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Application News

No. AD-0203

Bioanalysis / LCMS-8060

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

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❑ Overview

A bioanalytical MRM based method developed for quantitative analysis of Polymyxin B1, B2, B3 and Ile-B1 in human plasma samples on LCMS-8060. Trichloroacetic acid (TCA) was used as protein precipitation and ion-pairing reagent in this bioanalytical method development.

❑ 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 medicine 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 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.

❑ Experimental

The primary stock solution of polymyxin B1, B2, B3 and Ile-B1 (dissolved in the Milli-Q water, 1000 µg/mL) were further diluted to different working solutions ranging from 0.5 to 400 µg/mL. The calibration and quality control standards were prepared in human plasma contained mixed PB1, B2, B3 and Ile-B1 at final concentrations of 0.1, 0.25, 0.5, 1, 2.5, 3.75 and 5 µg/mL.

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

Table 1. Analytical conditions of four polymyxin B 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)

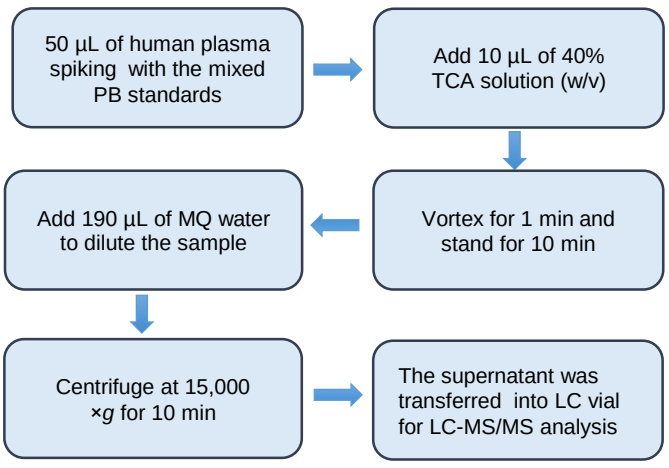

Figure 1. Procedure of human plasma sample preparation

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MRM method of polymyxin B

Polymyxin B1 and Ile-PB are structural isomers and polymyxin B2 and B3 are also isomers. The main precursors of these Polymyxin B 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 CE parameters for Polymyxin Bs on LCMS-8060

Compound	RT (min)	MRM Transition (m/z)		Q1 Pre Bias (V)	C.E. (V)	Q3 Pre Bias (V)
		Precursor	Product			
PB1	8.59	602.65 (2+)	241.20	-24	-24	-27
			101.10	-24	-35	-20
Ile-PB1	7.64	602.65 (2+)	241.15	-22	-24	-17
			101.15	-26	-35	-19
PB2	6.14	595.75 (2+)	227.20	-24	-20	-16
			101.10	-26	-40	-21
PB3	6.56	595.70 (2+)	227.15	-24	-24	-11
			101.05	-24	-33	-19

❑ Results and Discussion

Effects of TCA as ion-pairing reagent

Ion-pairing chromatography has been used in analysis of polar compounds with C18 columns. However, a drawback of this method is that the ion-pairing reagent present in the mobile phase may cause significant ion suppression and contamination in LC-MS/MS analysis. An alternative way was developed for the analysis of highly polar aminoglycoside [3], in which the ion-paring reagent was added only in the injected extract, while the mobile phases remain no addition of any ion-paring reagent. Trichloroacetic acid (TCA) was reported to be used as ion-pairing regent and also used in plasma precipitation at various concentration level. In this work, TCA was selected as the ion-pairing regent added to the injected extracts, but not in the mobile phase.

Moreover, Polymyxin B1 and Ile-PB are structural isomers and polymyxin B2 and B3 are also structural isomers. Therefore, it is expected to set up chromatographic conditions to be able to separate these isomers. Mixed standard sample with adding TCA in the sample was injected to LCMS-8060. As shown in Figure 2, desirable retentions with sufficient separation and good peak shape of the four polymyxin B were achieved on a Shim-pack GISS C18 column under the LC conditions.

The effects of trichloroacetic acid (TCA) concentration on the chromatographic behavior and protein precipitation extraction were addressed in our study. The results showed that 40% TCA solution worked best for the good efficiency in protein precipitation and achieved the baseline separation and symmetry peaks for all four forms of polymyxin B in this study.

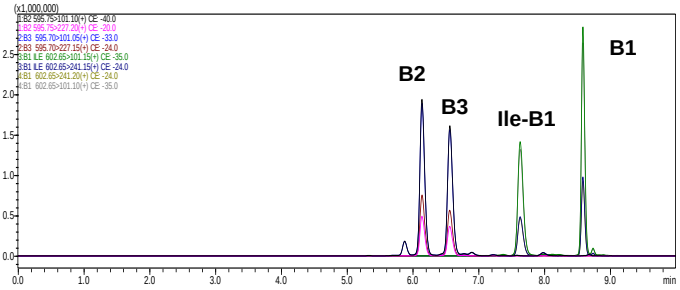

Figure 2. MRM chromatogram of mixed polymyxin B1, B2, B3 and Ile-B1 standard sample with 1 µg/mL TCA.

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, B2, B3 and Ile-B1 in the range of 0.1 ~ 5 µg/mL (shown in Fig. 3). The estimated LOD and LOQ for the four forms of polymyxin B in human plasma were shown in Table 3.

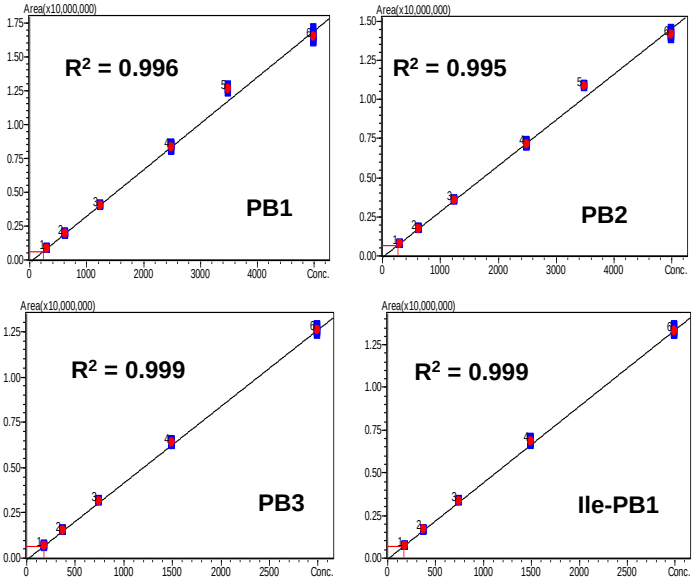

Figure 3. Calibration curves of PB1, PB2, PB3 and Ile-PB1 spiked in pooled human plasma on LCMS-8060

Table. 3 Linearity, LOD and LOQ of four polymyxin B in pooled human plasma

Compound	Range (µg/mL)	R^2	LOD (ng/mL)	LOQ (ng/mL)
PB1	0.1 – 5	0.996	0.85	2.57
PB2	0.1 – 5	0.995	2.14	6.49
PB3	0.1 – 5	0.999	1.30	3.93
Ile-PB1	0.1 – 5	0.999	1.12	3.39

Accuracy tests were performed by testing polymyxin B standards at low, medium and high concentration levels within their respective calibration ranges. The errors in accuracy were less than 20%, and precision was demonstrated to be less than 15% RSD (n=6).

Extraction recovery and matrix effect were evaluated and the results were shown in Table 4.

Table 4 Method performance for quantification of polymyxin B in human plasma on LCMS-8060

Compound	RT (min)	Conc. Level (µg/mL)	Accuracy (n=6)	RSD (%)	Conc. Level (µg/mL)	Matrix effect (%) (n=6)	Recovery (%) (n=2)
PB1	8.59 ± 0.02	5.0	97.2	2.2	5.0	121.6	91.2
		2.5	99.4	2.5	1.0	128.5	103.8
		0.63	98.1	2.5	0.1	–	58.9
PB2	6.14 ± 0.03	5.0	97.2	1.5	5.0	134.2	95.1
		2.5	98.2	1.9	1.0	115.1	104.0
		0.63	98.4	2.2	0.1	–	60.0
PB3	6.56 ± 0.03	3.0	99.7	1.5	5.0	109.8	93.2
		1.5	100.9	1.6	1.0	121.6	102.1
		0.38	100.0	2.4	0.1	–	65.3
Ile-B1	7.64 ± 0.04	3.0	99.4	1.7	5.0	123.0	90.6
		1.5	102.3	2.2	1.0	115.3	107.5
		0.38	99.7	2.8	0.1	–	60.5

❑ Conclusions

We described the development of an analytical method with trichloroacetic acid (TCA) used as both plasma protein precipitation reagent and ion-pairing reagent. Unlike the typical ion-pairing chromatography, TCA was not added to the mobile phases. The presence of TCA in the sample exhibits significant improvement in retention and separation of isomer pairs of polymyxin B1 and Ile-PB1, as well as polymyxin B2 and B3 with good peak shape in LC-MS/MS analysis. The quantification performance of polymyxin B1, B2, B3 and ile-B1 in pooled human plasma samples was evaluated including linearity, LOD, LOQ, accuracy, recovery and matrix effect. The advantages of this method is simple and avoiding ion-pairing reagent added to the mobile phase, allowing the analysis with a compatible LCMS mobile phase.

❑ Acknowledgement

We sincerely thank Dr. Lim Tze Peng, Department of Pharmacy, Singapore General Hospital, for providing polymyxin B1, B2, B3, Ile B1 standard solutions and valuble discussion.

❑ References

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