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Determination of colistin in human plasma, urine and other biological samples using LC–MS/MS

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Abstract

A liquid chromatography–tandem mass spectrometric (LC–MS/MS) method was developed to quantify colistin in human plasma and urine, and perfusate and urine from the isolated perfused rat kidney (IPK). Solid phase extraction (SPE) preceded chromatography on a Synergi Fusion-RP column with a mobile phase of acetonitrile, water and acetic acid (80/19/1) at 0.2 mL/min. Ions were generated using electrospray ionization and detected in the positive-ion mode. Multiple reaction monitoring was performed using precursor-product ion combinations. Calibration curves were linear from 0.028 μg/mL (human plasma, IPK perfusate and urine)/0.056 μg/mL (human urine) to 1.78 μg/mL (all four media) for colistin A sulfate; corresponding values for colistin B sulfate were 0.016/0.032 to 1.01 μg/mL. Accuracy and precision were within 10%. The LLOQ for colistin A sulfate was 0.028 μg/mL in human plasma, IPK perfusate and urine and 0.056 μg/mL in human urine; corresponding values for colistin B sulfate were 0.016 and 0.032 μg/mL. The low sample volume, short analysis time and low LLOQ are ideal for pre-clinical and human pharmacokinetic studies of colistin.

Keywords: Colistin; LC–MS/MS; SPE; Plasma; Urine

1. Introduction

Colistin (also known as polymyxin E) is an important member of the polymyxin group of cationic peptide antibiotics and was isolated from *Bacillus colistinus* in 1950 [1]. It features a cyclic heptapeptide and tripeptide side chain acylated at the N-terminus by a fatty acid (Fig. 1). At least thirty different components were found in commercially available colistin, 13 of which have been isolated and identified [2,3]. The major components are colistin A (polymyxin E1) and colistin B (polymyxin E2) which, together, account for more than 85% of total weight of the raw material [4]. The two substances differ in the length of the fatty acid side chain by one methylene group (Fig. 1).

Colistin has been available clinically since the 1960s as colistin methanesulfonate (CMS), in which all five free γ-amino groups of the L-α,γ-diaminobutyric acid (L-Dab) residues of colistin have been derivatized as their methanesulphonates [1]. At that time, clinical reports suggested a high incidence of toxicity from colistin and its use was quickly surpassed by other anti-pseudomonal reagents considered less toxic, such as aminoglycosides [5]. However, there has been a resurgent interest in its use for treating pulmonary infections caused by Gram-negative bacteria in cystic fibrosis (CF) patients. This is especially so for infections caused by multidrug-resistant (MDR) *Pseudomonas aeruginosa*, which accounts for more than 90% of the morbidity and mortality in CF patients [6]. Treatment of such infections is a considerable challenge because MDR *P. aeruginosa* is resistant to most of the currently used anti-pseudomonal antibiotics [7]. However, the efficacy of colistin against *P. aeruginosa* and its low incidence of resistance have been confirmed by many recent clinical trials [1]. In addition, numerous recent studies suggest that the toxicity of colistin is not as severe as previously believed [8]. Colistin is currently a last line of defense against infections caused by MDR Gram-negative organisms [9].

CMS is an inactive pro-drug of colistin [9]. It is hydrolyzed rapidly *in vivo* and *in vitro* and forms an extremely complex mixture of partially sulphomethylated derivatives as well as colistin [10]. It is now believed that colistin is responsible for the majority of the therapeutic and toxic effects after administration

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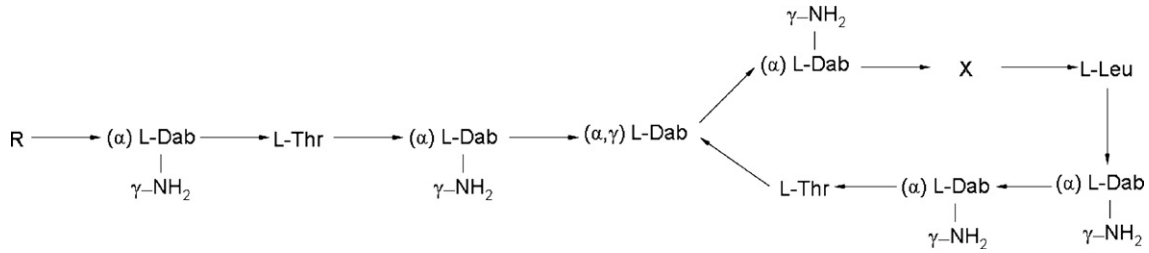

Fig. 1. Chemical structure of colistin and polymyxin B. Dab = L-α,γ-diaminobutyric acid. α and γ indicate the respective $–NH_2$ involved in the peptide linkage. Polymyxin B1: R = (+)-6-methyloctanoate, X = Phe; polymyxin B2: R = (+)-6-methylheptanoate, X = Phe; polymyxin E1 (colistin A): R = (+)-6-methyloctanoate, X = D-Leu; polymyxin E2 (colistin B): R = (+)-6-methylheptanoate, X = D-Leu.

of CMS. Thus, understanding the pharmacokinetic, pharmacodynamic and toxicodynamic behavior of colistin will be an important guide to its appropriate clinical use. Proper dosage regimens are necessary which take account of the pharmacokinetics and pharmacodynamics of colistin and its toxicity. To gain this knowledge requires a method for measuring colistin that is simple, rapid, specific and reliable; hence the purpose of this report. Unfortunately, after more than 40 years of clinical use, such information is limited due to the lack of robust analytical methods.

Existing analytical methods for colistin include microbiological assay [11], thin-layer chromatography (TLC) [12], immunological assay [13], capillary electrophoresis [14,15] and high-performance liquid chromatography (HPLC) [2–4,16,17]. Microbiological assays were widely used in the past for measuring the concentrations of 'total' colistin, but are time-consuming and lack specificity [12]. The complexity of preparing the immunogen of colistin for immunological determination of colistin in biological samples reduces the value of this technique [18]. TLC, capillary electrophoresis and HPLC have been applied for separation of the major components of colistin in the raw material [2,3,12,14,16]. However, due to its poor ultraviolet absorption and lack of native fluorescence, the analysis of colistin in biological media by HPLC poses problems. Decolin et al. [4] and Le Brun et al. [17] developed HPLC methods in which orthophthalaldehyde was employed as a derivatizing reagent. Since the derivatives were not overly stable, a complicated on-line derivatization procedure was required in such methods.

The HPLC assay developed more recently by Li et al. [19] was reported to be simple, selective and sensitive. The method included extraction of colistin from rat plasma and urine onto solid phase extraction (SPE) cartridges, followed by rapid and quantitative derivatization under mild conditions with fluorenyl-methyl chloroformate (FMOC-Cl). However, the requirement of time-consuming pre-column derivatization and relatively long chromatographic retention times are disadvantages [19].

Recently, two methods have been reported for quantifying colistin in milk and tissue homogenates using liquid chromatography–tandem mass spectrometry (LC–MS/MS) following SPE [20,21]. However, the methods were deemed to be far from satisfactory as they required large amounts of samples and, again, pretreatment of the samples was tedious and run times relatively long.

In order to measure the concentrations of colistin in a range of biological samples, including human plasma and urine and perfusate and urine collected from experiments with the isolated perfused rat kidney (IPK), we have developed a new method for quantifying colistin in different biological media using LC–MS/MS.

2. Experimental

2.1. Reagents and chemicals

Oasis HLB SPE cartridges (30 mg, 1 mL) were obtained from Waters (Waters, Milford, MA, USA). Colistin sulfate, polymyxin B sulfate and trichloroacetic acid (TCAA) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Polypropylene inserts were purchased from Supelco (Bellefonte, PA, USA). HPLC grade methanol, acetonitrile and acetic acid were purchased from BDH Laboratory Supplies (Poole, UK). Water was purified by a Milli-Q system (Millipore, Bedford, MA, USA). Plasma was obtained from the Australian Red Cross (Adelaide, Australia). Human urine was collected from healthy drug-free volunteers.

2.2. Sample pretreatment

An aliquot (200 μL) of human plasma or urine, or perfusate or urine from the IPK, was vortex-mixed in a polypropylene tube with 30 μL internal standard (5 μg/mL polymyxin B sulfate in water), 800 μL methanol–10% TCAA (50/50, v/v) solution, and centrifuged (4 °C, 1000 × *g*, 10 min). The supernatant was loaded onto an Oasis HLB SPE cartridge preconditioned with methanol followed by Milli-RQ water on a vacuum suction manifold (Supelco, Visiprep 24, Bellefonte, PA, USA). Impurities were removed with 1 mL water. Colistin and polymyxin B were eluted with 2 × 300 μL of methanol–water–acetic acid (80/19/1, v/v/v) and 50 μL of the combined eluent injected onto the LC–MS/MS.

2.3. Instrumentation

The LC–MS/MS system included an API 3000 triple quadrupole spectrometer (Applied Biosystems, Foster, Canada), a SIL-HTc autosampler, LC-10AD pump and DGU-14A degasser (all from Shimadzu, Kyoto, Japan). A Synergi Fusion-RP 2 μm column (20 mm × 2.0 mm, Phenomenex, Torrance,

CA, USA) equipped with a guard column (Phenomenex, Torrance, CA, USA) was used for partial separation of colistin from impurities. The mobile phase consisted of acetonitrile, water and acetic acid (80/19/1, v/v/v) delivered isocratically at 0.2 mL/min. Ions were generated using electrospray ionization (ESI) and detected in the positive-ion mode. Characteristic mass fragments of the identified precursor ions used for quantification were determined in multiple reaction monitoring (MRM) mode using precursor-product ion combinations. Optimal declustering potential (DP), collision energy potentials (CE), collision exit potentials (CXP) and focusing potential (FP) were determined based on the relative intensities of selected product ions. A dwell time of 150 ms, inter-channel delay of 10 ms and unit resolution were used during the experiment. Instrument settings, data acquisitions and processing were controlled by the software package Analyst (Version 1.4, Applied Biosystems, Foster, Canada). Nitrogen was used as nebulising (10 L/min), auxiliary (5 L/min), collision (10 L/min) and curtain (8 L/min) gas. Unit resolution was set for both Q1 and Q3.

2.4. Method validation

Appropriate volumes of three working standards of colistin sulfate in water (2.5, 10 and 80 μg/mL) were used to prepare calibration standards in drug-free medium (human plasma or urine, the IPK perfusate or urine), containing colistin sulfate at concentrations of 0.05, 0.10, 0.20, 0.40, 0.80, 1.6, 2.4 and 3.2 μg/mL. Quality control (QC) samples were prepared at 0.125, 0.25, 0.5, 1.0 and 2.0 μg/mL of colistin sulfate, similarly from a separate stock solution. Two linear calibration curves were constructed from the ratio of peak area of colistin A or B to that of internal standard and the nominated concentrations (vide infra) of colistin A or B sulfate in the calibration standards.

Neither colistin A nor B is commercially available at present. Thus it is not practically possible to quantify accurately their absolute amounts in biological samples by reference to calibration curves prepared from known amounts of the pure substances. Nevertheless, it has been reported that colistin A and B sulfates are the major components in commercially available colistin, although the proportion of colistin A and B sulfates differs between batches and manufacturers [4]. Therefore, the percentages of colistin A and B in the reference sample of colistin (as the sulphate) were estimated by HPLC analysis of a solution of colistin sulphate (1000 μg/mL) on an Ultrasphere C18 column (5 μm, 250 mm × 4.6 mm, Beckman, Berkley, CA, USA) using a mobile phase of acetonitrile–35 mM triethylamine (17:83, adjusted to pH 2.5 with 85% phosphoric acid) at a flow of 1.5 mL/min and detection at 210 nm [19]. The percentages of colistin A and B were calculated as the ratio of their respective peak area to the summed areas of all peaks. Colistin A and B in this batch were found to account for more than 87% of the total peak areas, and the percentages of A and B were calculated as $31.6 \pm 0.2\%$ and $55.8 \pm 0.6\%$ ($n = 4$), respectively. Apparent nominal concentrations of colistin A or B sulfate in each calibration standard and QC sample were calculated from the product of the concentration of colistin sulfate and its respective percentage. These apparent nominal values were used in constructing calibration curves, and for calculation of accuracy and precision.

The sum of the intensities of the peak areas of the selected product ions from the two transitions for each of colistin A, colistin B and the internal standard (polymyxin B1) were calculated, and used to generate calibration curves of the ratio of the intensities of colistin A or B to polymyxin B1 versus concentration of colistin A or B. Calibration curves were weighted according to $1/y$ (y = analyte peak area/internal standard peak area). Intra-day accuracy and precision were assessed from six consecutive analyses of the QCs of colistin sulfate prepared at five different concentrations in the four different media; inter-day accuracy and precision was obtained from consecutive analyses of the same batch of QC samples on four separate occasions. Accuracy (Diff%) was expressed as the quotient of the percentage difference in concentration (the calculated concentration minus the nominal concentration) and the nominal concentration. Precision was expressed as the coefficient of variation (%CV). The lower limit of quantification (LLOQ) of the assay was defined as the lowest concentration of the standard curve sample which could be measured with an intra-day accuracy and precision within 15% using six replicates [22].

2.5. IPK study

The right kidneys from five male Sprague–Dawley rats were isolated and perfused according to the protocol described by Wang et al. [23]. Colistin sulfate was added into the IPK circuit system to achieve an initial concentration of 2 μg/mL. After a 5-min period of equilibration, urine samples were collected over 5-min intervals and perfusate samples at the mid-time of each interval. Concentrations of colistin A and B sulfates in perfusate and urine were determined using the method described above.

3. Results and discussion

3.1. LC–MS/MS

The needle voltage and the source temperature of the turbo ion spray were optimized at 5500 V and 350 °C, respectively, according to the intensities of the precursor ions. The optimized DP, CE, CXP and FP are shown in Table 1.

Colistin sulfate and polymyxin B sulfate (1 μg/mL) were infused in to the triple quadrupole mass spectrometer to study the precursors spectra of both (as shown in Fig. 2),

Table 1
The optimum potential settings for the analysis of major components colistin A, colistin B and polymyxin B1 in tandem mass spectrometry

Analyte	MRM ions (*m/z*)	DP (V)	CE (V)	CXP (V)	FP (V)
Colistin A	585.7 → 101.2	61	47	9	330
	585.7 → 241.4	63	33	7	330
Colistin B	578.8 → 101.2	59	49	9	330
	578.8 → 227.3	61	31	7	330
Polymyxin B1	602.7 → 241.4	68	33	7	330
	602.7 → 233.4	67	42	7	330

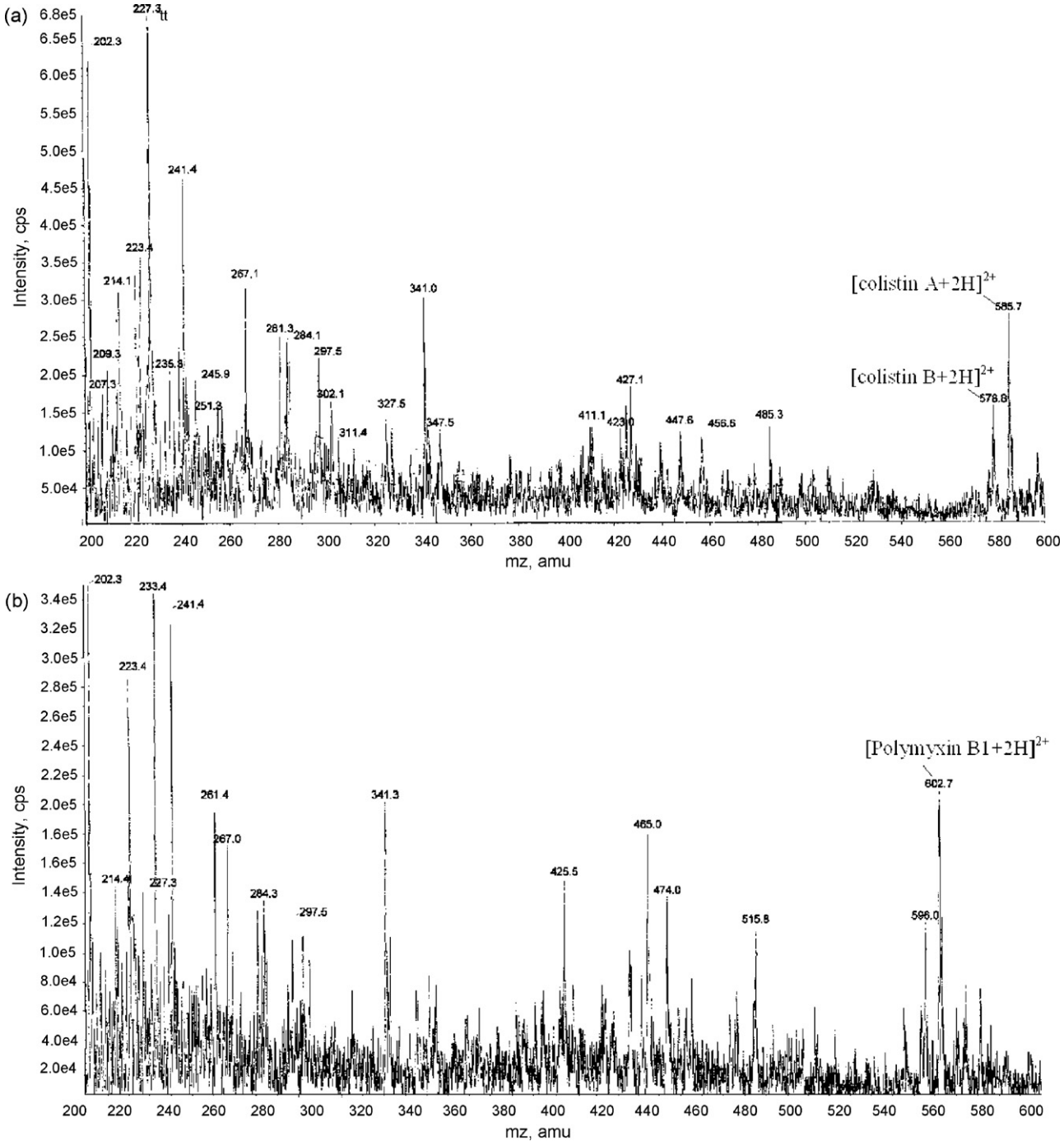

Fig. 2. Full mass spectra of (a) colistin sulfate (1 μg/mL) and (b) polymyxin B sulfate (1 μg/mL) infused into the triple quadrupole mass spectrometer.

as well as the dominant daughter ions for MRM. The singly charged colistin and polymyxin B were barely observable in their respective spectra (data not shown). The doubly charged ions ([colistin A + 2H]$^{2+}$ at *m/z* 585.7 and [colistin B + 2H]$^{2+}$ at *m/z* 578.8) were found to be the dominant species, which is consistent with a previous study using ESI as the ionization source [20]. The *m/z* 101.2 and *m/z* 241.4 were found to be the dominant daughter ions for colistin A and *m/z* 101.2 and *m/z* 227.3 for colistin B. The fragment ions at *m/z* 101.2 represents [L-Dab-γ-NH_2]$^+$, *m/z* 241.4 represents [6-methyloctanoicacid-L-Dab-γ-NH_2 + H]$^+$ and *m/z* 227.3 represents [6-methylheptanoicacid-L-Dab-γ-NH_2 + H]$^+$ [24].

Neither polymyxin B1 nor B2 is commercially available. Thus, commercially available polymyxin B sulfate containing a mix of the two was used as the internal standard. However, only polymyxin B1 was quantified by monitoring *m/z* 602.4 → 241.4 and *m/z* 602.4 → 233.4. The *m/z* 602.4 corresponds to the doubly charged polymyxin B1 ([polymyxin B1 + 2H]$^{2+}$) [25], while the product with *m/z* 233.4 corresponds to the singly charged di-

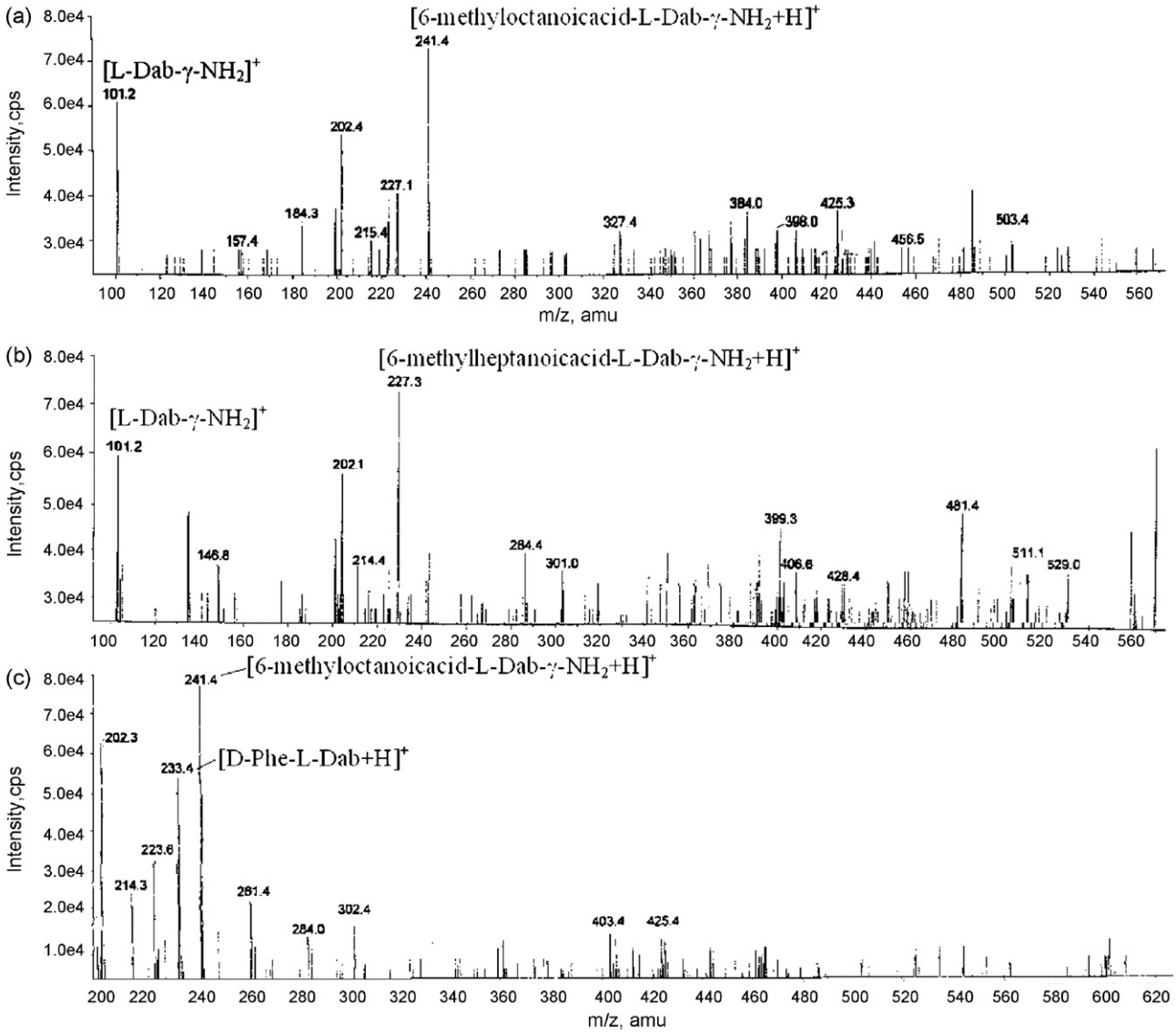

Fig. 3. Daughter ions spectra of (a) [colistin A + 2H]$^{2+}$ at *m/z* 585.7, (b) [colistin B + 2H]$^{2+}$ at *m/z* 578.8 and (c) [polymyxin B1 + 2H]$^{2+}$ at *m/z* 602.7 under MRM conditions.

peptide [D-Phe-L-Dab + H]$^{+}$, where L-Dab-γ-NH_2 has lost its γ-NH_2 group (as shown in Fig. 3).

3.2. Estimating the proportion of colistin A and B in the raw material

The percentages of colistin A and B in the raw material were calculated from the corresponding ratio of their peak areas after HPLC of an aqueous solution of colistin sulphate with detection by UV absorbance. The ratio of the peak areas of colistin A to B (1.76 ± 0.02, $n = 4$) differed from the ratio of the response to colistin A and B (1.59 ± 0.05, $n = 5$) after injection of an aqueous solution of colistin sulfate (10 μg/mL,) onto the LC–MS/MS. The congeners of colistin differ only in the length of fatty acid moiety by one methylene group or in amino acids in the peptide moiety by substitution of serine for threonine or an alkyl amino acid for leucine in colistin A and B (two minor congeners have methionine substituted for leucine) [24]. Therefore, it was assumed that these two analytes and the other colistin congeners within the raw material had the same response factor with UV detection. Hence the percentages of colistin A and B in the raw material after HPLC-UV analysis were used for generating calibration curves of apparent nominal concentrations against their respective responses relative to polymyxin B1.

3.3. Sample pretreatment

Direct injection onto an LC–MS/MS system after protein precipitation was used to quantify colistin in milk [20], which contains proteins, carbohydrates, lipids and smaller solutes. However, our in-house experience has shown that such a practice leads to a rapid loss of sensitivity and variable suppression of the MS response; therefore, prior extraction of the samples was necessary. During optimization of the pretreatment procedure, we found it very important to use TCAA for treat-

ing samples prior to SPE. Indeed, TCAA has been used in a number of studies as an effective reagent for protein precipitation and breaking drug–protein bonds. In our preliminary tests, acetic acid at various concentrations in water or methanol were tested, but none of them gave reproducible recovery of colistin. However, 10% TCAA solution mixed with an equal volume of methanol as the protein precipitating reagent gave satisfactory results with human plasma and IPK perfusate and was also used with urine. The use of TCAA is supported by several previous reports. For example, Sin et al. [20] used acetic acid in acetonitrile at various concentrations for treating samples of milk and tissue homogenates but failed to gain a satisfactory peak for colistin, while 4% TCAA in acetonitrile was found to be a suitable precipitating reagent for recovering colistin from these samples [20]. In addition, Decolin et al. [4] found that the addition of methanol to TCAA solution was necessary to improve recoveries of colistin from solid tissues. In our study, 40% methanol in the supernatant after centrifugation may increase the recovery of colistin, as less polar impurities would be washed out during loading and colistin A and B retained on the sorbent. The colistin was then eluted by 2× 300 μL methanol–water–acetic acid (80:19:1, v/v/v). Further elution revealed negligible amounts of colistin retained by the SPE sorbent.

3.4. Chromatographic conditions

Polymyxin B1 was chosen as the internal standard as it has a structure and chemical properties similar to colistin. Therefore, it is not surprising that polymyxin B and colistin A or B have almost identical retention times. However, with the LC–MS/MS settings described above in MRM mode, such overlapping signals do not pose any interference when quantifying either colistin A or B. Fig. 4 shows an example of a typical chromatogram for colistin at the LLOQ in human plasma. When each blank medium was prepared and analyzed without added internal standard, no significant interfering peak was observed (chromatogram not shown).

Colistin is a mixture of compounds, with two major components (colistin A of MW 1169, colistin B of 1155); they possess hydrophilic (heptapeptide ring) and hydrophobic (fatty acid chain) moieties (Fig. 1). During preliminary work, tailing and peak broadening were observed for colistin on an MS chromatogram when the mobile phase contained a low percentage of acetonitrile. Such a phenomenon, probably due to the terminal amine groups and the long fatty acid moiety, was also observed by Wan Eric et al. [21]. These workers used gradient elution to improve peak shape. However, we were able to alleviate the tailing and improve the shape with a different column and a simpler isocratic mobile phase containing an increased proportion (80%) of acetonitrile. When analyzing colistin by capillary zone electrophoresis, a mobile phase of low pH was found to reduce the absorption to the capillary wall, which presumably occurred via its five free amino groups [15]. We also observed an improvement in peak shape when 1% acetic acid was included in the mobile phase.

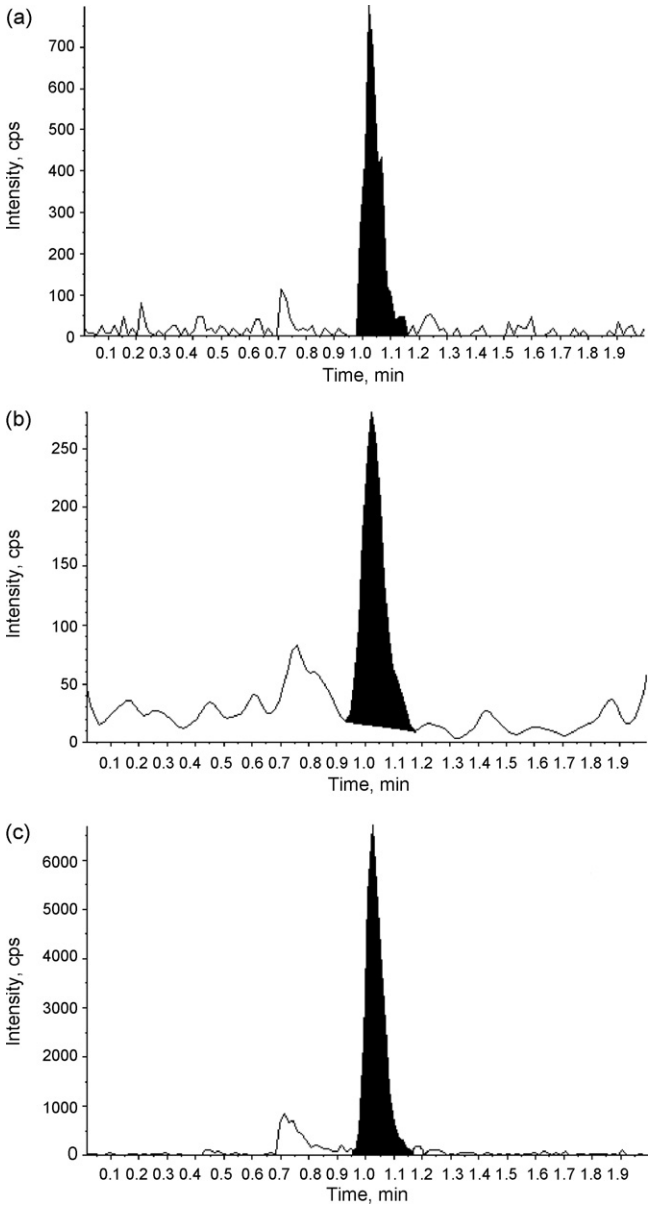

Fig. 4. Typical LC–MS/MS chromatograms of colistin A (a), colistin B (b) and the internal standard (c) in drug-free human plasma spiked with colistin sulfate at 0.05 μg/mL, corresponding to apparent nominal concentrations for colistin A and B sulfates of 0.028 and 0.016 μg/mL, respectively.

3.5. Method validation

The LLOQ for colistin A sulfate was calculated as 0.056 μg/mL in human urine and 0.028 μg/mL in human plasma, IPK urine and perfusate, while the corresponding values for colistin B sulfate were 0.032 and 0.016 μg/mL. The equations for the calibration curves of colistin A sulfate in human plasma, urine, IPK perfusate and urine ($n = 4$, slope and intercept presented as mean values) were $y = 3.84x + 0.011$, $y = 2.29x - 0.030$, $y = 3.55x + 0.003$ and $y = 3.26x - 0.016$, respectively; the corresponding equations for colistin B sulfate were $y = 3.11x + 0.020$, $y = 2.83x - 0.071$, $y = 3.20x + 0.062$ and $y = 3.01x + 0.009$. The linearity of the assay was achieved over the range of (0.028/0.056)–1.78 μg/mL and

Table 2
Intra- and inter-day precision (%CV) of the assay for QC samples containing different concentrations of colistin sulfate

Analyte	Medium	Intra-day CV (%, $n=6$)					Inter-day CV (%, $n=4$)				
		0.07 μg/mL	0.14 μg/mL	0.28 μg/mL	0.56 μg/mL	1.12 μg/mL	0.07 μg/mL	0.14 μg/mL	0.28 μg/mL	0.56 μg/mL	1.12 μg/mL
Colistin A sulfate	Plasma	4.0	3.7	4.8	2.8	4.0	6.2	5.8	5.5	3.4	3.7
	Urine	1.7	6.1	6.0	2.5	2.7	5.5	5.6	4.6	1.5	2.3
	Perfusate	5.0	5.6	3.6	4.0	4.3	3.6	2.7	3.1	3.8	3.8
	Perfusion urine	3.9	3.4	1.6	2.4	2.0	3.9	3.6	4.8	3.2	2.5
Analyte	Medium	Intra-day CV (%, $n=6$)					Inter-day CV (%, $n=4$)				
		0.04 μg/mL	0.08 μg/mL	0.16 μg/mL	0.32 μg/mL	0.63 μg/mL	0.04 μg/mL	0.08 μg/mL	0.16 μg/mL	0.32 μg/mL	0.63 μg/mL
Colistin B sulfate	Plasma	4.2	3.7	4.3	6.0	5.2	7.4	6.0	2.4	3.8	4.2
	Urine	4.2	4.9	4.7	2.8	1.8	6.0	6.2	4.5	2.6	3.9
	Perfusate	5.4	4.7	5.3	5.8	5.0	5.9	5.6	4.8	3.7	3.9
	Perfusion urine	2.3	2.6	2.1	3.6	1.8	4.1	4.0	4.0	5.2	2.8

Table 3
Intra- and inter-day accuracy (Diff%) of the assay for QC samples containing different concentrations of colistin sulfate

Analyte	Medium	Intra-day (%, $n=6$)					Inter-day (%, $n=4$)				
		0.07 μg/mL	0.14 μg/mL	0.28 μg/mL	0.56 μg/mL	1.12 μg/mL	0.07 μg/mL	0.14 μg/mL	0.28 μg/mL	0.56 μg/mL	1.12 μg/mL
Colistin A sulfate	Plasma	4.4	4.2	5.2	5.9	4.0	4.8	5.1	4.5	2.5	3.5
	Urine	1.7	7.1	3.9	3.9	3.6	5.7	5.1	6.0	5.7	3.9
	Perfusate	4.4	8.4	3.8	3.0	5.2	4.1	5.8	3.0	4.0	6.0
	Perfusion urine	3.1	2.6	1.7	2.2	1.5	3.8	2.5	3.8	3.0	1.9
Analyte	Medium	Intra-day (%, $n=6$)					Inter-day (%, $n=4$)				
		0.04 μg/mL	0.08 μg/mL	0.16 μg/mL	0.32 μg/mL	0.63 μg/mL	0.04 μg/mL	0.08 μg/mL	0.16 μg/mL	0.32 μg/mL	0.63 μg/mL
Colistin B sulfate	Plasma	4.2	3.7	4.3	6.0	5.2	7.1	5.1	2.1	3.2	3.7
	Urine	4.6	4.3	4.9	2.2	1.8	5.1	5.2	4.6	2.3	3.0
	Perfusate	4.9	5.4	4.4	5.6	5.6	5.9	4.3	4.0	3.2	4.3
	Perfusion urine	2.2	2.0	2.0	3.0	1.8	5.3	3.5	3.4	3.8	3.2

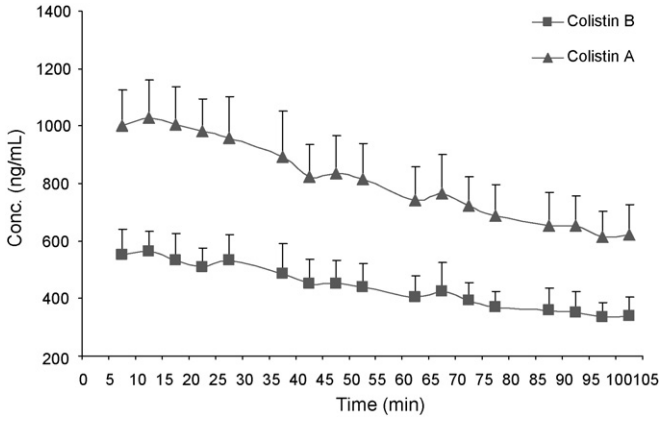

Fig. 5. Perfusate concentration (mean ± SD) profiles of colistin A and B sulfates in the IPK study ($n = 5$).

(0.016/0.032)–1.01 μg/mL for colistin A and B sulfates, respectively, with coefficients of correlation greater than 0.998. After back-calculating the concentrations of colistin A and B sulfates in all calibration standards from the derived calibration curve, accuracy and precision were within 15% (data not shown). Intra-day/inter-day accuracy and precision of the assay for QC samples were within 10% (Tables 2 and 3).

3.6. Application in a pre-clinical pharmacokinetic study

This analytical method has been successfully used for determining the concentrations of colistin in perfusate and urine samples from a pharmacokinetic study in the IPK. Fig. 5 shows a rapid decrease in the concentrations of both colistin A and B sulfates in perfusate.

4. Conclusion

A new LC–MS/MS method has been developed for measuring colistin in biological samples. The method includes protein precipitation with a mixture of TCAA and methanol, extraction of colistin A and B with SPE cartridges, elution with a mixture of methanol, water and acetic acid, followed by injection onto the LC–MS/MS. Importantly, there was no need to derivatize colistin, making sample pretreatment simple and rapid. Furthermore, the small volumes of sample required and very short run times make this method very useful and powerful for analyzing a large number of samples. This method has been successfully used for measuring the concentrations of colistin in IPK perfusate and urine in a pharmacokinetic study examining the disposition of colistin in the isolated organ. To the best of our knowledge, this is the first paper describing a method for measuring colistin in human plasma and urine using LC–MS/MS.

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