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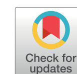
Development of an UPLC-MS/MS method coupled with in-source CID for quantitative analysis of PEG-PLA copolymer and its application to a pharmacokinetic study in rats



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ABSTRACT

Poly(ethylene glycol)-*block*-poly(lactic acid) (PEG-PLA) is a biocompatible and amphiphilic block copolymer composed of a hydrophilic PEG block and a hydrophobic PLA block, which can self-assemble into micelles in water. It is one of the most commonly used biodegradable polymers for drug encapsulation, drug solubilization and drug delivery. Due to the complexity and heterogeneity of PEG-PLA, the precise analysis of this polymer is a great challenge. This study reports an application of an UPLC tandem mass spectrometry coupled with in-source collision induced dissociation (CID) technique for the analysis of a model compound mPEG2000-PDLLA2500-COOH, which could be dissociated in source and generate a series of fragment ions corresponding to its subunits. These surrogate ions including PLA-specific and PEG-specific fragment ions could be further broken into specific product ions in collision cell. Finally, the ion transition at *m/z* 505.0 → 217.0 was selected for the quantitation of mPEG2000-PDLLA2500-COOH. This assay achieved a lower limit of quantitation (LLOQ) of 0.05 μg/mL with only 30 μL rat plasma. The linear range is 0.05 to 5 μg/mL. Intraday and interday accuracy and precision were within ± 12.1%. The method was successfully applied to the pharmacokinetic study of mPEG2000-PDLLA2500-COOH in rats. The results revealed that LC-MS/MS coupled with in-source CID is a sensitive and specific strategy for analysis of PEG-PLA. This method can be potentially extended to the analysis of other pharmaceutical polymer excipients.

1. Introduction

The clinical applications of chemotherapeutic drugs are often limited by their toxicity, side effects, poor aqueous solubility and drug resistance. It has been a hotspot in pharmaceutical research to design an ideal drug delivery system to reduce toxicity and improve efficiency. Many new delivery systems such as polymers, liposomes or micelles have been extensively studied and applied to reduce toxicity and side effects, enhance tumor tissue targeting, prolong blood circulation time, and avoid being swallowed by reticular endothelial system (RES) [1–4]. Among all nanoscale drug carriers, polymeric micelle, self-assembled from biocompatible and biodegradable amphiphilic block copolymers into core-shell nano-structures in aqueous medium, is considered as a promising drug delivery vehicle for tumor targeted delivery of lipophilic drugs [5–7]. In the unique core-shell structures, the hydrophobic core is surrounded by a hydrophilic outer shell extending into the aqueous milieu, which provides a stable interface between the micelle core and the aqueous environment.

Poly(lactide acid) (PLA) is a kind of polymer material with good biocompatibility, biodegradability, bioabsorbability, low immune response, low toxicity and mechanical strength, which can be used as clinical and pharmaceutical high molecular materials [8]. However, the application especially for intravenous administration of PLA is limited due to its hydrophobicity and long degradation time. Therefore, PLA has usually been copolymerized with typical hydrophilic polyethylene glycol (PEG) to change its properties. Biocompatible PEG-PLA amphiphilic diblock copolymer as a novel drug carrier has become more popular in the current decade [9]. As the most commonly used

Abbreviations: PEG-PLA, Poly(ethylene glycol)-block-poly(lactide acid); mPPC, mPEG2000-PDLLA2500-COOH; CID, collision induced dissociation; LLOQ, lower limit of quantitation; PPT, protein precipitation; LLE, liquid-liquid extraction; SPE, solid-phase extraction; AUC_{0-t}, area under the plasma concentration-time curve; $t_{1/2}$, plasma elimination half-life; HPLC-MS/MS, high performance liquid chromatography tandem mass spectrometry; IS, internal standard; ESI, electro-spray ionization; QC, quality control

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hydrophilic block with excellent hydrophilicity, biocompatibility and "stealth" property, PEG could protect the drug-loaded PLA core from protein absorption and cell adhering, thereby prolonging blood circulation time [10]. For hydrophobic drugs, the hydrophilic segment PEG in PEG-PLA can increase the stability and solubility of the drug, and the hydrophobic segment PLA can increase the drug loading rate through hydrophobic interaction. It has been proven that PEG-b-PLA NPs are able to pass the blood brain barrier, and due to this skill, they are one of the most favorable drug carriers for the CNS [11]. In recent years, several PEG-PLA micelles have been approved for clinical trials or even on market. Genexol^{PM}, a paclitaxel formulation based on PEG-PLA copolymer micelles, was approved in South Korea in 2007 for the treatment of breast, lung, and ovarian cancers [12]. Moreover, it is currently under clinical trials in USA [13].

Despite the increased popularity of PEG-PLA micelles in the field of nanomedicine, their application is often limited due to their potential toxicity and long-term side effects. A series of studies have proven that PEG-b-PLA micelles could cause neuroendocrine disrupting effect in the neonatal female rats. Monika Dvořáková et al. indicated that administration of PEG-b-PLA to infantile rats induced a significant reduction in antioxidant capacity and the elevation of catalase and SOD activities compared to controls [11]. Rollerova et al. showed that short time neonatal exposure to polymeric PEG-b-PLA NPs (20 mg.kg b.w.$^{-1}$) may alter the course of in vivo luteinizing hormone releasing hormone stimulated luteinizing hormone secretion in adult female Wistar rats [14]. Besides that, as a pharmaceutical excipient, the dynamic behavior of PEG-PLA could directly affect the release, distribution and excretion of the loaded drugs in vivo. Therefore, a full characterization of the fate and pharmacokinetic behavior of PEG-PLA micelles after administration is necessary to interpret their preclinical and clinical toxicity.

As a polydisperse polymer with high molecular weight, PEG-PLA has countless molecular weights even in a narrow range of PDI. A number of analytical methods have been applied to the analysis of these kind of polymers, including colorimetry, radiolabeling, ELISA, and high performance liquid chromatography (HPLC) [15–23]. However, these methods have certain limitations and drawbacks such as poor specificity and reproducibility, long run time, low sensitivity, large sample volume, and complex sample preparation. The development of high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) has made it possible for quantitative analysis of polymeric excipients. However, due to the heterogeneous nature and the multiple charge states observed for PEG-PLA, direct analysis of PEG-PLA in LC-MS/MS with traditional MRM scan mode is really challenging. To the best of our knowledge, there is no study for determination of PEG-PLA in biological samples by LC-MS/MS has been reported so far. In this study, choosing mPEG2000-PDLLA2500-COOH (mPPC, 2-kDa linear methoxyl poly(ethylene glycol) conjugated with 2.5-kDa poly (D, L-(±)-lactide acid)) as a model compound, we first report a novel quantitative assay utilizing LC-MS/MS coupled with collision-induced dissociation in the ionization source (in-source CID) and MRM scanning mode for analysis of PEG-PLA in biological samples. The method is selective and sensitive. In-source CID, caused by declustering potential added between the orifice and the skimmer, could lead to the fragmentation of parent ions and should be avoided in conventional MRM method [24,25]. However, in this study, we use this technique to produce some specific surrogate ions of mPPC from its numerous precursor ions. Undergoing in-source CID, mPPC could generate a series of unique PEG-specific and PLA-specific ions after HPLC separation. These surrogate ions were subjected to a further CID in collision cell, then the surrogate ions to their fragment ions transitions can be used for the analysis of PEG-PLA in biological samples. MRM provides high sensitivity and specificity for PEG-PLA. Surrogate ions generated by CID in electrospray ionization (ESI) source allows ion population in all charge states for fragmentation without parent ions isolation, and therefore generally creates much higher intensity of the surrogate ions signals. Gong et al. reported the quantitative method for PEG and PEGylated proteins in animal tissues by LC–MS/MS coupled with in-source CID [26]. On account of the lower sensitivity of PEG related ions, the summation of the signals from five transitions was used to improve assay sensitivity, which made the quantification process more complicated. In this study, PLA-related ions with higher response intensity were selected for analysis of PEG-PLA. And the sensitivity of one ion transition could meet the requirement for quantification of this copolymer in plasma. The method was successfully applied to a pharmacokinetic study of mPPC in rats. The results proved that LC-MS/MS with in-source CID and MRM is a sensitive and selective strategy for analysis of mPPC. It is believed that the in-source CID with MRM technique applied in this study can be potentially extended to the analysis of other polymers.

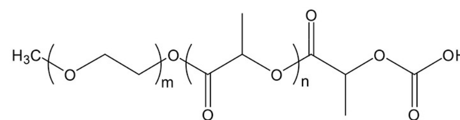

Fig. 1. Chemical structure of mPEG2000-PDLLA2500-COOH.

2. Materials and methods

2.1. Chemical and reagents

Linear mPPC were provided by Xi'an ruixi Biological Technology Co. Its structure is shown in Fig. 1. Oxcarbazepine (purity > 98%) for use as internal standard (IS), HPLC grade acetonitrile and isopropanol were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Ultra-high purity water was prepared by a Milli-Q System. Formic acid was analytical grade and used without further purification.

2.2. UPLC-MS/MS conditions

Ultra-high performance liquid chromatography (UPLC) was performed using an Acquity UPLC system (Waters Corp., MA, USA) with auto-sampler maintained at 4 °C. Separation of mPPC was conducted on a ZORBAX RRHD 300SB-C8 column (2.1 × 50 mm I.D, 1.8 μm, 300 Å, Agilent, USA) maintained at 40 °C at a flow rate of 0.3 mL/min. Mobile phases consisted of 0.1% formic acid in deionized water as aqueous phase (A) and 50% isopropanol in acetonitrile as organic phase (B). The gradient elution was performed as follows: 0–1.0 min 10% B; 1.0–3.0 min 10 → 40% B; 3.0–3.5 min 40 → 90% B; 3.5–5.5 min 90% B; 5.5–5.6 min 90 → 10% B; 5.6–7.0 min 10% B.

Mass detection used a Qtrap 6500 mass spectrometer (Sciex, Toronto, Canada) equipped with an electro-spray ionization (ESI) source and operated in the positive ion mode. Analyst software (AB SCIEX, version 1.6) was used to acquire and process the data. The positive electro-spray ionization was performed at 500 °C with an ion spray voltage of 5500 V. The pressures of ion source nebulizer gas, heater gas and curtain gas were 50, 50 and 35 psi, respectively. Declustering potentials and collision energies were 280 V and 26 eV for mPPC and 50 V and 25 eV for IS, respectively. Multiple reaction monitoring with in-source CID of mPPC and IS utilized the transitions at m/z 505.0 → 217.0 and m/z 253.3 → 208.2, respectively.

2.3. Preparation of calibration standards and quality control (QC) samples

Stock solutions of mPPC and IS (all in 1 mg/mL) were prepared in acetonitrile-water (50:50, v/v). Calibration standards were prepared by diluting stock solutions with blank rat plasma to final concentrations of 0.05, 0.1, 0.3, 0.5, 1.0, 3.0 and 5.0 μg/mL. QC samples were prepared in the similar way at concentrations of 0.1, 0.5 and 3.0 μg/mL. The working solution for IS (100 ng/mL) was prepared by diluting an aliquot of stock solution with acetonitrile-water (50,50, v/v). All mPPC and IS solutions were stored at 4 °C before use.

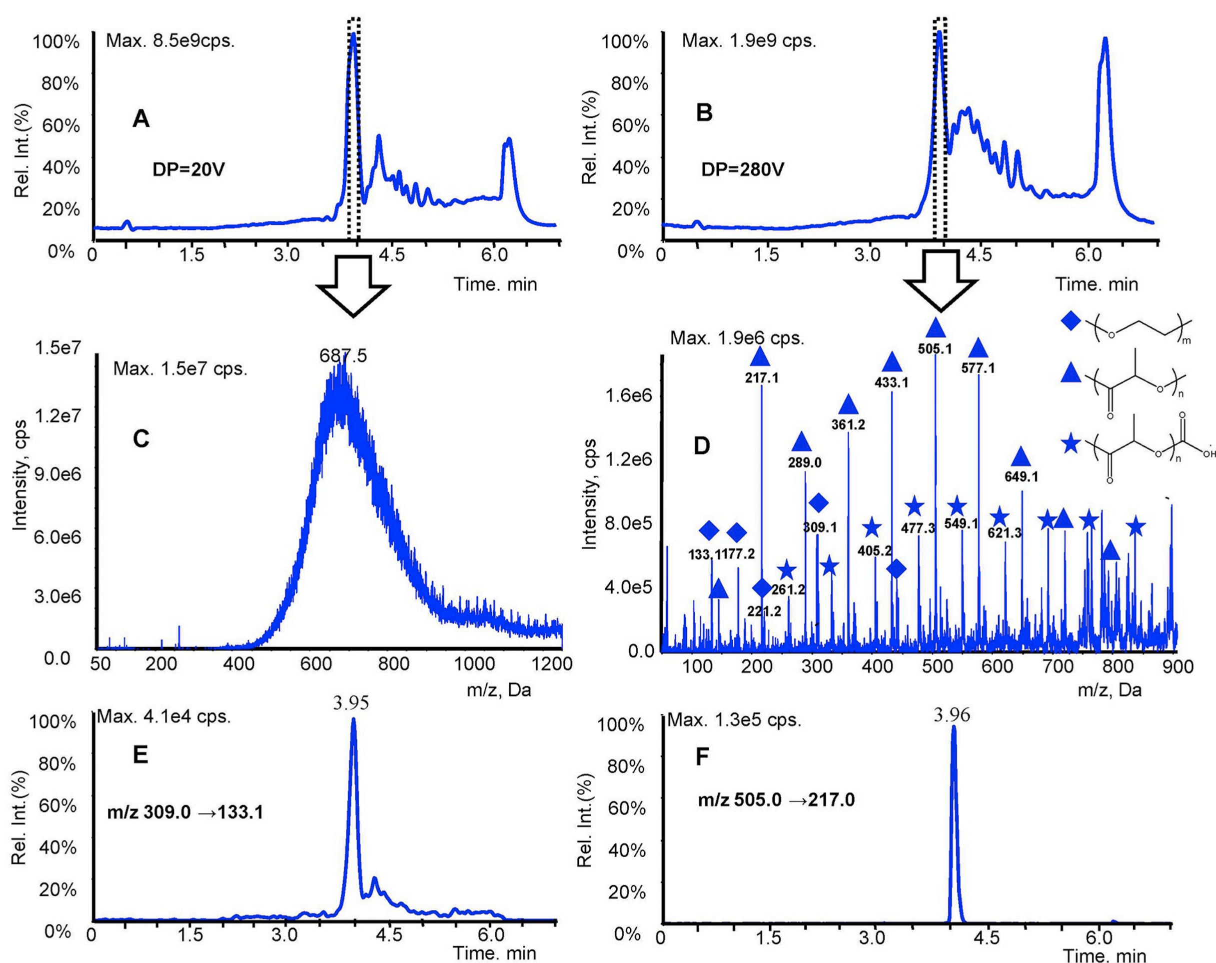

Fig. 2. Mass condition optimization for mPEG2000-PDLLA2500-COOH with a Qtrap 6500 mass spectrometer: TIC of Q1 chromatograms of mPEG2000-PDLLA2500-COOH (10 μg/mL) at DP 20 V (A) and 280 V (B), respectively; in-source CID/Q1 mass spectra from 3.85 min–4.05 min of mPEG2000-PDLLA2500-COOH at DP 20 V (C) and 280 V (D), respectively; representative in-source CID/MRM chromatograms for mPEG2000-PDLLA2500-COOH (1 μg/mL) at mass transition of *m/z* 309.0 to *m/z* 133.1 (E) and *m/z* 505.0 to *m/z* 217.0 (F) at DP 280 V.

2.4. Sample preparation

A 30 μL aliquot of plasma (calibration standards, QC samples and plasma samples from rats dosed with mPPC) was mixed with 20 μL IS working solution and 300 μL acetonitrile in the centrifuge tubes. The mixture was vortex mixed for 1 min and centrifuged at 13,800*g* for 5 min at 4 °C. 2 μL of the supernatant was injected into the UPLC-MS/MS for analysis.

2.5. Assay validation

Specificity was assessed by analyzing blank rat plasma samples obtained from 6 rats. Calibration curves were assessed by weighted linear regression ($1/x^2$) based on the analyte-IS peak area ratios versus analytes' concentrations. The lowest concentration on the calibration curve is taken as the lower limit of quantitation (LLOQ). Intra- and inter-day accuracy and precision were evaluated by analysis of six replicate QC samples on three validation days. The precision expressed as relative standard deviation (RSD(%)) should be within ± 15% and the accuracy expressed as relative error (RE(%)) within ± 15% except at the LLOQ where not exceed ± 20% was accredited. To cover the applied dilution of the rat plasma samples, 6 replicates of 10-fold dilution of samples (ten times of the highest QC concentration) with rat plasma were used to evaluate the dilution integrity. The carry-over effects were evaluated by analyzing extracted blank rat plasma sample immediately after the highest concentration of the calibration curve. The extraction recovery was evaluated by comparing peak areas of mPPC and IS in QC samples with the mean peak areas resulting from their correspondence post-extracted spiked samples. Matrix effect was assessed by comparing peak areas in blank plasma samples spiked with mPPC and IS with those obtained by their corresponding neat standard QC solution. The stability of mPPC in rat plasma was assessed by analysis of triplicate QC samples at each of the low, mid and high concentrations kept at room temperature for 2 h, at −80 °C for 30 days and after three freeze/thaw cycles (−20 °C to room temperature). Stability of processed samples stored under auto-sampler conditions for 4 h was also investigated.

2.6. Pharmacokinetic study

SD rats (n = 6, 3 males, 3 females, weight 200 ± 10 g) were obtained from the Liaoning Changsheng biotechnology Co. Animal welfare and experimental procedures and related ethical regulations were carried out according to the Guidance for the Care and Use of Laboratory Animals of Dalian University of Technology. After a 24 h fasting, each rat was administered a single tail vein intravenous injection of 5 mg/kg mPPC. Dosing volume administered was 5 mL/kg. Blood samples (50 μL) were collected from ophthalmic veins into heparinized tubes before dosing and at 0.083, 0.167, 0.333, 0.5, 1, 2, 3, 4, 6, 8 and 10 h after dosing. Plasma was separated by centrifugation at 13800*g* at 4 °C for 5 min and stored at −80 °C until analysis. Pharmacokinetic parameters were calculated using the software DAS 3.0.

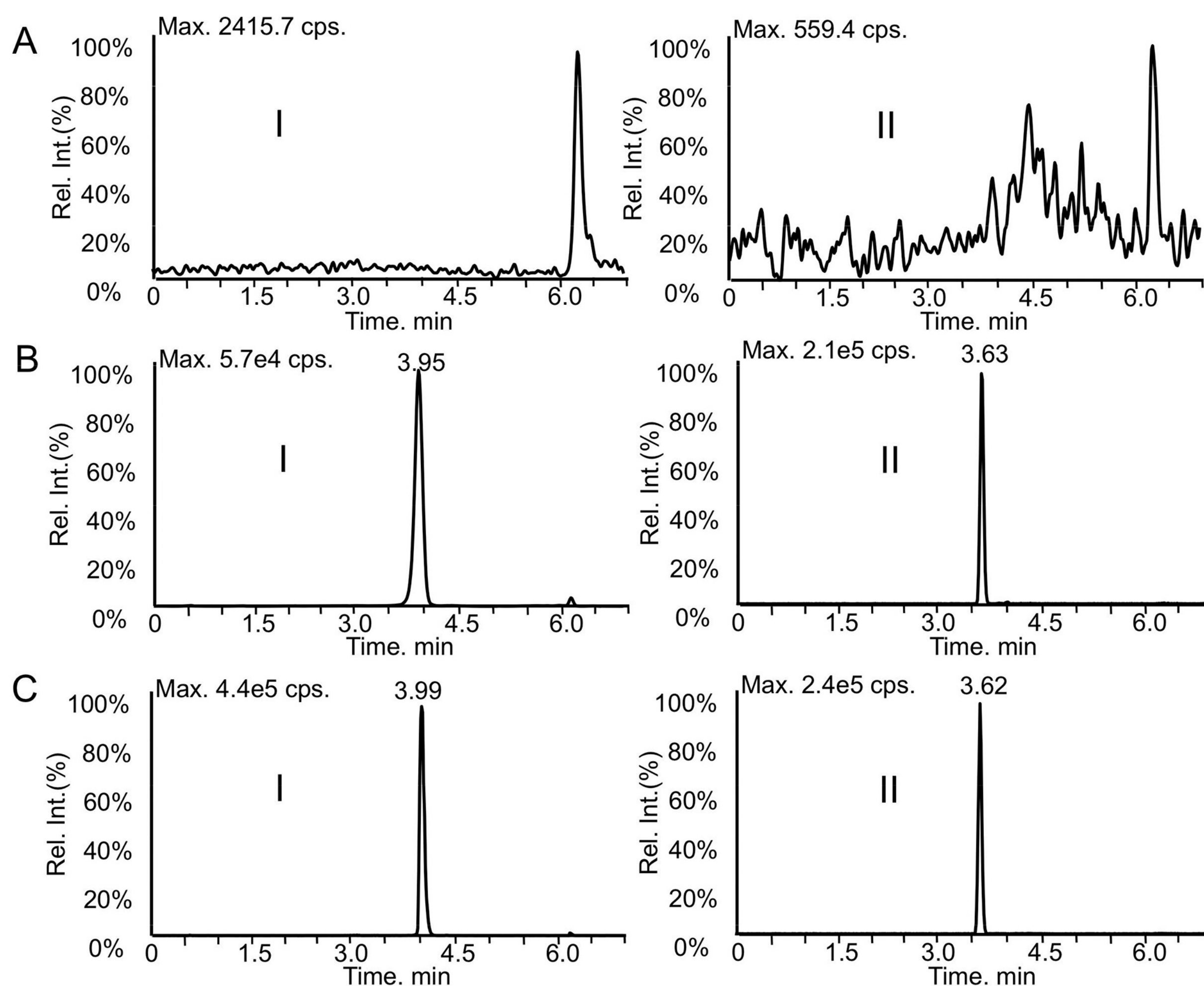

Fig. 3. Representative in-source CID/MRM chromatograms for mPEG2000-PDLLA2500-COOH at mass transition of *m/z* 505.0 to *m/z* 217.0 (I) and oxcarbazepine (II) with Qtrap 6500 mass spectrometer in (A) blank rat plasma sample, (B) blank plasma sample spiked with PEG-PLA(0.05 μg/mL) and oxcarbazepine (0.01 μg/mL) and (C) a rat plasma sample detected 2 h after intravenous injection of 5.0 mg/kg of mPEG2000-PDLLA2500-COOH with oxcarbazepine (0.01 μg/mL) as IS added.

3. Results and discussion

3.1. Optimization of MS conditions

During the optimization of mass spectrometry conditions, traditional direct infusion with a syringe pump was not suitable because it couldn't separate the high molecular impurities from the small molecular polymer which would cause interfere for the selection of surrogate ions. In this study, UPLC-MS/MS was used to optimize the mass spectrometer parameters on a Qtrap 6500 mass spectrometer (Fig. 2A, B). As seen in Fig. 2C, using the ESI source in positive ion mode coupled with in-source CID, most of the ions were normally distributed in the range of *m/z* 500 to *m/z* 900(carried multiple charges), indicating that polymers cannot be efficiently broken at DP 20 V. Compared with it, the fragment ions associated with its structure were appeared at a high DP of 280 V (Fig. 2D). It indicated that under collision-induced dissociation in the ionization source, the polymeric species were dissociated and a series of PEG-specific and PLA-specific ions were generated. As we know, PEG is a synthetic polymer composed of repeating ethylene oxide subunits. It has been reported that in the mass spectra, PEG could be broken into fragments of different structural units, with corresponding *m/z* at 89.05971 (2 monomers), 133.08592 (3 monomers), 177.11214 (4 monomers) and so on [27]. The adjacent fragments had a *m/z* difference of 44 Da. In Fig. 2D, *m/z* 133.2, 177.1, 221.2, 309.4 and 353.3 are a series of regular fragments with a difference of 44 Da between adjacent fragments. It means that these ions are PEG-specific fragment ions. In the same way, PLA is a synthetic polymer composed of repeating lactate subunits(72 Da). In Fig. 2D, *m/z* 73.2, 145.1, 217.1, 289.1, 361.2, 433.1, 505.1, 577.1, 649.2 and 721.0 are a series of regular fragments with a difference of 72 Da between adjacent fragments, which means that these kinds of ions are PLA-specific fragment ions. In this manuscript, the model compound we used is mPPC, a PEG-PLA amphiphilic diblock copolymer. The end of the PLA chain is modified by a carboxyl group, therefore some carboxy-modified PLA fragment ions (*m/z* 189.1, 261.1, 333.3, 405.2, 477.3, 549.1, 621.2 and 693.2) can also be found in Fig. 2D. Theoretically, the end of PEG chain is modified by methyl groups, so there should be some mPEG-specific fragment ions. Due to the low intensity of these kinds of ions, the detail information was not shown in this manuscript. In further study, above ions with higher response were chosen as “parent ion” and transmitted into the collision cell where the collision energy was optimized to obtain product ions with a high signal.

In Fig. 2E and F, two mass transition of *m/z* 309.0 to *m/z* 133.1 and *m/z* 505.0 to *m/z* 217.0 at DP 280 V were present with the same retention time with the TIC of Q1 chromatograms (Fig. 2A and B). The responses were linear over a range of concentrations for both ion pairs. All these indicated that the PEG-PLA can be quantitatively analyzed with the two ion pairs. Due to the lower sensitivity and higher baseline of mass transition *m/z* 309.0 to *m/z* 133.1, finally the mass transition *m/z* 505.0 to *m/z* 217.0 was selected for the quantitation of mPPC. Other parameters in mass spectrometer including spray voltage, gas and temperature in ion source and collision energy were also optimized to

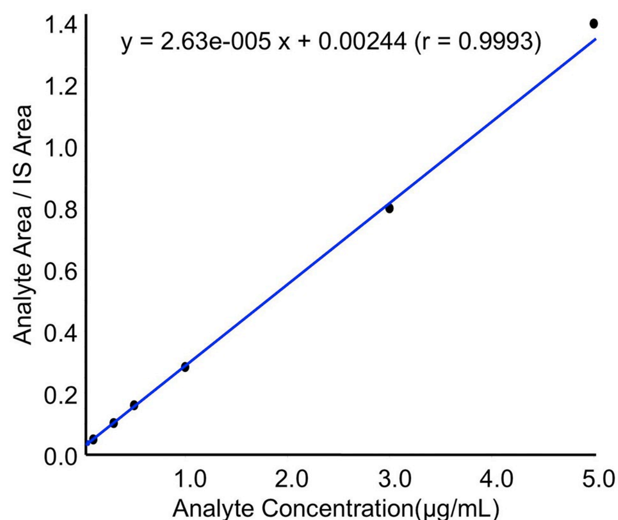

Fig. 4. Representative standard curve of mPEG2000-PDLLA2500-COOH at mass transition of m/z 505.0 to m/z 217.0 in rat plasma (the concentration of oxcarbazepine is 0.01 μg/mL).

give a better sensitivity for determination.

3.2. Optimization of chromatographic conditions

Most macromolecular polymers have large molecular weights ranging from thousands to hundreds of thousands dalton, and their particle sizes are very large. Conventional C18 column has been widely used in chromatographic analysis because of its good separation ability for various compounds. Most commercially available C18 chromatographic columns have pore sizes between 60 Å and 120 Å, which may be blocked by macromolecules with large particle sizes. Moreover, the high molecular polymer has a strong binding ability with the C18 stationary phase resulting in a long retention time on the C18 column, which is not suitable for high throughput analysis. Finally, in order to shorten and rationalize the analysis time for the analysis of mPPC, ZORBAX RRHD 300SB-C8 chromatographic column with a large pore size of 300 Å was chosen for the experiments.

In order to obtain better separation effect and symmetrical peak shape, a series of organic reagents such as methanol, acetonitrile and isopropyl alcohol were evaluated as organic phases. Methanol was excluded due to its poor elution capacity. As we know, macromolecular polymers are mixtures of different chain length polymers. Due to the complex molecular weight of mPPC, the chromatographic peak was too broad and the peak shape was asymmetric while using acetonitrile as organic phase. To solve these problems, isopropanol with stronger elution ability was added into acetonitrile as organic phase. When the volume proportion of the two reagents was 1:1, together with the inclusion of 0.1% formic acid in water as aqueous phase and a suitable gradient elution, good chromatographic peak shape and satisfactory retention with enhanced signal intensity of mPPC could be obtained.

3.3. Sample preparation

Sample preparation is an important procedure before biological samples injected into an UPLC-MS/MS system. Several sample preparation methods including protein precipitation (PPT), liquid-liquid extraction (LLE) and solid-phase extraction (SPE) were tested to obtain an efficient method with better recovery of mPPC. Contrast with PPT, the recoveries of LLE and SPE were limited. Furthermore, the time required for sample processing was shorter as protein precipitation provides simple operation procedures. Compared with methanol, perchloric acid, isopropanol, acetonitrile was chosen for protein precipitation due to its superior recovery and high efficiency of removing interference of biological matrix.

3.4. Assay validation

Specificity is the evaluation of whether the biological matrix interferes with the analysis of the compounds to be tested. In the study, no interfering peaks were observed in the blank rat plasma at the retention times of mPPC and IS, suggesting no interference from endogenous substances (Fig. 3). Calibration curves showed good linear relationship in the concentration range of 0.05–5 μg/mL of mPPC in rat plasma with regression equation calculated at a weighting factor of $1/(x^2)$ (Fig. 4). As shown in Table 1, intra and inter-day precisions were < 10.1% and < 12.1%, respectively, with accuracy < ± 10%, within accepted limits (± 15%) at various concentrations of QC samples, indicating that the method was accurate and reproducible. Assay of the diluted samples also gave satisfactory results with calculated concentrations in the range 85–115% of the nominal concentrations. No obvious carry-over effect was observed. The results of recoveries and matrix effects (Table 2) were consistent at different concentration, showing that ion suppression and enhancement was not significant for mPPC across the concentration range. Stability evaluations showed that mPPC were stable under all the evaluated conditions (Table 3).

Table 1
Accuracy and precision results for the quantitation of mPEG2000-PDLLA2500-COOH in rat plasma.

Concentration(μg/mL)		RSD(%)		RE(%)
Nomial conc	Mean found conc	Intra-day	Inter-day	
0.05(LLOQ)	0.0508 ± 0.00527	10.1	12.1	1.68
0.1	0.0965 ± 0.00701	5.50	10.5	−3.54
0.5	0.472 ± 0.0281	5.98	10.8	−5.66
3.0	3.02 ± 0.154	0.759	2.96	5.77

Table 2
Extraction recoveries and matrix effects for mPEG2000-PDLLA2500-COOH in rat plasma.

Concentration(μg/mL)	Recovery(%)	Matrix effect(%)
0.1	113 ± 5.83	87.4 ± 7.43
0.5	92.9 ± 2.05	112 ± 7.03
3.0	110 ± 3.78	92.7 ± 0.528

Table 3
Stability of mPEG2000-PDLLA2500-COOH in rat plasma and processed samples under different storage conditions.

Nominal conc. (μg/mL)	Plasma samples stored at room temperature for 2 h		Processed samples stored at −4 °C for 30 days		Processed samples stored at room temperature for 4 h		Processed samples freeze/thaw stability (3 cycles)	
	Mean ± SD	RE (%)	Mean ± SD	RE (%)	Mean ± SD	RE (%)	Mean ± SD	RE (%)
0.1	0.107 ± 0.00211	6.67	0.104 ± 0.00414	3.70	0.105 ± 0.00103	5.33	0.0997 ± 0.00474	−0.331
0.5	0.438 ± 0.0180	−12.4	0.436 ± 0.0104	−12.9	0.490 ± 0.0183	−1.93	0.476 ± 0.0232	−4.87
3.0	2.67 ± 0.0763	−11.2	2.68 ± 0.0913	−10.8	2.91 ± 0.0651	−2.89	2.73 ± 0.162	−9.00

Table 4
Pharmacokinetic parameters of mPEG2000-PDLLA2500-COOH in rats after intravenous administration of 5 mg/kg mPEG2000-PDLLA2500-COOH injection.

Parameters	Mean ± SD
$t_{1/2}$ (h)	1.31 ± 0.155
C_0 (μg/mL)	25.3 ± 2.78
$AUC_{(0\text{-}t)}$(μg·h/mL)	44.9 ± 2.95
$AUC_{(0\text{-}\infty)}$(μg·h/mL)	45.1 ± 3.06
$MRT_{(0\text{-}t)}$(h)	1.79 ± 0.0140
CL(L/h/kg)	0.111 ± 0.00800

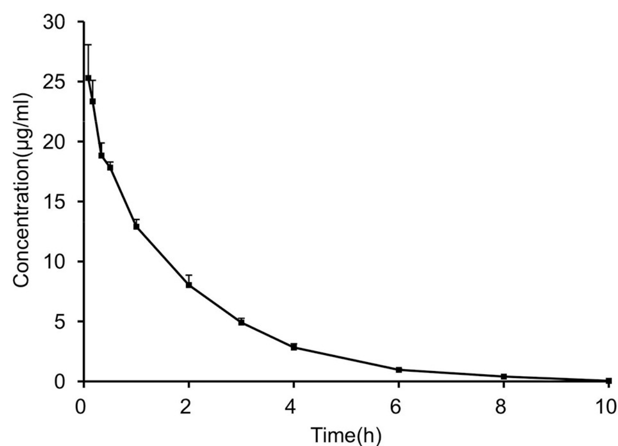

Fig. 5. Mean plasma concentration–time curves of mPEG2000-PDLLA2500-COOH after a single caudal vein intravenous injection of 5 mg/kg mPEG2000-PDLLA2500-COOH to rats (data are mean ± SD, n = 6).

3.5. Pharmacokinetic study

The UPLC-MS/MS method with in-source collision induced dissociation was successfully applied to the pharmacokinetic study of mPPC in rats after a single caudal vein intravenous injection of 5 mg/kg mPPC. Mean pharmacokinetic parameters for mPPC in rat plasma (n = 6) are summarized in Table 4. The mean plasma concentration-time curve of mPPC is shown in Fig. 5. The extrapolated drug concentration (C_0) of mPPC was 25.3 ± 2.78 μg·mL^{-1}. The area under the plasma concentration-time curve ($AUC_{0\text{-}t}$) of mPPC was 44.9 ± 2.95 μg·h·mL^{-1}. As shown in Fig. 5, mPPC had a clear elimination phase after intravenous administration. The concentration of mPPC was reduced to 49 ng/mL at 10 h postdose indicating an almost complete elimination. The fast elimination may relate to the block broken between PEG and PLA as the plasma elimination half-life ($t_{1/2}$) of mPPC (1.31 ± 0.155 h) was smaller than that of PEG400 ($t_{1/2}$ = 2 h) [28]. And it will be helpful for the rapid release and therapeutic effects of the loaded drugs.

4. Conclusions

This work reports the first application of a UPLC tandem mass spectrometry coupled with in-source CID strategy for quantitative analysis of PEG-PLA in rat plasma. The advantages of this method include short analysis time (7 min per sample) and high sensitivity using only 30 μL rat plasma (LLOQ, 0.05 μg/mL). In this study, mPPC dissociated in source and generated a series of surrogate ions corresponding to its subunits. PEG-PLA could be detected by its specific fragment ions related to PLA and PEG. The method was successfully applied to the pharmacokinetic study of mPPC in rats. This study proves that UPLC-MS/MS with CID in source is a sensitive and specific strategy for analysis of PEG-PLA in biological samples. It is believed that the technique applied in this work can be potentially extended to the analysis of other synthetic polymers.

Declaration of Competing Interest

None.

Acknowledgements

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