46	4.23	5207.31 \pm 77.67	1.49
20 h-at room temperature	84.34 \pm 8.00	9.49	2853.62 \pm 32.43	1.14	152.24 \pm 4.46	2.93	5199.28 \pm 64.20	1.23
46 h-in autosampler	91.21 \pm 5.48	6.01	2258.01 \pm 35.17	1.56	159.5 \pm 6.09	3.82	3948.25 \pm 61.87	1.57
30 h-4 °C refrigerator	80.68 \pm 2.21	2.74	2826.52 \pm 29.15	1.03	151.07 \pm 3.85	2.55	5153.22 \pm 36.33	0.71
One to three freeze-thaw cycles under freezing at -20°C	91.85 \pm 8.97	9.77	2238.22 \pm 24.78	1.11	155.91 \pm 2.22	1.42	4014.27 \pm 7.23	0.18
One to three freeze-thaw cycles under freezing at -80°C	93.69 \pm 3.15	3.37	2308.27 \pm 37.94	1.64	159.32 \pm 3.23	2.03	4089.37 \pm 73.70	1.80

Table 6 Dilution Integrity of the Method for Determination of Colistin A and Colistin B(n=6)

Batch	Colistin A			Colistin B		
	Nominal Concentration (ng/mL)	Actual Concentration (ng /mL)	Accuracy (%)	Nominal Concentration (ng/mL)	Actual Concentration (ng /mL)	Accuracy (%)
1	1698.76	1678.655594	-1.18	3000	2933.9346	-2.20
2	1698.76	1707.874073	0.54	3000	2920.1759	-2.66
3	1698.76	1708.991481	0.60	3000	2954.0034	-1.53
4	1698.76	1625.114872	-4.34	3000	2854.3636	-4.85
5	1698.76	1697.126669	-0.10	3000	2898.956	-3.37
6	1698.76	1679.941898	-1.11	3000	2934.4472	-2.19

Application

Our validated HPLC-MS/MS has been applied to quantitatively determine the colistin concentrations of plasma samples obtained from 9 patients in the ward of PLA General Hospital. The clinical data of the 9 patients was showed in Table 7, including basic demographic characteristics, administration, daily dose (IU), and pathogenic bacteria. The ages of the patients range from 24–94 years old with three females and six males. They were all infected with *Acinetobacter baumannii*. Then all patients had treatment with colistin sulfate by intravenous drips 50 million IU, q12 h. The results of quantitative determination showed that the trough level of concentrations ranged from 0.235 to 1.019 $\mu\text{g mL}^{-1}$ and the peak level of concentrations ranged from 1.132 to 2.622 $\mu\text{g mL}^{-1}$. Our results were consistent with previous study³⁰ which showed the trough concentration of colistin sulfate ranged from 0.15 to 2.51 $\mu\text{g mL}^{-1}$, the peak concentration ranged from 0.89 to 4.56 $\mu\text{g mL}^{-1}$. The results of previous study and our detection indicated that large individual differences exist during the clinical application of colistin sulfate. However, there are no sufficient clinical data on the dose-effect relationship to ensure the safe clinical application of colistin except a few clinical research.^{31–33} Hence, due to this condition, it is essential to achieve the correct individualized dosing of colistin by therapeutic drug monitoring (TDM), then finally to ensure its efficacy and safety. Considering that colistin TDM should be widely used in clinical practice, a rapid and simple method for the quantitative determination of colistin should be developed.

In our study, we developed and validated the quantitative determination method of colistin with direct protein precipitation for sample pre-treatment which is simplicity of operator, time-saving and cheaper than previous determination methods. This method will be helpful in launching pharmacokinetic/pharmacodynamic (PK/PD) investigations of colistin to ensure the correct individualized dosing. Physicians and pharmacists could conduct prompt clinical dose adjustments based on TDM data of colistin to achieve optimal clinical benefit and avoid adverse effects. Although LC-MS has high sensitivity and selectivity, its complexity and sample preparation requirements pose challenges. Future methods like immunoassays could offer simpler operation and lower costs for rapid screening of large samples.

Table 7 The Colistin Concentrations in the Plasma of 9 Patients

Patients	Age	Sex	Administration	Pathogenic Bacteria	Dosage (Million/IU)	Trough Concentrations ($\mu\text{g mL}^{-1}$)	Peak Concentrations ($\mu\text{g mL}^{-1}$)
1	71	female	ivgtt	Acinetobacter	50	1.019	2.622
2	92	Male	ivgtt	baumannii	50	0.676	1.132
3	73	Female	ivgtt		50	0.141	1.663
4	54	Female	ivgtt		50	0.164	1.363
5	24	Male	ivgtt		50	0.376	2.038
6	25	Male	ivgtt		50	0.235	1.703
7	84	Male	ivgtt		50	0.305	1.038
8	88	Male	ivgtt		50	0.598	1.796
9	94	Male	ivgtt		50	0.799	1.407

Conclusions

We developed and validated a rapid HPLC-MS/MS method with a simpler sample pre-treatment procedure to quantify colistin concentrations in human plasma. In our method, the analyte in the plasma was purified and enriched by protein precipitation, which is a simpler way than nitrogen blow-drying and reconstitution or solid-phase extraction. Furthermore, the method has been successfully applied to clinical practice for determining the optimal and safe dose of colistin required for each patient.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Ainsworth GC, Brown AM, Brownlee G. Aerosporin, an antibiotic produced by *Bacillus aerosporus* Greer. *Nature*. 1947;159:4060):263. doi:10.1038/160263a0
2. Benedict RG, Langlykke AF. Antibiotic activity of *Bacillus polymyxa*. *J Bacteriol*. 1947;54(1):24.
3. Jones TS. The chemical basis for the classification of the polymyxins. *Biochem J*. 1948;43(2):xxvi.
4. Kwa A, Kasiakou SK, Tam VH, Falagas ME. Polymyxin B: similarities to and differences from colistin (polymyxin E). *Expert Rev Anti Infect Ther*. 2007;5(5):811–821. doi:10.1586/14787210.5.5.811
5. Nang SC, Azad MAK, Velkov T, Zhou QT, Li J. Rescuing the Last-Line Polymyxins: achievements and Challenges. *Pharmacol Rev*. 2021;73(2):679–728. doi:10.1124/pharmrev.120.000020
6. Matar KM, Al-Refai B. Quantification of colistin in plasma by liquid chromatography-tandem mass spectrometry: application to a pharmacokinetic study. *Sci Rep*. 2020;10(1):8198. doi:10.1038/s41598-020-65041-w
7. Ross S, Puig JR, Zaremba EA. Colistin: some preliminary laboratory and clinical observations in specific gastroenteritis in infants and children. *Antibiot Annu*. 1959;7:89–100.
8. Falagas ME, Michalopoulos A. Polymyxins: old antibiotics are back. *Lancet*. 2006;367(9511):633. doi:10.1016/s0140-6736(06)68241-x
9. Poirel L, Jayol A, Nordmann P. Polymyxins: antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. *Clin Microbiol Rev*. 2017;30(2):557–596. doi:10.1128/cmr.00064-16
10. Rychlickova J, Kubickova V, Suk P, Urbanek K. Challenges of colistin use in ICU and therapeutic drug monitoring: a literature review. *Antibiotics*. 2023;12(3). doi:10.3390/antibiotics12030437
11. Ordooei Javan A, Shokouhi S, Sahraei Z. A review on colistin nephrotoxicity. *Eur J Clin Pharmacol*. 2015;71(7):801–810. doi:10.1007/s00228-015-1865-4
12. Falagas ME, Kasiakou SK. Toxicity of polymyxins: a systematic review of the evidence from old and recent studies. *Crit Care*. 2006;10(1):R27. doi:10.1186/cc3995
13. Kim EJ, Oh J, Lee K, et al. Pharmacokinetic characteristics and limited sampling strategies for therapeutic drug monitoring of colistin in patients with multidrug-resistant gram-negative bacterial infections. *Ther Drug Monit*. 2019;41(1):102–106. doi:10.1097/ftd.0000000000000572
14. Leroy P, Decolin D, Nicolas S, Archimbault P, Nicolas A. Residue determination of two co-administered antibacterial agents--cephalexin and colistin--in calf tissues using high-performance liquid chromatography and microbiological methods. *J Pharm Biomed Anal*. 1989;7(12):1837–1846. doi:10.1016/0731-7085(89)80201-8
15. Ahmed H, Elbarbry F, Clark B. Mixed micellar electrokinetic chromatographic analysis of colistin, polypeptide antibiotic, using laser-induced fluorescence detection. *Am J Anal Chem*. 2012;03(03):233–241. doi:10.4236/ajac.2012.33031
16. Elverdam I, Larsen P, Lund E. Isolation and characterization of three new polymyxins in polymyxins B and E by high-performance liquid chromatography. *J Chromatogr*. 1981;218:653–661. doi:10.1016/s0021-9673(00)82091-9
17. Orwa JA, Van Gerven A, Roets E, Hoogmartens J. Liquid chromatography of polymyxin B sulphate. *J Chromatogr A*. 2000;870(1–2):237–243. doi:10.1016/s0021-9673(99)00936-x
18. Chepyala D, Tsai IL, Sun HY, Lin SW, Kuo CH. Development and validation of a high-performance liquid chromatography-fluorescence detection method for the accurate quantification of colistin in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2015;980:48–54. doi:10.1016/j.jchromb.2014.12.015
19. Li J, Milne RW, Nation RL, et al. A simple method for the assay of colistin in human plasma, using pre-column derivatization with 9-fluorenylmethyl chloroformate in solid-phase extraction cartridges and reversed-phase high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl*. 2001;761(2):167–175. doi:10.1016/s0378-4347(01)00326-7
20. Wang Y, Yu X, Chen C, et al. Development of UPLC-MS/MS method for the determination of colistin in plasma and kidney and its application in pharmacokinetics. *J Pharm Biomed Anal*. 2023;233:115440. doi:10.1016/j.jpba.2023.115440

21. Giovana C, LUCiana ED, Nathalie TF, Thais AC. Microbiological assay for the determination of colistin sulphate. *Curr Anal Chem.* 2014;10:433–448.
22. Fu Q, Li X, Zheng K, et al. Determination of colistin in animal tissues, egg, milk, and feed by ultra-high performance liquid chromatography-tandem mass spectrometry. *Food Chem.* 2018;248:166–172. doi:10.1016/j.foodchem.2017.12.029
23. Mead A, Gillard N, Robert C, et al. Determination of colistin in luminal and parietal intestinal matrices of chicken by ultra-high-performance liquid chromatography-tandem mass spectrometry. *J Vet Pharmacol Ther.* 2021;44(6):982–985. doi:10.1111/jvp.13022
24. Zhao M, Wu XJ, Fan YX, Guo BN, Zhang J. Development and validation of a UHPLC-MS/MS assay for colistin methanesulphonate (CMS) and colistin in human plasma and urine using weak-cation exchange solid-phase extraction. *J Pharm Biomed Anal.* 2016;124:303–308. doi:10.1016/j.jpba.2016.02.045
25. Mead A, Richez P, Azzariti S, Pelligand L. Pharmacokinetics of colistin in the gastrointestinal tract of poultry following dosing via drinking water and its bactericidal impact on enteric *Escherichia coli*. *Front Vet Sci.* 2021;8:698135. doi:10.3389/fvets.2021.698135
26. Li J, Milne RW, Nation RL, et al. Use of high-performance liquid chromatography to study the pharmacokinetics of colistin sulfate in rats following intravenous administration. *Antimicrob Agents Chemother.* 2003;47(5):1766–1770. doi:10.1128/aac.47.5.1766-1770.2003
27. Ouchi S, Matsumoto K, Okubo M, Yokoyama Y, Kizu J. Development of HPLC with fluorescent detection using NBD-F for the quantification of colistin sulfate in rat plasma and its pharmacokinetic applications. *Biomed Chromatogr.* 2018;32(5):e4167. doi:10.1002/bmc.4167
28. Hanai Y, Matsuo K, Kosugi T, et al. Rapid, simple, and clinically applicable high-performance liquid chromatography method for clinical determination of plasma colistin concentrations. *J Pharm Health Care Sci.* 2018;4:22. doi:10.1186/s40780-018-0119-x
29. Pike R. U.S. Department of health and human services food and drug administration center for drug evaluation and research; 2008.
30. Y-C M, X-K W, Yang X-L, Zhang Z-Q. Simultaneous determination of colistin sulfate and tigecycline in human plasma by liquid chromatography-tandem mass spectrometry method. *BMC Chemistry.* 2024;18(1). doi:10.1186/s13065-023-01109-8
31. Katip W, Rayanakorn A, Sornsuvit C, et al. High-loading-dose colistin with nebulized administration for carbapenem-resistant *Acinetobacter baumannii* pneumonia in critically ill patients: a retrospective cohort study. *Antibiotics.* 2024;13(3):287. doi:10.3390/antibiotics13030287
32. Katip W, Uitrakul S, Oberdorfer P. Clinical efficacy and nephrotoxicity of the loading dose colistin for the treatment of carbapenem-resistant *Acinetobacter baumannii* in critically ill patients. *Pharmaceutics.* 2021;14(1):31. doi:10.3390/pharmaceutics14010031
33. Katip W, Rayanakorn A, Oberdorfer P, Taruangsri P, Nampuan T. Short versus long course of colistin treatment for carbapenem-resistant *A. baumannii* in critically ill patients: a propensity score matching study. *J Infect Public Health.* 2023;16(8):1249–1255. doi:10.1016/j.jiph.2023.05.024

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