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Original Article

Automated radiosynthesis of [^{11}C]CPPC for in-human PET imaging applications

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Abstract: The macrophage colony-stimulating factor 1 receptor (CSF1R) is almost exclusively expressed in microglia, representing a biomarker target for imaging of microglia availability. [^{11}C]CPPC has specific binding affinity to CSF1R and suitable kinetic properties for *in vivo* PET imaging of microglia. However, previous studies reported a low radiochemical yield, motivating additional research to optimize [^{11}C]CPPC radiochemistry. In this work, we report an automated radiosynthesis of [^{11}C]CPPC on a Synthra MeIPlus module with improved radiochemical yield. The final [^{11}C]CPPC product was obtained with excellent chemical/radiochemical purities and molecular activity, facilitating high-quality in-human PET imaging applications.

Keywords: [^{11}C]CPPC, radiosynthesis, automation, PET imaging, radiopharmaceutical

Introduction

Positron Emission Tomography (PET) imaging is a powerful tool for identification, characterization, and diagnosis of diseases and disorders [1-3]. Using targeted radiopharmaceuticals, PET imaging can map the distribution and concentration of receptor systems and other molecular targets to identify and localize physiological and pathophysiological underpinnings of human diseases [4-6]. In the human brain, the macrophage colony-stimulating factor 1 receptor (CSF1R) is almost exclusively expressed in microglia, representing a target for imaging of microglia availability [7-9]. Several PET radiopharmaceuticals targeting CSF1R have been developed and investigated [10-16]. Among these, [^{11}C]CPPC demonstrated excellent selective CSF1R affinity in IC_{50} of 0.8 nM and 5 nM for CSF1R inhibition assay and bone marrow derived macrophage proliferation assay, respectively [17]. [^{11}C]CPPC also showed 30-120% increased specific bindings in animal models [12], and high uptakes at brain regions with CSF1R expressing at the first-in-human PET imaging [18]. These preliminary results indicate that [^{11}C]CPPC is a suitable radiotracer for translation studies of the microglial availability in humans. Previous studies reported a low radiochemical yield [19], motivating additional research to optimize [^{11}C]CPPC radiosynthesis. In this work, we report an improved radiosynthesis of [^{11}C]CPPC with excellent radiochemical yield and purity that meet all needs of in-human PET imaging (**Figure 1**).

Materials and methods

General

Unless otherwise stated, reagents, solvents, and chemicals were purchased from commercially available vendors and used without further purification. The 5-cyano-N-(4-(4-methylpiperazin-1-yl)-2-(piperidin-1-yl)phenyl)furan-2-carboxamide (CPPC) reference standard and 5-cyano-N-(4-(piperazin-1-yl)-2-(piperidin-1-yl)phenyl)furan-2-carboxamide (pre-CPPC) precursor were synthesized in-house following the reported method [12]. Prior to use, tC18 cartridge was conditioned with 5 mL ethanol and 5 mL deionized water, respectively. Radioactivity of [^{11}C]CPPC product was determined with a Capintec® CRC-712M dose calibrator (Capintec, Inc., Florham Park, NJ, USA).

Chromatographic method

Reverse-phase semi-preparative high-performance liquid chromatography (HPLC) purification was carried out on an RNplus Research module (Synthra, Hamburg, Germany). The reverse-phase semi-preparative HPLC was conducted on a Synergi 4 μm Fusion-RP 80 Å LC Column (4 μm, 250 × 10 mm; Phenomenex, Torrance, CA) with MeCN/0.1 M $HCOONH_4$ (v/v = 50/50) mobile phase at a flow rate of 6 mL/min. The retention times of pre-CPPC and [^{11}C]CPPC were 4-6 and 9-11 min, respectively.

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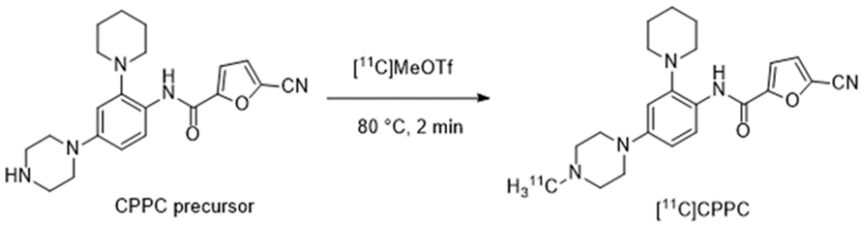

Figure 1. Scheme of [^{11}C]CPPC radiosynthesis.

Reverse-phase analytical analyses were performed on an Acquity ultra-performance liquid chromatography (UPLC) system (Waters, Milford, MA, USA). The chemical and radiochemical purities analyses were conducted on an analytical ACQUITY BEH C18 column (1.7 μm, 2.1 × 100 mm; Waters, Milford, MA, USA) at the UV wavelength of 254 nm. The sample injection volume was 10 μL, and the mobile phase was a mixture of MeCN/0.05 M $HCOONH_4$ (v/v = 45/55) with a flow rate of 0.3 mL/min. The retention times of pre-CPPC and CPPC reference standard were 1.4 and 2.4 min, respectively.

RNplus research module configuration

Synthra RNplus research module configuration and setup for [^{11}C]CPPC production are detailed in **Figure 2**. In the module configuration, vials A1, C1-C3, bottle D1, and reaction vessels 1 were used for [^{11}C]CPPC radiosynthesis, purification, and formulation.

Radiosynthesis, purification, and formulation of [^{11}C] CPPC

Radiosynthesis of [^{11}C]CPPC was performed on a Synthra MeIPlus Research module with the methylation of the precursor with [^{11}C]CH_3OTf, which was converted from [^{11}C]CO_2. [^{11}C]CO_2 was generated by $^{14}N(p, \alpha)^{11}C$ reaction with a 16.5 MeV GE PETtrace 800 cyclotron, and then delivered to the Synthra MeIPlus module. [^{11}C]CO_2 was trapped at -190°C and reduced to [^{11}C]CH_4 at 350°C by passing through a nickel catalyst column under hydrogen flow. [^{11}C]CH_4 was further converted to [^{11}C]CH_3I by iodination at 740°C under a pump-driven circulation. The formed [^{11}C]CH_3I was trapped in a Pora-pak Q column at room temperature, and released from the column at 200°C. [^{11}C]MeI was simultaneously converted to [^{11}C]CH_3OTf by passing through a AgOTf column at 200°C.

Next, [^{11}C]CH_3OTf was delivered into the reaction vessel, in which the pre-CPPC precursor dissolved in DMSO (300 μL) was preloaded. The reaction mixture was heated to 80°C for 2 minutes, followed by 1 mL HPLC eluent dilution, and transferred to a semi-preparative HPLC column for separation (**Figure 3**). The [^{11}C]CPPC fraction was collected, diluted with deionized water, and concentrated on a solid-phase extraction tC18 cartridge. Followed by washing with 10 mL sterile water, [^{11}C]CPPC was eluted with 1.5 mL ethanol from tC18 cartridge, reconstituted in 10 mL normal saline, and passed through a sterile 0.22 μm Millex-GV filter into a product vial. A sample of ~0.5 mL was taken from the finished final product for quality control (QC) tests following United States Pharmacopeia (USP) and GMP guidelines.

Results and discussions

Radiosynthesis

Three consecutive batches for the validation of [^{11}C]CPPC production were completed successfully on the Synthra MeIPlus module. The total synthesis time of each production was 60 min from the start of bombardment (SOB). The final [^{11}C]CPPC product was obtained in 5.1 ± 0.1 GBq (137 ± 3 mCi) at the end of synthesis (EOS), with 20 min irradiations at 55 μA. The radiochemical yields were 30.8 ± 0.6% decay corrected to the end of bombardment (EOB) based on the starting [^{11}C]CO_2 activity of 66 ± 0.1 GBq (1785 ± 3 mCi). The mean volumes of formulated [^{11}C]CPPC product were 10.8 ± 0.1 mL.

Quality control

QC results demonstrated that the [^{11}C]CPPC product met all release criteria for human use, as shown in **Table 1**. All three batch products were clear, colorless solutions, and free from particulate matter. The pH and half-life values were within the ranges of 4.0-7.0 and 18.3-22.4 min, respectively. From analytical HPLC results (**Figures 4** and S1, S2, S3), the radiochemical purities of [^{11}C]CPPC were 100% and the total unidentified chemical impurities were 0.24 ± 0.11 μg/mL. The concentration of non-radiochemical mass of CPPC were 0.96 ± 0.05 μg/mL, and the molecular activities were 194 ± 14 GBq/μmol (5238 ± 368 mCi/μmol) at EOS. The radionuclidic purities were determined by a Multi-Channel Analyzer (MCA) and no long half-life radioisotopes were detected after overnight decay. The concentrations of residual solvents in the product were below the limits of the release criteria. The integrity of the final filter was demonstrated by a bubble-point filter test. The formulated products were sterile and nonpyrogenic from the sterility and endotoxin results. Stability tests of [^{11}C]CPPC were performed at 1 hour after EOS, showing no significant changes (Table S1).

Discussion

[^{11}C]CPPC has shown selective CSF1R affinity and suitable kinetic properties for *in vivo* PET imaging applications. Due to the 20 min half-life of carbon-11, a high-yield radiosynthesis of [^{11}C]CPPC is essential for in-human investigations. To improve the radiochemical yield, we explored various radiolabeling conditions by testing the solvents, concentrations of precursors and temperatures (data not shown). As a result, the best radiolabeling yields were obtained using DMSO with two minutes heating at 80°C. We completed the automation on the Synthra MeIPlus module, and performed three consecutive valida-

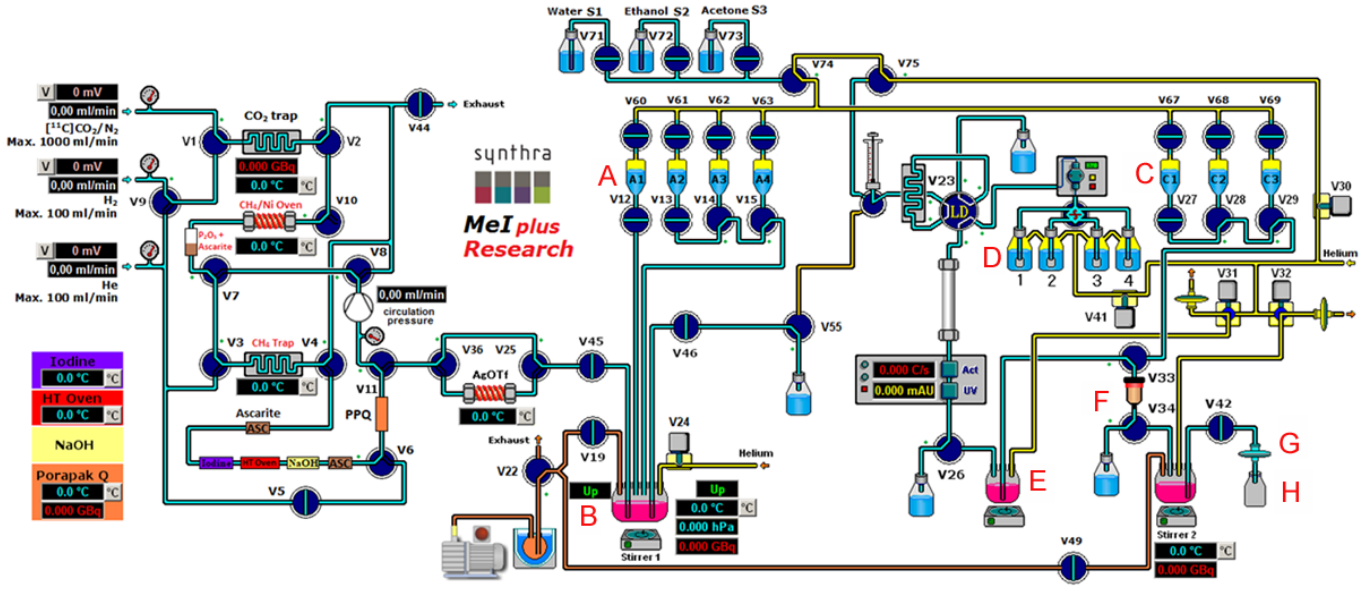

Figure 2. Diagram and setup of Synthra RNplus Research module for [^{11}C]CPPC production. A1: 1 mL MeCN/0.1 M $HCOONH_4$ (v/v = 50/50); B: 1 mg pre-CPPC in 0.3 mL DMSO preloaded in reaction vessel; C1: 10 mL of sterile water; C2: 1.5 mL of ethanol; C3: 4 mL of 0.9% NaCl; D1: HPLC eluent (MeCN/0.1 M $HCOONH_4$ (v/v = 50/50)); E: 40 mL of water and 1 mL ascorbic acid solution (100 mg/mL); F: tC18 Sep-Pak cartridge; G: Millex-GV filter; H: 30 mL product vial with preloaded 6 mL of 0.9% NaCl.

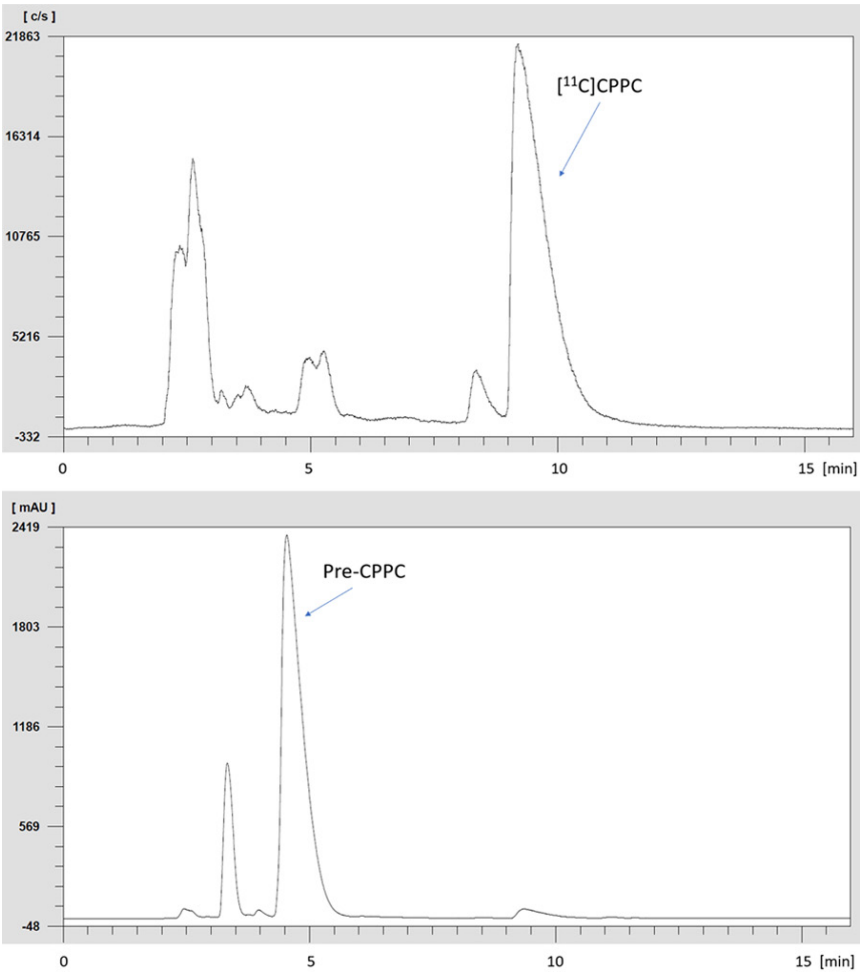

Figure 3. Representative reverse-phase semi-preparative HPLC chromatograms for [^{11}C]CPPC purification (Top: radioactivity channel and bottom: UV channel).

tion runs. The final [^{11}C]CPPC product was obtained in 5.1 ± 0.1 GBq (137 ± 3 mCi) at the end of synthesis (EOS) with a typical irradiation of 20 min at 55 μA. In the reported method [19], only 3.1 ± 0.6 GBq (83 ± 16 mCi) of [^{11}C]CPPC were obtained with 20-27 minutes beam at 60 μA. The overall yield in our method is significantly improved even with shorter beam time. If needed, more activity in the final product can be achieved by increasing the bombardment time to 30 min. Nevertheless, the current yield is sufficient for a 20 mCi dose preparation at 40 min after EOS.

Loop-radiochemistry was reported to give high radiochemical yield with less activity loss during transfer. However, the attempt to transfer this radiochemistry to loop-method failed. We found that the pre-CPPC precursor in DMSO was blown out of the HPLC loop within 30 seconds at flowrates as low as 5 mL/min. Considering that the typical [^{11}C]MeI/MeOTf delivery requires 2-3 minutes at 10-15 mL/min, the current HPLC loop on the module is not suitable for loop-radiochemistry.

Fluorine-18 has a half-life of 109.8 min and decays by 96.7% positron emission, and therefore is widely used for the development of radiopharmaceuticals.

Table 1. Summary of QC results from three [^{11}C]CPPC validation runs

QC Test	Acceptance Criteria	Result		
		Run 1	Run 2	Run 3
Appearance	Clean, colorless and no particles	Pass	Pass	Pass
Concentration (mCi/mL)	≥ 5 mCi/mL @ EOS	12	13	13
Filter integrity	Bubble point: ≥ 345 KPa (50 psi)	Pass	Pass	Pass
Radionuclidic identity	Half-life (min): 18.3-22.4	20.3	20.4	20.2
Radionuclidic purities	≥ 99.5% observed gamma emission should correspond to 0.511 MeV	Pass	Pass	Pass
pH	pH value: 4.0-7.0	4.5	4.5	4.5
Radiochemical purity	[^{11}C]CPPC peak: ≥ 90%	100%	100%	100%
Chemical purity	CPPC mass: ≤ 10 μg/mL	1.0	1.0	0.9
	Total impurities: ≤ 10 μg/mL	0.1	0.4	0.3
Molecular activity	≥ 22.2 GBq/μmol at EOS	181	187	213
Chemical purity: residual solvent	Ethanol ≤ 15% (w/v)	9.1%	9.1%	9.1%
	MeCN ≤ 0.04% (w/v)	0.006%	0.009%	0.006%
	DMSO ≤ 0.5% (w/v)	0.00%	0.00%	0.00%
Pyrogen test	LAL Endotoxins test: < 175 EU/vial	Pass	Pass	Pass
Sterility test	No growth after 2 weeks incubation	Pass	Pass	Pass

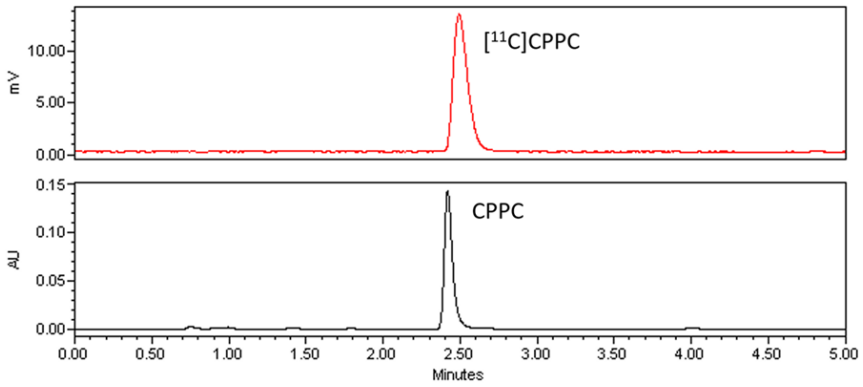

Figure 4. Representative analytical reverse-phase HPLC chromatograms of [^{11}C]CPPC and reference co-injection (Top: radioactivity channel and bottom: UV channel).

The fluorine-18 labeled [^{11}C]CPPC derivative, 5-cyano-N-(4-(4-(2-[^{18}F]fluoroethyl)piperazin-1-yl)-2-(piperidin-1-yl) phenyl)furan-2-carboxamide ([^{18}F]FCPPC), has been developed and showed promising properties in preclinical evaluation [20]. Targeting for clinical translations of [^{18}F] FCPPC, we have synthesized the precursor and reference standard. The validation of automated [^{18}F]FCPPC production is under development and will be reported later.

Conclusions

An efficient automated radiosynthesis of [^{11}C]CPPC was developed with high radiochemical yield and great repeatability. The QC results exhibited excellent chemical/radiochemical purities and molecular activity, facilitating high-quality in-human PET imaging applications.

Acknowledgements

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Disclosure of conflict of interest

None.

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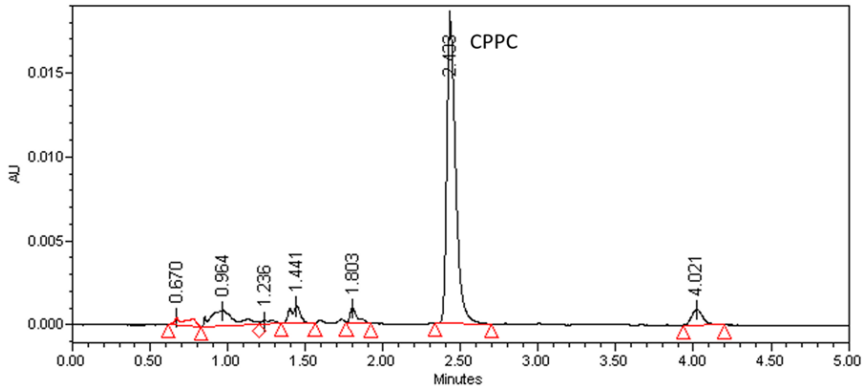

Figure S1. Representative analytical HPLC chromatograms of [^{11}C]CPPC product (UV channel).

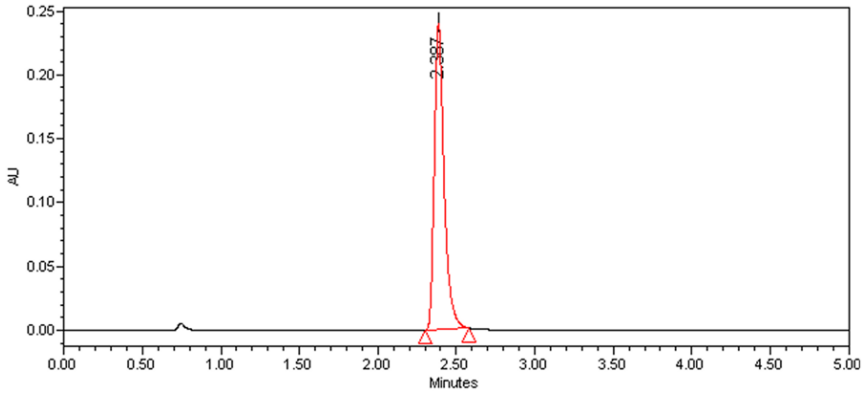

Figure S2. Representative analytical HPLC chromatograms of CPPC reference (10 μg/mL).

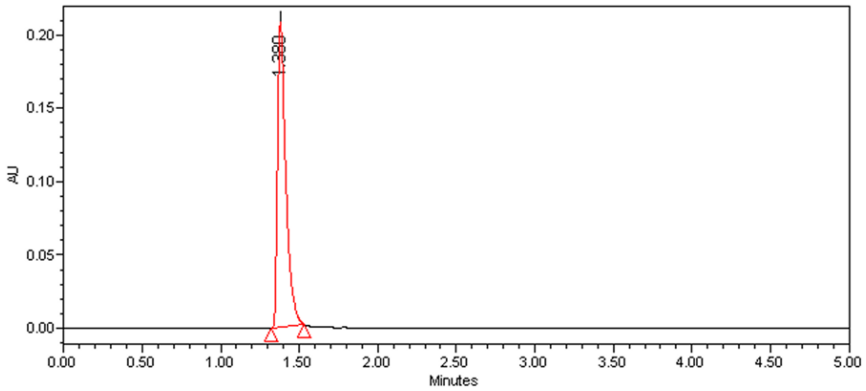

Figure S3. Representative analytical HPLC chromatograms of pre-CPPC (10 μg/mL).

Table S1. Summary stability results at 1 hour after EOS of [^{11}C]CPPC validation runs

QC Test	Acceptance Criteria	Result		
		Run 1	Run 2	Run 3
Appearance	Clean, colorless and no particles	Pass	Pass	Pass
Radionuclidic identity	Half-life (min): 18.3-22.4	20.4	20.4	20.3
pH	pH value: 4.0-7.0	4.5	4.5	4.5
Radiochemical purity	[^{11}C]CPPC peak: ≥ 90%	100%	100%	100%
Chemical purity	CPPC mass: ≤ 10 μg/mL	1.0	1.0	0.9
	Total impurities: ≤ 10 μg/mL	0.1	0.4	0.3
Pyrogen test	LAL Endotoxins test: < 175 EU/vial	Pass	Pass	Pass
Sterility test	No growth after 2 weeks incubation	Pass	Pass	Pass