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ORIGINAL ARTICLE

Development and validation of high-performance liquid chromatography method for analysis of β-CCT and its related substances

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KEYWORDS

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HPLC;
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Analytical;
Forced degradation

Abstract Compound 2β-carbomethoxy-3β-(4-chlorophenyl)tropane (β-CCT) is a key intermediate for the synthesis of some clinical dopamine transporter (DAT) imaging agents. Potential impurities from synthesis process of β-CCT and degradation during storage might have detrimental effect on the final imaging agents. Thus, it is necessary to guarantee the quality of β-CCT. In this study, a rapid, sensitive and accurate high-performance liquid chromatography (HPLC) method was developed and validated for the analysis of β-CCT and its related substances. The chromatographic separation was achieved on a reverse-phase phenomenex™ Gemini C18 column with an isocratic mobile phase consisted of methanol, water and TFA (30:70:0.1 v/v/v). The flow rate was 1.0 mL/min at 30 °C and samples were monitored at 220 nm. The method was validated concerning system suitability, linearity, accuracy, precision, specificity, robustness and stability. The limit of detection (LOD) and the limit of quantification (LOQ) of β-CCT were 0.5 and 1.5 μg/mL, respectively. The linearity range of β-CCT was 1.5–450 μg/mL with a good linear correlation coefficient (R^2 = 0.9999) between the peak response and concentration. Specificity investigation through forced degradation experiments displayed that β-CCT was stable in acidic, thermal and photolytic degradation conditions, but significantly unstable in alkaline and oxidative conditions. With the developed chromatographic method, possible impurity α-CCT from synthetic process and potential

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degradation products could be well separated from β-CCT. Good recovery and precision were manifested in the assay method. These results indicated that the present method would be suitable for not only the quality assurance of β-CCT in regular production sample assays but also the monitoring and determination of its related substances.

1. Introduction

Dopamine transporter (DAT) is a transmembrane protein and a crucial regulator of dopamine (DA) neurotransmission in the central nervous system. The lack of DAT is a primary cause of aberrant DA regulation (Liu et al., 2011; Qiao et al., 2012; Thal et al., 2019). Many researches have confirmed that DAT is associated with several neurodegenerative and neuropsychiatric diseases, including Parkinson's disease (Nicastro et al., 2018), schizophrenia (Sekiguchi et al., 2019) and depressive disorder (Camardese et al., 2014). In recent years, the application of radiolabeled DAT ligands for positron emission tomography (PET) and single-photon emission tomography (SPECT) imaging is becoming more and more important in clinic for diagnosing or monitoring these neurological diseases (Palermo et al., 2019; Suridjan et al., 2019). Up till now, most DAT imaging agents are based on tropane derivatives structure labelled with radioactive nuclides (Booth et al., 2015; Jeon et al., 2020; Haseli et al., 2021), such as ^{99m}Tc-TRODAT-1 (Shinto et al., 2014) for SPECT, [^{18}F]FECNT (van der Zande et al., 2016) and [^{18}F]FECNT-d$_4$ (Cao et al., 2021) for PET and so on (Naumiec et al., 2015).

Compound 2β-carbomethoxy-3β-(4-chlorophenyl)tropane (β-CCT, Fig. 1) is a key synthetic intermediate for the above-mentioned DAT imaging agents (Meegalla et al., 1997; Xing et al., 2000; Chung et al., 2001; Pijarowska-Kruszyna et al., 2014; Naumiec et al., 2015). In the pharmaceutical industry, it is very essential to control the purity of key intermediates to ensure the quality of the final medicine products (Xiao et al., 2007). As reported, β-CCT is usually synthesized from (R)-(-)-anhydroecgonine methyl ester and 4-chlorophenyl magnesium bromide by Michael addition reaction (Fig. 1), in which 2α-carbomethoxy-3β-(4-chlorophenyl)tropane (α-CCT, Fig. 1) is a main byproduct. Clark et al. firstly reported that α-CCT was accompanied at a ratio of 1:3 (2α: 2β) (Clarke et al., 1973). Afterwards, Lifen Xu et al. improved the synthesis method to give a ratio of 1:7 (2α: 2β) (Xu et al., 1996). Although most α-CCT in the reaction might be removed by chromatography as reported, it might still remain as a small amount of impurity in β-CCT. α-CCT is a diastereoisomer of β-CCT product, with the orientation of the methoxycarbonyl at the C-2 position in opposite direction from the plane (Fig. 1), which was reported to be inactive to DAT (Clarke et al., 1973; Xu et al., 1996; Zeng et al., 2006). In addition, it can be expected that the epimers will undergo a highly similar manner in the following reactions, resulting in diastereomeric impurities in the final active pharmaceutical ingredients (APIs). Furthermore, the other potential impurities in the key intermediates may be carried over throughout the subsequent synthesis steps. Thus, it is necessary to develop an efficient analytical method to carry out quality control on the key intermediate to guarantee the purity and imaging effects of the final DAT imaging agents (Xiao et al., 2007).

Currently, high-performance liquid chromatography (HPLC) has been considered as one of the most useful techniques for the determination and quantification of APIs, including its key intermediates. To our knowledge, there was no available reported HPLC investigation for the analysis of β-CCT. In this study, we have developed HPLC method for assaying β-CCT and its related substances for quality control of DAT imaging agent.

2. Materials and methods

2.1. Materials

β-CCT and α-CCT (process impurity) were produced and purified carefully in our laboratory as previously reported by Xu et al. (Xu et al., 1996) and Clarke et al. (Clarke et al., 1973). 2β-Carboxyl-3β-(4-chlorophenyl)tropane (β-CXCT), the related substance as described in the following forced degradation experiment, was prepared in our laboratory by hydrolysis of β-CCT in aqueous dioxane according to the literature (Carroll et al., 1995). HPLC grade methanol (MeOH, 99.9%) was purchased from J&K Science (Shanghai, China). HPLC grade trifluoroacetic acid (TFA ≥ 99.5%) and triethylamine (TEA ≥ 99.9%) were procured from Energy Chemical (China). Water used for the mobile phase was purified by the Milli-Q Advantage A10 water purification system (Millipore, USA). All laboratory glassware and pipette tips were calibrated with high pure water at ambient temperature throughout the analytical studies.

2.2. Instrumentation and chromatographic conditions

A Waters Empower 3 system (Waters Corporation, USA) equipped with a Waters 1525 binary pump, a column oven, a Waters 2707 auto-injector and a Waters 2489 Ultraviolet

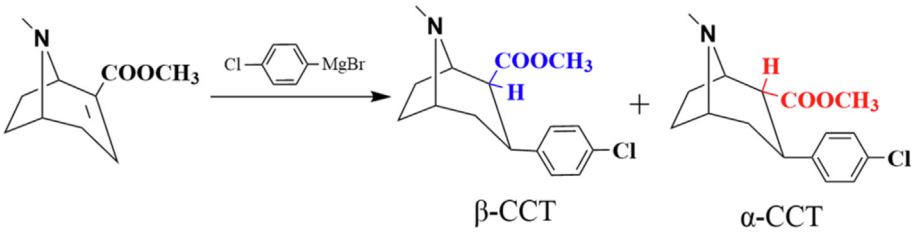

Fig. 1 Synthesis reaction of 2β-carbomethoxy-3β-(4-chlorophenyl)tropane (β-CCT), in which impurity 2α-carbomethoxy-3β-(4-chloroph enyl)tropane (α-CCT) was accompanied.

(UV) detector was used. The chromatographic separation of samples and method validation were carried out on a reverse-phase phenomenex™ Gemini C18 column (150 × 4. 6 mm, particle size 5 μm 118 Å) with the mobile phase consisting of methanol, water and TFA (30:70:0.1 v/v/v). The column temperature was maintained at 30 °C and the flow rate of mobile phase for isocratic elution was 1.0 mL/min. The detection wavelength was set at 220 nm to monitor analytes. The total completed analysis time was 30 min and the injection volume was 10 μL for analytes. All obtained results were analyzed using Empower™ 3 software.

2.3. Validation of the HPLC method

Method parameters were validated for system suitability test, specificity, the limit of detection (LOD), the limit of quantification (LOQ), linearity, accuracy, precision, robustness and stability.

2.3.1. System suitability test

System suitability test is an integral part of the liquid chromatographic technique and various parameters of system suitability are assessed to verify that the analytical system works properly. The test was conducted in six replicate injections of 300 μg/mL (β-CCT). Parameters of the peak area, theoretical plates, retention time and tailing factor of analytes were studied by the results of the system suitability test to ensure the system for the intended use.

2.3.2. Specificity

Specificity is an essential characteristic of HPLC for assessing the analyte in the presence of its potential impurities. In this study, the specificity was evaluated by spiking the process impurity α-CCT and forced degradation products. The forced degradation experiment was performed as follow: The test of acid and basic hydrolysis was performed in 0.10 M HCl and 0.10 M NaOH, respectively at room temperature for 72 h; Oxidation experiments were carried out at room temperature for 72 h with 3 mg solid compound β-CCT dissolved in 3% H_2O_2 solution; For thermal stability, solid samples were kept in heat chambers at 60 °C for 72 h; For the photo-stability test, solid β-CCT was exposed to light for an integrated near ultraviolet energy of 200 w hr m^{-2} and 1.2 million lux hours of visible light, according to ICH (ICH 1996).

2.3.3. Detection limit and quantitation limit

LOD is the concentration of analyte at a signal-to-noise ratio (S/N) of 3:1. LOQ is defined as the minimum concentration produced by S/N of 10:1, so that the analyte could be reliably quantified. The LOD and LOQ samples of β-CCT and its related substances were prepared by diluting each standard stock solution with methanol to a suitable concentration.

2.3.4. Range

The range of β-CCT and its related substances is from LOQ to the specification concentration. The concentration range of β-CCT, α-CCT (Imp-A) and β-CXCT (Imp-B) was in the range of 1.5–450 μg/mL, 1.5–30 μg/mL and 1.2–30 μg/mL, respectively.

2.3.5. Linearity

The linearity of the optimized method was established by injecting six different solutions with the concentration range from LOQ level to the specification level into the HPLC system. The linear relationship was plotted by the measured peak area against concentration data and was analyzed by least-squares linear regression analysis.

2.3.6. Accuracy

The accuracy of an analytical approach expresses the closeness of the expected value to the measured value. The accuracy of β-CCT and its related substances was assessed in recovery studies with samples of 80%, 100% and 120% of the test concentration. Each concentration was determined in triplicate for 3 days. The accuracy (recovery) was determined by the following formula:

% Recovery = 100 × Experimental concentration/Prepared concentration

2.3.7. Precision

The precision of the proposed method was evaluated by determining the intra-assay and inter-assay precision. Each precision level of β-CCT (220, 320, 420 μg/mL), α-CCT (Imp-A; 9, 13, 17 μg/mL) and β-CXCT (Imp-B; 9, 13, 17 μg/mL) was obtained by diluting the stock solution. For intra-day precision, three concentration samples of β-CCT and its related substances were analyzed on the same day, whereas for inter-day precision, samples were assessed during two consecutive days. The peak area of analytes was recorded and then the relative standard deviation (RSD%) was calculated with the acceptance criteria $\leq 2.0\%$ ($n = 6$).

2.3.8. Robustness

The robustness of the method was studied by assessing the effect of deliberate small changes in method parameters to indicate its reliability for normal application. In the present study, robustness of the method was established by testing influence of small changes in the composition of mobile phase (±2%), flow rate (±0.05 mL/min), wavelength (±5 nm) and temperature (±5 °C).

2.3.9. Stability indication

The solution stability of β-CCT and its related substances was monitored after storage for 0, 4, 8, 12, 24, 36 and 72 h at ambient laboratory temperature. The mobile phase stability was also estimated for 0, 4, 8, 12, 24, 36 and 72 h by freshly prepared testing sample solutions.

3. Results and discussion

3.1. Optimization and development for chromatographic conditions

The analysis method on β-CCT and its related substances has been developed and validated. One of the main objectives in the method development was to achieve good separation with the fast possible runtime and high possible sensitivity. For this purpose, the method was optimized with various parameters such as stationary phase, wavelength, the ratio of mobile phase, column temperature and flow rate.

In the chromatographic method development, the selection of stationary phase is an important aspect. Reversed-phase C18 column is usually used as a stationary phase. The retention mechanism of compounds separated on the C18 column is mainly because of hydrophobic interactions. β-CCT and its related substances showed different affinities to the reversed-phase C18 column owing to their structural differences i.e. orientation of methoxycarbonyl at the C-2 position (Fig. 1) making the carboxyl group at the C-2 position in β-CXCT more polar than the methoxycarbonyl group in β-CCT. Thus, good resolution was obtained with β-CXCT being eluted first, followed by β-CCT and α-CCT.

The proper wavelength selection was one of the most important conditions to keep the baseline noise minimum and achieve optimum system suitability parameters, since inappropriate wavelength would lead to low absorptivity of analytes. It was found that β-CCT and its related substances displayed a UV absorbance maximum at approximately 220 nm, as shown in Fig. 2. Furthermore, the baseline gained under this wavelength was smooth to meet HPLC performance. Thereby, the detection wavelength was set at this condition throughout the analysis.

In the present research, methanol was used as an organic modifier for its easily commercial availability and good solubility of β-CCT and related substances. The ratio of the mobile phase is a considerable parameter in liquid chromatography. Fig. 3 summarizes the effect of methanol ratios in the mobile phase. Generally, decreased proportion of methanol in the mobile phase will prolong the attachment of β-CCT and its related substances on the C18 column. As shown in Fig. 3, the retention time had a small fluctuation when methanol percentage was set in the range of 25–30%, in which 30% methanol gave good elution property and peak symmetry to separate all the compounds. To improve interactions with the chromatographic column, acidic (eg. TFA) or basic reagent (eg. TEA) were commonly used as additives in the mobile phase. In this research, TFA showed a more stable baseline and a better peak pattern, as compared with TEA. In short, the methanol, water and TFA at a portion of 30:70:0.1 (v/v/v) were chosen as the mobile phase for analysis. In addition, the flow rate and the column temperature were also investigated to obtain better separation effect. We obtained low baseline noise

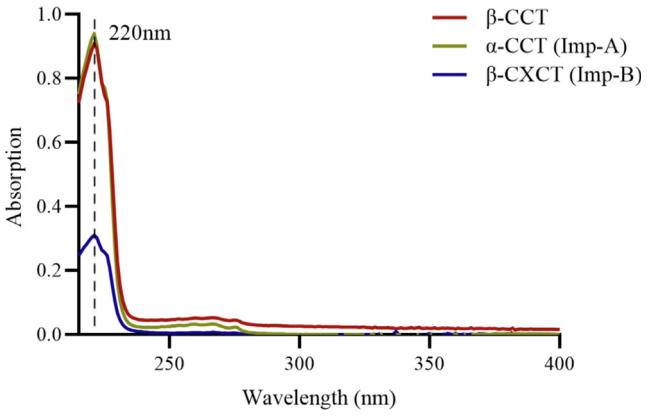

Fig. 2 The UV-wavelength scanning of β-CCT and its related substances.

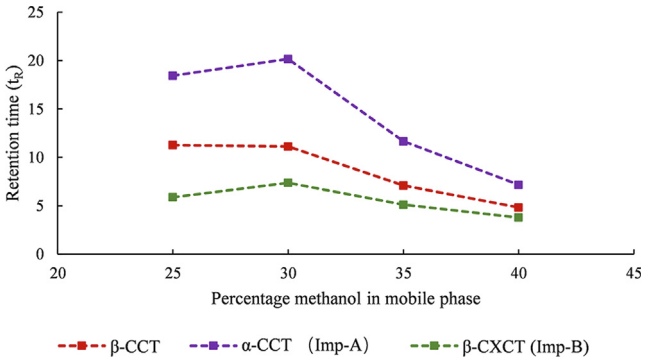

Fig. 3 Effect of percentage methanol in mobile phase on the retention time of β-CCT and its impurities.

and optimal system adaptability parameters at a flow rate of 1.0 mL/min under the optimal temperature of 30 °C.

In summary, the optimized method exhibited satisfactory resolution and separation in a short period. A representative chromatogram of the separation of β-CCT and its related substances (α-CCT and β-CXCT) is presented in Fig. 4.

3.2. Method validation

3.2.1. System suitability test

The system suitability test was evaluated by calculating tailing factor ($T < 1.2$), number of theoretical plates ($N > 2400$) and RSD% < 2.0% of β-CCT (300 μg/mL). The RSD% of the standard peak areas of β-CCT was 1.96% in six replicate injections. More detailed results of β-CCT and its related substances are shown in Table 1. The results of the system's suitability provide good information about the state of the HPLC apparatus and the separation efficiency.

3.2.2. Specificity

The specificity of the developed method for β-CCT quantification was performed in the presence of its potential impurity α-CCT (Imp-A) which originates from the synthesis processes of β-CCT (Xu et al., 1996) and other possible impurities produced on forced degradation. The thermal, photolytic, alkaline, acid and oxidative forced degradation conditions to β-CCT samples was applied (Table 2) and the degradation of β-CCT in these conditions was studied. Each forced degradation condition was compared to a blank solution and tested in triplicate. Experiment results demonstrated that exposure of β-CCT to acid, photolytic and thermal degradation showed an almost full recovery (99.52 ~ 100.18%) without significant degradation. Obvious degradation was found under alkaline and hydrogen peroxide conditions (89.80 ~ 94.48%). Therefore, β-CCT was stable under acidic, thermal and photolytic conditions, but obviously degraded under alkaline and oxidative conditions. The chromatogram of each forced degradation condition as presented in Fig. 5. The detected degradation product was satisfactorily resolved from β-CCT with resolution factors > 1.5 without the interferences from blank mobile phases. The peak area of degradation products increased with the extension of the destruction time. Notably, the alkaline and hydrogen peroxide conditions gave the same degradation product peak with the same retention time of approximately 7.6

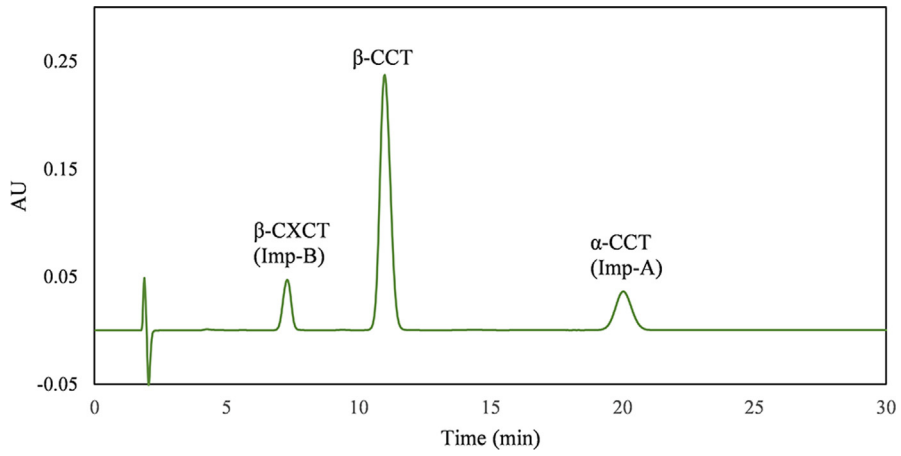

Fig. 4 A representative chromatogram of impurity-spiked β-CCT solution.

Table 1 LOD, LOQ, regression analysis of calibration graphs and system suitability parameters for β-CCT and its related substances for the proposed HPLC.

Parameter	β-CCT	α-CCT (Imp-A)	β-CXCT (Imp-B)
LOD (μg/mL)	0.5	0.5	0.4
LOQ (μg/mL)	1.5	1.5	1.2
Concentration range (μg/mL)	1.5–450	1.5–30	1.2–30
Slop (b)	19,735	23,268	22,919
Intercept (a)	4631	6681	2283
Correlation coefficient	0.9999	0.9997	0.9997
System suitability parameters			
A_r [a]	5,973,696	62,497	124,306
N[b]	3583.33	6286.50	2494.83
t_r[c]	11.26	20.85	7.49
R_s[d]	5.83	11.45	5.83
T_f[e]	1.10	1.02	0.95
R_e[f]	1.96	1.03	1.15

[a] Peak area; [b] Number of theoretical plates; [c] Retention time, in minutes; [d] Resolution, between two compounds adjacent to each other; [e] Tailing factor; [f] Repeatability, the relative standard deviation of the peak area.

Table 2 Forced degradation conditions applied to β-CCT.

Forced degradation condition	Solvent	Temperature	Standing Time	Degradation product
Acid hydrolysis	Hydrochloric acid at 0.1 M	Room temperature	72 h	None
Alkaline hydrolysis	Sodium hydroxide solution at 0.1 M	Room temperature	72 h	β-CXCT (Imp-B)
Oxidation	H_2O_2 aqueous solution at 3%	Room temperature	72 h	β-CXCT (Imp-B)
Thermal	Solide state	60 °C	72 h	None
Photolysis	Solide state	25 °C	10 days	None

9 ± 0.08 min. Fractions of the major degradation products were collected in pure form and analyzed by mass spectrometry, which gave its m/z $[M + H]^+ = 280.2, 282.2$ (Fig. 5). We speculated the degradation product might be β-CXCT, exact mass 279.1, 281.1 (Fig. 6). To confirm this, we synthesized β-CXCT in our lab according to the reported procedure (Carroll. et al., 1991) and carefully purified by recrystallization and compared the synthesized β-CXCT with the degradation product at the same developed HPLC condition. The same retention time and peak characteristics confirmed our speculation. Although alkaline degradation and hydrogen peroxide degradation products are the same, their mechanisms may be different. The ester groups are easy to hydrolyze under alkaline conditions. According to the Baeyer-Villiger reaction, the process of H_2O_2-induced degradation may produce organic peroxides in which the O-O bond is unstable (Terent'ev et al., 2008; Ni et al., 2016; Le-tao et al., 2018) and the degradation product β-CXCT may be produced. Moreover, we also found a small amount of β-CXCT in the β-CCT samples after long term storage at room temperature. Consequently, β-CXCT is a potential degradation impurity of β-CCT. Based on the above studies, potentially related substances were tracked

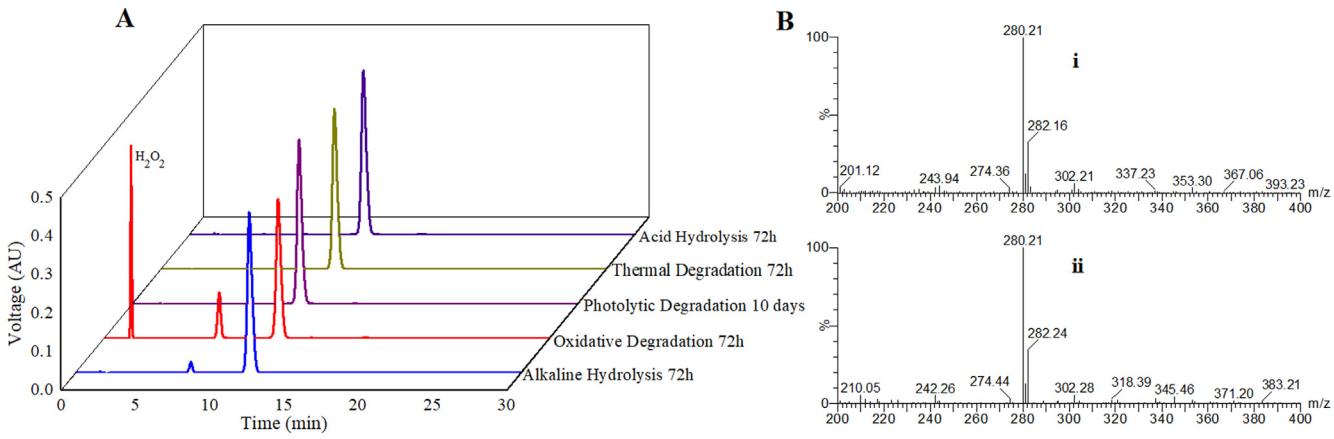

Fig. 5 A: HPLC chromatograms of β-CCT under alkaline, oxidative, photolytic, thermal, and acid conditions. B: The mass spectra for structure identification of degradants (i: Mass spectrum of the degradation product under hydrogen peroxide condition; ii: Mass spectra of the degradation product under alkaline conditions).

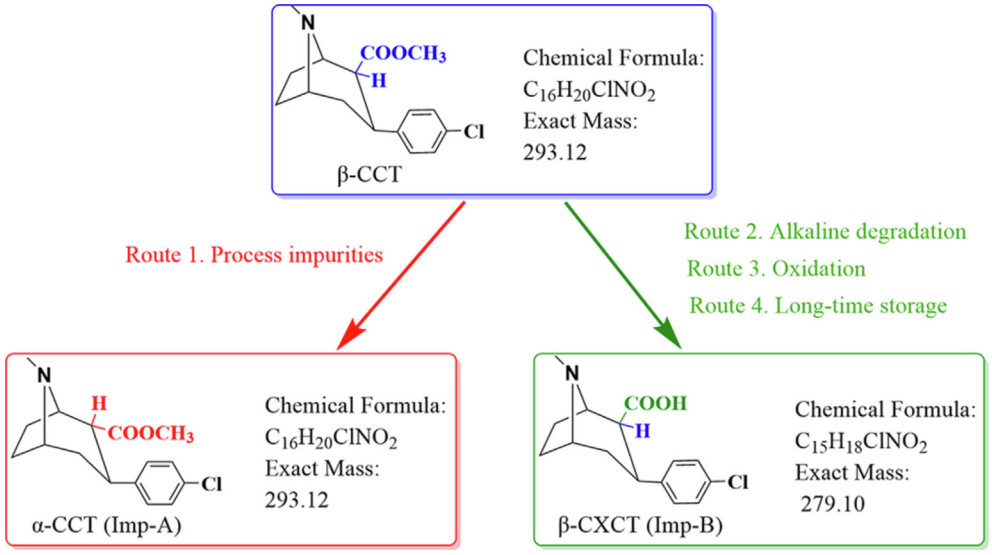

Fig. 6 Routes of potentional impurities of β-CCT and their relationship. Route 1: In the Michael addition reaction of synthesizing β-CCT; Route 2: Degradation under the alkaline condition; Route 3: Degradation under the oxidation condition; Route 4: Storage for more than three years.

and the formation pathway were described in Fig. 6. The entire evidence further confirmed that the specificity of the developed analytical method for the intended use.

3.2.3. Limit of detection and quantitation

The LOD and LOQ of β-CCT and its related substances were determined by injecting a series of diluted solutions with known concentrations. The lowest concentration could be reliably detected by the comparsion of measured signal with baseline noise signal. Consequently, the LOD and LOQ values of β-CCT were 0.5 μg/mL and 1.5 μg/mL, respectively. The results demonstrated that this method was sufficiently sensitive, as listed in Table 1.

3.2.4. Linearity

Linearity was described in terms of the calibration curve. The curve of β-CCT and its related substances was obtained by plotting the mean peak areas (μV · s) against the corresponding concentrations (μg/mL). Equation of the calibration curve for β-CCT (in the range of 1.5–450 μg/mL), α-CCT (Imp-A; in the range of 1.5–30 μg/mL) and β-CXCT (Imp-B; in the range of 1.2–30 μg/mL) were $y = 19735x - 4631$, $y = 23268x - 6681$ and $y = 22919x + 2283$, respectively. Each linear regression coefficient was found ≥ 0.9997 for calibration curve, displaying excellent correlations between the peak area and concentration (Table 1).

3.2.5. Accuracy

Recovery experiments were performed to evaluate the accuracy of the method by analyzing three different concentrations of samples in triplicate for three consecutive days. The percentage recoveries were calculated from calibration curves and recovery rates of β-CCT, α-CCT (Imp-A) and β-CXCT (Imp-B) were at the range of 99.19–101.68%, 98.38–100.36%

and 98.42–101.03%, respectively, more detailed information is given in Table 3. All the recoveries were within the limit of acceptance (98.00–102.00%). These results confirmed that recoveries of the assay method were accurate enough.

3.2.6. Precision

The precision of the developed method was tested by analyzing intra- and inter-day studies in six replicates of three different concentration solutions. The data collected and processed on two consecutive days are presented in Table 3. The Fisher F test on the variances (F_{calc} = 1.026, $F_{0.05/2}$ = 19, p = 0.9898) evidenced that the factor of the day of assay had no significant contribution to the variation in results. A paired *t*-test (t_{calc} = 1.406, $t_{0.025/2}$ = 4.303, p = 0.2950) verified that the p-value was greater than 0.05, demonstrating sample averages of the intra-day and inter-day data had no significant difference. Statistical analysis of the data revealed that mean values of the sample peak areas in two successive days were found no significant difference at the 95% of probability level. Repeated injections, repeated analyses and the RSD% of inter- and intra-day precision were at the range of 0.18–1.35% (RSD% < 2.0%). According to the above statistical analysis, the overall variability of the measured value displayed minimal changes were within the acceptable range during the intra- and inter-day studies and the acceptability criteria were thus met.

3.2.7. Robustness

The robustness of the method was studied by deliberate small changes in chromatographic parameters. The tailing factor was found between 1.0 and 1.2. The resolution was > 2.0 and the theoretical plates were found between 3037 and 3973. All retention time remained in the acceptance range on the whole (Fig. 7), the optimum was as following: the mobile phase con-

Table 3 The data of accuracy were obtained by the recovery analysis of β-CCT and its related substances (mean ± SD, n = 3). The data of precision were obtained by the relative standard deviation of peak areas (RSD %, n = 6).

Analytes	Concentration (μg/mL)	Accuracy			Precision		
		Recovery (98–102%, n = 3)			Concentration (μg/mL)	RSD% ≤ 2% (Area, n = 6)	
		Day 1	Day 2	Day 3		Intra-day	Inter-day
β-CCT	240	100.06 ± 0.07	99.25 ± 1.27	99.91 ± 0.57	220	0.33	1.05
	300	99.80 ± 1.47	101.11 ± 0.56	99.22 ± 0.93	320	0.48	0.60
	360	100.52 ± 0.74	101.68 ± 0.24	99.19 ± 0.59	420	0.72	1.35
α-CCT (Imp-A)	9.6	99.86 ± 1.01	99.52 ± 0.91	100.36 ± 0.18	9.0	0.18	0.35
	12.0	98.81 ± 0.23	99.46 ± 0. 23	98.38 ± 0.29	13.0	0.38	0.28
	14.4	98.66 ± 0.44	98.98 ± 0.86	99.39 ± 0.27	17.0	0.21	0.37
β-CXCT (Imp-B)	9.6	99.33 ± 0.57	100.04 ± 1.39	99.85 ± 0.21	9.0	0.89	0.44
	12.0	101.03 ± 0.13	100.41 ± 0.58	99.44 ± 0.30	13.0	0.65	0.42
	14.4	98.57 ± 0.52	99.33 ± 0.32	98.42 ± 0.12	17.0	0.41	0.34

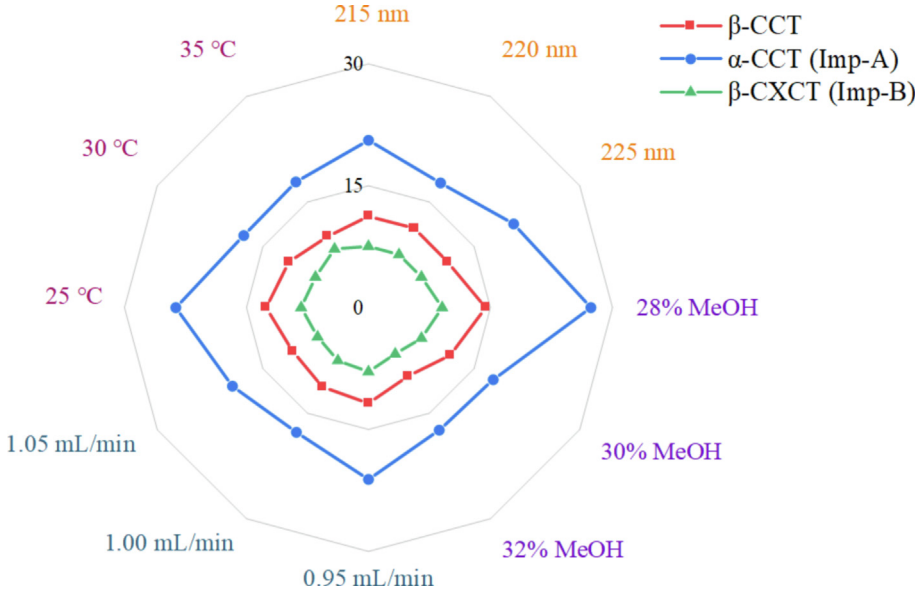

Fig. 7 Robustness study for the β-CCT and its related substances on the distribution of retention time.

Table 4 Marketed formulation analysis.

Batches number	β-CCT%	α-CCT (Imp-A) %	β-CXCT (Imp-B) %
1	99.42 ± 0.67	0.18 ± 0.02	–
2	99.16 ± 0.28	0.62 ± 0.04	–
3	99.22 ± 1.51	–	0.66 ± 0.002

taining 30% MeOH, column temperature of 30 °C and the flow rate of 1.0 mL/min. Compared with the optimal conditions, the retention time of 28% methanol in the mobile phase was slightly worse. The RSD% calculated among three different wavelengths about the retention time and the number of theoretical plates were less than 2.0%. The results demonstrated that the peaks of β-CCT and its related substances were not significantly affected by any of the small modifications in chromatographic conditions. Therefore, the developed method is robust enough to maintain reliable results.

3.2.8. Stability studies

Stability studies were conducted to assess the stability of β-CCT and its related substances at different time intervals. No significant variations were observed in their chromatograms, peak areas, retention time, or tailing factors during this period. These experiment results verified that sample solutions and mobile phases were stable for at least 72 h when stored at room temperature.

3.3. Assay of samples

To further validate the application of this method, three batches of samples at a concentration of 300 μg/ mL were analyzed under the working conditions. As shown in Table 4, the content of β-CCT was > 98.0% in all batches. Interestingly, the third batch, which had been stored for more than three years, displayed a small amount (0.15%) of β-CXCT, similar to the forced degradation experiment under alkaline hydrolysis and hydrogen peroxide conditions described above. Hence, the method could be used not only for daily analysis but also for the potential determination of samples in long-term stability experiments.

4. Conclusions

In summary, an HPLC method was developed and validated for the simultaneous detection and quantifications of the key intermediate β-CCT of DAT imaging agents and its related substances. The proposed method has been validated in terms of system suitability, specificity, linearity, accuracy, precision and robustness. Forced degradation of β-CCT displayed that this intermediate was stable under photolytic, thermal and acid conditions, whereas it was sensitive under oxidation and alkaline conditions. Therefore, β-CCT should be stored to avoid oxidation and alkalinity environment. This method provides a practicable tool for the quality control of β-CCT to ensure the quality of final DAT imaging agents.

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