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TP 118

Bioanalytical Method for Quantification of Polymyxin B1, B2, B3 and Isoleucine-polymyxin B1 in Human Plasma

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1. Overview

- A simple bioanalytical MRM based method developed for quantitative analysis of Polymyxin B1, B2, B3 and Ile-B1 in human plasma sample on Shimadzu LCMS-8060
- Trichloroacetic acid is successfully used as an protein precipitation and ion-pairing reagent for the bioanalytical method development

2. Introduction

Polymyxin B (PB) is widely used as a last selection of infection therapy due to the emergence of multi-drug resistant bacteria. The commercial formulation of PB is a chemical mixture containing over 30 polymyxin B polypeptides. It was reported that there were variations in the composition of PB components in different products [1]. The different components may not exhibit equivalent pharmacological activity and toxic propensity [2]. Therefore, monitoring of all the main forms of polymyxin B is needed for accurate assessment of their pharmacokinetic properties and toxicity. Thus, we develop a simple, sensitive and selective LC/MS/MS method for the quantitation of four main forms of polymyxin B in human plasma including PB1, PB2, PB3 and Ile-PB1, which account for more than 95% of the polypeptides in commercial formulation.

3. Experimental

Polymyxin B1, B2, B3 and Ile-B1 standards were dissolved in the Milli-Q water (primary stock solution, 1000 µg/mL) and diluted to different working solutions to create the calibration curves and quality control standards, ranging from 0.5 to 100 µg/mL.

Polymyxin Bs pre-spiked in human plasma were extracted by protein precipitation with 40% trichloroacetic acid solution. The procedure is shown in Figure 1. Briefly, 10 µL of 40% (w/v) trichloroacetic acid solution was added to 50 µL of plasma samples and mixed vigorously for 1 min. After precipitation for 10 min, 190 µL of MQ water added to the sample for dilution. After centrifugation at 15,000×*g* for 10 mins, the supernatant was transferred into a HPLC glass vial. The obtained sample was injected to LCMS-8060 for analysis. The analytical conditions on LC-MS /MS 8060 are shown in Table 1.

Table 1. Analytical conditions of polymyxin Bs on LCMS-8060

Column	Shim-pack GISS C18 (100 mm. x 2.1mm I.D., 1.9µm)
Flow Rate	0.4 mL/min
Mobile Phase	A : 0.01% Trifluoroacetic acid (TFA) in milli-Q water with 0.5% Formic acid B : 0.01% Trifluoroacetic acid (TFA) in Acetonitrile (ACN) with 0.5% Formic acid
Elution Mode	Gradient elution, LC program 10 minutes 14%B (0.01 min to 1.00 min)→25%B (2.00 min) →28% B (7.00 min to 8.00 min) →22%B (9.00min)
Oven Temp.	40ºC
Injection Vol.	20 µL

Interface	ESI
MS Mode	MRM, Positive
Heat Block temp.	350ºC
DL temp.	250ºC
Interface temp.	350 ºC
Nebulizing gas flow	Nitrogen, 3.0 L/min
Drying gas flow	Nitrogen, 10.0 L/min
Heating gas flow	Zero air, 10 L/min
CID gas	270 kPa (Ar)

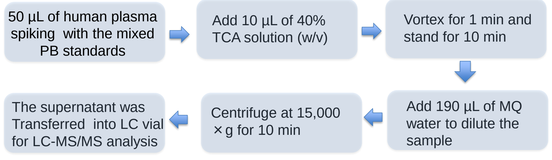

Figure 1. The procedure for human plasma sample preparation

MRM method of polymyxin Bs

Polymyxin B1 and Ile-PB are structural isomers and polymyxin B2 and B3 are also isomers. Therefore, it is expected to set up chromatographic conditions to be able to separate the isomers. As shown in Table 2 and Figure, the LC method established can separate the isomer pairs completely under the conditions. The main precursors of Polymyxin Bs are doubly-charged. MRM optimization was performed using an Automated MRM optimization program in LabSolutions. The details of the MRM parameters obtained are shown in Table 2.

Table 2. MRM transitions and compound-dependent MS parameters for Polymyxin Bs on LCMS-8060

Compound	RT (min)	MRM Transition		Pause time (msec)	Dwell time (msec)	Q1 Pre Bias (V)	C.E. (V)	Q3 Pre Bias (V)
		Precursor	Product					
Polymyxin B1	8.59±0.02	602.65 (2+)	241.20	3	20	-24	-24	-27
			101.10	3	20	-24	-35	-20
Ile-PB1	7.64±0.04	602.65 (2+)	241.15	3	20	-22	-24	-17
			101.15	3	20	-26	-35	-19
Polymyxin B2	6.14±0.03	595.75 (2+)	227.20	3	20	-24	-20	-16
			101.10	3	20	-26	-40	-21
Polymyxin B3	6.56±0.03	595.70 (2+)	227.15	3	20	-24	-24	-11
			101.05	3	20	-24	-33	-19

4. Results and Discussion

4.1 Effects of TCA as ion-pairing reagent added in samples to separation and peak shape of polymyxin Bs

Ion-pairing chromatography has been applied normally with addition of an ion-paring reagent in the mobile phase. An alternative way is used recently, where the ion-paring reagent is only deposited on the column brought with sample injection. This was preferred especially for LC-MS/MS analysis. Successful analytical methods of this kind of ion-pairing chromatography were reported to achieve desirable retention and resolution on a reversed phase column with so-called LC-MS friendly mobile phase without addition of ion-pairing reagent [3]. TCA is selected as the ion-pairing regent in this work. It is added to only the samples, but not in the mobile phase. It was observed that with TCA added to sample only, the LC separation of the polymyxins Bs was improved significantly in peak shape and tailing effect.

Using this simple sample preparation with TCA added only in protein crashing of plasma sample, desirable retention and good resolution of the four polymyxin Bs were achieved on a Shim-pack GISS C18 (100 mm. x 2.1mm I.D., 1.9µm) column with mobile phases without TCA. The effect of the different concentration of trichloroacetic acid on the protein precipitation and the chromatographic behavior were investigated. The results showed that 40% TCA solution works best for the good efficiency in protein precipitation and achieved the baseline separation and symmetry peaks for all four forms of polymyxin Bs.

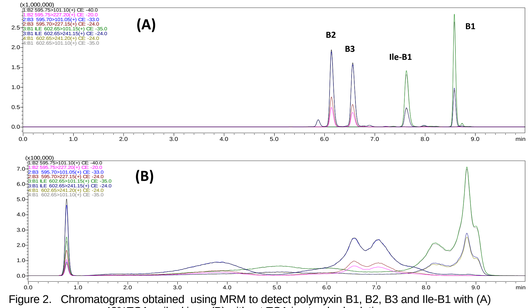

Figure 2. Chromatograms obtained using MRM to detect polymyxin B1, B2, B3 and Ile-B1 with (A) 40%TCA spiked in or (B) without TCA in standard solutions.

4.2 Performance evaluation for quantitation of polymyxin Bs in human plasma

Linearity, LOD and LOQ: Respectable linearities (R^2 >0.995) were achieved for polymyxin B1 and B2 in the range of 0.1 ~ 5 µg/mL, PB1 and Ile PB1 in the range of 0.05 ~ 3.75 µg/mL. Calibration linear ranges were different for the four forms of polymyxin B because of their different concentration level in commercial formulation of polymyxin B.

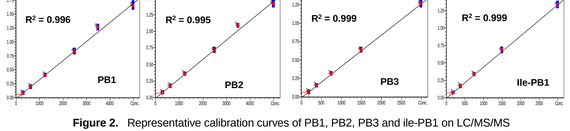

Figure 2. Representative calibration curves of PB1, PB2, PB3 and ile-PB1 on LC/MS/MS

The LOD and LOQ for the four forms of polymyxin B standards ranged from 0.32 to 5.40 µg/mL and 1.13 to 17.99 µg/mL, respectively as shown in Table 3.

Accuracy tests were performed by testing polymyxin Bs standards at low, medium and high concentration levels within their respective calibration ranges. The errors in accuracy were less than 20%, and prevision is demonstrated was less than 15% RSD. ***Extraction recovery and matrix effect*** were evaluated and the results were shown in Table 4.

Table 3. Linearity, LOD and LOQ of polymyxin Bs in human plasma

Compound	Range (µg/mL)	R^2	LOD (ng/mL)	LOQ (µg/mL)
PB1	0.1 – 5	0.996	5	0.01
PB2	0.1 – 5	0.995	5	0.01
PB3	0.05 – 3.75	0.999	5	0.01
Ile-PB1	0.05 – 3.75	0.999	5	0.03

Table 4. Method performance for the quantification of polymyxin Bs in human plasma

Compound	RT (min)	Conc. Level (µg/mL)	Accuracy (n=6)	RSD (%)	Conc. Level (µg/mL)	Matrix effect	Recovery (%) (n=2)
PB1	8.59 ± 0.02	5	97.18	2.19	5	121.63	91.16
		2.5	99.4	2.53	1	128.48	103.82
		0.625	98.08	2.53	0.1	–	58.93
PB2	6.14 ± 0.03	5	97.23	1.52	5	134.16	95.10
		2.5	98.20	1.86	1	115.13	104.03
		0.625	98.37	2.21	0.1	–	60.04
PB3	6.56 ± 0.03	3	99.70	1.47	5	109.84	93.15
		1.5	100.93	1.61	1	121.61	102.08
		0.375	100.04	2.37	0.1	–	65.34
Ile-B1	7.64 ± 0.04	3	99.43	1.70	5	123.01	90.58
		1.5	102.28	2.20	1	115.27	107.47
		0.375	99.67	2.81	0.1	–	60.46

5. Conclusions

We described the development of an analytical method with trichloroacetic acid (TCA) used as a precipitation reagent and ion-pairing reagent added to plasma samples only. This novel and simple LC-MS/MS method exhibits excellent separation for the isomers of polymyxin B1 and Ile-PB1, as well as polymyxin B2 and B3 and with good peak shape. The quantification performance of polymyxin B1, B2, B3 and ile-B1 in human plasma samples was evaluated including linearity, LOD, LOQ, accuracy, recovery and matrix effect. One additional advantage of the method is avoiding ion-pairing reagent in the mobile phase, allowing the analysis with a compatible LCMS mobile phase.

Acknowledgement

We thank Dr. Lim Tze Peng (Department of Pharmacy, Singapore General Hospital) for providing polymyxin B1, B2, B3, Ile B1 standard solutions.

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