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Research Article

Efficient synthesis, characterization, and establishment of reference standards of major candesartan cilexetil impurities for pharmaceutical quality control

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ABSTRACT

Process- and degradation-related impurities of candesartan cilexetil present significant challenges in pharmaceutical quality control due to their structural similarity to the parent drug and the limited availability of certified reference standards. This study aimed to develop efficient and cost-effective synthetic routes for five important impurities of candesartan cilexetil (A, B, D, F, and G), followed by structural characterization and establishment of reference standards suitable for pharmaceutical analysis. Controlled acidic and alkaline hydrolysis of candesartan cilexetil produced impurities B and G in high yields (88% and 95%, respectively). Impurities F and D were obtained from candesartan cilexetil and impurity B, respectively via alkylation reactions with average yields of approximately 40%, while impurity A was synthesized via a one-step esterification of impurity G with a yield of up to 70%. Structural identities were confirmed using nuclear magnetic resonance and high-resolution mass spectrometry. A validated reverse-phase high-performance liquid chromatography method demonstrated excellent specificity, linearity ($R^2 > 0.998$), precision (RSD < 0.52%), and accuracy (98.0–101.9%). Homogeneity testing and assigned value determination were conducted in accordance with ISO and ICH guidelines through interlaboratory comparison. The certified purity values ranged from 98.2% to 99.1% with low uncertainty. The developed workflow provides a practical, scalable, and economically feasible approach for producing impurity reference standards, supporting impurity profiling, stability testing, and routine pharmaceutical quality control of candesartan cilexetil and related formulations.

Keywords:
Synthesis; Candesartan; Impurities; Reference standard

1. INTRODUCTION

High blood pressure, also known as hypertension, is a chronic medical condition characterized by persistently elevated arterial pressure, and it represents a major global health burden. It causes severe cardiovascular complications such as stroke, heart failure, coronary heart disease, and myocardial infarction and increases the risk of brain, kidney, and other diseases, especially the severity of COVID-19[1], leading to premature deaths worldwide. Globally, about 1.39 billion adults have hypertension, of which low- and middle-income countries account for two-thirds[2]. In Vietnam, the proportion of adults with high blood pressure is rising, reaching nearly half of the population, and new cases are increasingly observed at younger ages. To date, various pharmaceutical groups have been developed for cardiovascular disease in general and for high blood pressure. Among these, angiotensin II receptor antagonists, commonly known as the sartan group,

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have been frequently prescribed[3]. Candesartan cilexetil (CDC) is an orally administered prodrug that undergoes hydrolysis in the gastrointestinal tract to form the active metabolite, candesartan[4]. However, CDC contains multiple process- and degradation-related impurities that may impact drug safety and quality.

The control of impurities is vital to ensure the purity, quality, safety, and effectiveness of drug products, particularly for chronic therapies, since certain impurities have been reported to possess mutagenic properties[5]. For instance, impurity G in CDC was identified as a carcinogenic, mutagenic, and reproductive toxicant[6]. The International Council for Harmonization (ICH) has set guidelines concerning impurities in new drug substances and pharmaceutical products[7]. Most impurities are related to the manufacturing process and may occur during the synthesis of the active pharmaceutical ingredient (API), such as impurities A and G produced during CDC synthesis. Furthermore, some impurities, including impurities B, D, and F, can emerge as degradation impurities due to the breakdown of the API under storage or stress conditions[8]. These impurities exist in trace amounts and frequently exhibit physicochemical similarity to the parent API, making their isolation and characterization difficult. It is also noted that byproducts generated during synthesis may persist in subsequent manufacturing steps and lead to secondary impurities[9,10].

The impurities must be strictly regulated, as stipulated in the British Pharmacopoeia 2025 (BP 2025)[11], the European Pharmacopoeia 11.6 (EP 11.6)[12], and the United States Pharmacopoeia 2025 (USP 2025)[13]. Robust impurity profiling requires reliable analytical methods, along with high-quality reference standards. Kumar et al. (2012) reported a chromatographic procedure using the UV detector at 254 and 210 nm[14]. This procedure was considered suboptimal for the chromatographic system due to the use of an inorganic salt solution in the mobile phase. Subsequently, Srinivas et al. (2012) developed a reversed-phase ultra-performance liquid chromatography (UPLC) method at 210 nm, using a mobile phase containing trifluoroacetic acid[15]. This approach enabled the detection of impurities A and B with a limit of detection (LOD) of 0.016%; however, it still involved the use of a highly toxic reagent.

The paucity of accessible impurity standards poses a major challenge for pharmaceutical manufacturers, especially in regions where international sourcing is associated with high cost, long lead times, and complex import regulations[16]. Therefore, there is a compelling need for a standardized, efficient, and economically feasible and scalable synthetic approach. Although synthetic routes have been reported for some CDC impurities, most describe low yields without information on chromatographic purity of the synthesized compounds, such as impurity A[17] and impurities B, D and F[18]. Notably, limited reports are available on the synthesis and establishment of impurity G reference standards.

Although several studies have reported the identification and synthesis of individual candesartan cilexetil impurities, a comprehensive workflow integrating efficient synthesis, structural characterization, to the best of our knowledge, analytical validation, homogeneity assessment, and assignment of certified reference values remain limited. Furthermore, the availability of certified impurity reference standards is restricted by high cost and limited accessibility, particularly in developing countries. Therefore, this study aimed to develop a systematic and practical approach for synthesizing major candesartan impurities. The purity of synthesized impurities was confirmed by high-performance liquid chromatography with photodiode array detection (HPLC-PDA) and subsequently assessed for establishing certified reference standards. This work provides a sustainable and reliable solution for pharmaceutical quality control applications not only for candesartan cilexetil but also for related sartan pharmaceuticals.

2. MATERIALS AND METHODS

2.1. Materials and instruments

All reagents and solvents were purchased from Fisher Scientific or Merck and were used as received without further purification. CDC raw material (batch 5668-21-184, content 100.0% as an anhydrous substance) was obtained from Zhejiang Huahai Pharmaceutical Co., Ltd. Standards of impurities included impurity A (Lot No. 1-AXF-48-5, TRC Canada), B (Lot No. LRAD4059, Sigma–Aldrich RTC, USA), D (Lot No. 2-YEN-11-2, TRC Canada), F (Lot No. 19-MGG-126-2, TRC Canada), and G (Lot No. 1-LWJ-97-1, TRC Canada). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker spectrometer (Germany) at 500 MHz. Mass spectra were recorded on the Shimadzu IT-TOF mass spectrometer (Japan).

2.2. Methods

2.2.1. Synthesis

Five impurities of candesartan cilexetil (CDC) were synthesized from CDC as the common precursor through optimized reaction pathways. Impurity G resulted from alkaline hydrolysis[19], whereas impurity B was obtained from acidic hydrolysis[18]. Impurity D was synthesized via ethylation of impurity B using

ethyl bromide[18], and impurity F was formed through a similar ethylation applied directly to CDC[18], thus highlighting the step-by-step nature of the process. The formation process of product A involves the esterification of product G in the presence of 4-dimethylaminopyridine (DMAP)[20]. Reaction progress was monitored by thin-layer chromatography (TLC) using silica gel 60 F254 plates (0.063 to 0.200 mm). Purification was performed by recrystallisation and/or silica gel flash column chromatography. Structural confirmation was achieved using high-resolution electrospray ionization mass spectrometry (HRESIMS) and NMR spectroscopy.

Synthesis of impurity A from impurity G: Impurity G (440 mg, 1 mmol) was dissolved in anhydrous dichloromethane (DCM, 15 mL) with N,N-dimethylformamide (DMF, 5 mL). Then, 4-(dimethylamino) pyridine (DMAP, 10 mg), ethanol (20 µL, 0.4 mmol), and N, N'-dicyclohexylcarbodiimide (DCC, 206 mg, 1 mmol) were added to the reaction mixture at 0 ºC. The reaction mixture was stirred for 5 minutes at 0 ºC, followed by 3 hours at 20 ºC. The precipitated urea byproduct was then filtered off, and the filtrate was concentrated in vacuo. The residue was redissolved in DCM, washed twice with 0.5 N HCl and saturated NaHCO3 solution, dried over Na2SO4, filtered, and concentrated in vacuo. The product was purified by column chromatography using ethyl acetate-n-hexane (1:1) as eluent.

Synthesis of impurity B from CDC: CDC (610.7 mg, 1 mmol) was dissolved in DCM (25.0 mL), then concentrated HCl (1 mL) was added. The mixture was stirred at 50 ºC for 5 hours. After solvent removal, the resulting solid was filtered, washed with cold water, and dried under vacuum at 60 °C for 3 hours.

Synthesis of impurity D from impurity B: A mixture of impurity B (200.2 mg, 0.34 mmol), acetone (10 mL), sodium carbonate (95.1 mg, 0.90 mmol) and ethyl bromide (55.0 µL, 0.73 mmol) was stirred at 50 – 55 ºC for 3 hours. After cooling, water was added to quench the reaction. The precipitated product was filtered off, washed with acetone, and concentrated. Purification was performed by silica gel chromatography using n-hexane-ethyl acetate (1:1) as the eluent.

Synthesis of impurity F from CDC: A mixture of CDC (125.4 mg, 0.2 mmol), acetone (30 mL), and sodium carbonate (44.8 mg, 0.4 mmol) was stirred at room temperature for 1 hour, followed by the addition of ethyl bromide (22.5 µL, 0.30 mmol). The reaction was continued at 50 – 55 °C for 3 h. After cooling and water quenching, the product was filtered, washed, concentrated, and purified by silica gel chromatography (n-hexane/acetone, 5:1).

Synthesis of impurity G from CDC: CDC (1 g, 1.64 mmol) was suspended in absolute ethanol (40 mL) and 15% KOH solution. The mixture was heated at 55 – 65 ºC for 5 hours and cooled in an ice bath. The solution was acidified to pH 1–2 with hydrochloric acid to induce precipitation. The solid was filtered, washed with water, recrystallized from cold methanol, and dried under vacuum at 60 °C for 3 h.

2.2.2. Method validation and establishment of impurity reference standards

Method validation was performed using an Agilent HPLC 1260 photodiode-array detector (PDA). Compounds were separated on a Gemini C18 column (150 x 4.6 mm, 5µm) at the detection wavelength of 254 nm. Injection volume was 10 µL. Samples were eluted using the mobile phase listed in Table 1, in the gradient program and at the flow rate of 0.8 mL/min[8,14,17].

The analytical method for the synthesized impurities was established and validated according to ICH guidelines (ICHQ2(R1), 2022)[22], including system suitability, specificity, linearity, precision (intra-day and inter-day), accuracy, LOD and LOQ. The establishment of the impurity reference standards was based on the principle of homogeneity[23], with the assigned values[24] obtained from three independent laboratories, conforming to the rigorous standards of Good Laboratory Practice (GLP) or ISO/IEC 17025[25], ensuring the highest quality of the research.

3. RESULTS AND DISCUSSION

3.1. Synthesis

3.1.1. Impurity A

Impurity A was synthesized from impurity G through Steglich esterification, achieving a yield of 70% as an off-white solid. TLC analysis (DCM-methanol, 8:1) showed *Rf* value of 0.45.

The FT-IR spectrum showed excellent agreement of synthesized impurity A with its reference standard (Figure S1), confirming structural identity and purity of the product. FT-IR (cm^{-1}): 1712 (C=O), 1614 (aromatic C–C), 1548–1574 (N–O), 1037–1215 (C–N), 3026–3068 (aromatic C–H), 2864–2982 (aliphatic C–H). HRMS (ESI) *m/z* calculated for $C_{26}H_{25}N_6O_3$ ($[M+H]^+$) = 469.1983, found 469.2003 (Figure S2). ^{1}H-NMR (MeOD, 500 MHz), δ (ppm): 7.64 (*dd*; *J* = 8.0, 1.0 Hz; 1H; H_{Ar}); 7.53 (*dd*; *J* = 8.0, 1.0 Hz; 1H, H_{Ar}); 7.43 – 7.49 (*m*; 2H; H_{Ar}); 7.36 – 7,39 (*m*; 2H; H_{Ar}); 7.18 (*t*; *J* = 8.0 Hz; 1H; H_{Ar}); 7.00 (*d*; *J* = 8.0 Hz; 2H; H_{Ar}); 6.79 (*d*, *J* = 8.0 Hz; 2H; H_{Ar}); 5.57 (*s*, H14); 4.60 (*q*; *J* = 7.0 Hz; 2H; H23); 4.21 (*q*; *J* = 7.5 Hz; 2H; H25); 1.47 (*t*; *J* = 7.0 Hz; 3H; H24); 1.23 (*t*; *J* = 7.5 Hz; 3H; H26) (Figure S3). ^{13}C-NMR (MeOD, 125 MHz), δ (ppm): 180.3 (C22); 167.7 (C1); 142.8, 142.5, 141.7, 136.9, 136.7, 132.1, 131.9, 131.2, 130.9, 130.5, 129.9, 128.0,

Table 1. Mobile phase composition and gradient elution program.

Time	Solution A: Acetonitrile and water (57:43) in glacial acetic acid[13]	Solution B: Acetonitrile and water (90:10) in glacial acetic acid[13]
0	100	0
3	100	0
33	0	100
33.1	100	0
40	100	0

127.0, 124.9, 122.2, 122.1 (C_{Ar}); 68.3 (C25); 62.5 (C23); 47.8 (C14); 14.8 (C26); 14.5 (C24) (Figure S4).

Alternative approaches to synthesize impurity A (an ester) and impurity G (an acid) were also investigated. Particularly, impurity A was synthesized by Fischer esterification of impurity G with ethanol, catalyzed by a Lewis acid. In this method, thionyl chloride ($SOCl_2$) was used to convert G into the chloride acid derivative, which is more reactive toward absolute ethanol. However, this method was unsuccessful. Finally, the Steglich esterification reaction, a mild and efficient method, was applied to synthesize impurity A from bulky acids containing unstable functional groups such as impurity **G**. This reaction proceeds via carbodiimide-based condensation of a carboxylic acid with ethanol, catalyzed by DMAP, forming the ester moiety.

Raman et al. (2011) synthesized impurity A via the reaction of a mixture of ethyl 1-[(2'-cyanobiphenyl-4-yl)methyl]-2-ethoxy-1*H*-benzimidazol-7-carboxylate (10 g), (n-$C_4H_9)_3SnCl$ (23 g), NaN_3 (4.5 g), *N, N*-diisopropylethylamine (2.1 g) in xylene (50 mL), without reporting the process yield[12]. In comparison, their method required greater amounts of toxic chemicals and a longer time of reaction (up to 18 to 20 hours, compared to only 3 hours in our procedure). Using the same mobile phase for purification, our product achieved a higher purity (98.5%), compared to theirs (97%).

3.1.2. Impurity B

Impurity B was obtained in 88% yield and 99.0% purity through controlled acidic hydrolysis of CDC. The product appeared as a white solid with Rf = 0.65 (DCM–MeOH–AcOH, 180:20:1).

The FT-IR spectrum of synthesized impurity B was highly consistent with that of the reference standard (Figure S5). FT-IR (cm^{-1}): 1680 (C=C), 1751 (C=O), 1602 (aromatic C–C), 1545–1566 (N–O), 1066–1207 (C–N), 3026–3134 (aromatic C–H), 2860–2939 (aliphatic C–H). MS (ESI) *m/z* calculated for $C_{31}H_{30}N_6O_6$ ($[M\text{-}H]^-$) = 581.2154, found 581.2166 (Figure S6). ^{1}H–NMR (MeOD, 500 MHz), δ (ppm): 7.64 – 7.67 (*m;* 2H; H_{Ar}); 7.55 – 7,56 (*m;* 1H; H_{Ar}); 7.51 (*d; J* = 8.0 Hz; 1H; H_{Ar}); 7.42 (*dd; J* = 8.0, 1.0 Hz; 1H; H_{Ar}); 7.33 (*dd; J* = 7.5, 1.0 Hz; 1H; H_{Ar}); 7.14 (*t; J* = 8.0 Hz; 1H; H_{Ar}); 7.05 (*br*; 4H; H_{Ar}); 6.82 (*q; J* = 5.5 Hz; 1H; H23); 5.46 (*q*; *J* = 16.0 Hz; 2H; H14a); 4.61 – 4,66 (*m*; 1H; H26); 1.88 – 1.91 (*m*; 2H; CH_2); 1.71 – 1.77 (*m*; 2H; CH_2); 1.47 (*d; J* = 5.5 Hz; 3H; H24); 1.41 – 1.47 (*m*; 2H, H_{Ar}); 1.29 – 1.43 (*m*; 2H; CH_2) (Figure S7). ^{13}C–NMR (MeOD, 125 MHz), δ (ppm): 165.2 (C22); 157.5 (C1); 154.0 (C25); 143.0 (C15); 139.7 (C3); 139.7, 138.1, 132.5, 131.9, 131.7, 131.3, 130.4, 129.9, 128.9, 128.3, 124.5, 124.3, 122.3, 115.6, 114.7 (aromatic carbons); 93.4 (C23); 78.6 (C26); 46.4 (C14); 32.4, 32.3, 26.2, 24.5 (methylene); 19.7 (C24) (Figure S8).

Havlíček et al. (2009) synthesized impurity B from candesartan cilexetil (2 g), using acetone (16 mL), water (4 mL) and concentrated hydrochloric acid (0.2 mL)[21]. Compared with our process, their yield was similar to ours (87.9% and 88%, respectively), but the reaction time was slightly longer than ours (5 hours and 6 hours, respectively). Mohan et al. (2009) produced impurity B via the degradation of candesartan cilexetil tablets (50 g) in DCM (250 mL) and hydrochloric acid (25%) at 25–30 °C for 24 hours[18]. Although this method obtained the highest yield (up to 90%) and was easy to follow, it was more time-consuming and did not report the product purity, compared with our synthetic procedure.

3.1.3. Impurity D

Impurity D was prepared from impurity B via ethylation using ethyl bromide, yielding 44% of a white solid with 98.5% purity (Rf = 0.37, hexane–ethyl acetate 1:1).

The FT-IR spectrum of the synthesized impurity D showed a high degree of similarity to that of the reference standard (Figure S9). FT-IR (cm^{-1}): 1703–1744 (C=O ester), 1602 (aromatic C–C), 1666 (C=O), 1037–1192 (C–N), 3024–3055 (aromatic C–H), 2860–2986 (aliphatic C–H). HRMS (ESI) *m/z* calculated for $C_{33}H_{35}N_6O_6$ ($[M+H]^+$) = 611.2613, found 611.2589 (Figure S10). ^{1}H-NMR (Acetone-d_6, 500 MHz), δ (ppm): 7.79 (*dd; J* = 7.5, 1.5 Hz; 1H; H_{Ar}); 7.56 (*dt; J* = 7.5, 1.5 Hz; 1H; H_{Ar}); 7.54 (*dt*; J = 7.5, 1.5 Hz; 1H; H_{Ar}); 7.42 (*dd; J* = 8.0, 1.0 Hz; 1H; H_{Ar}); 7.34 – 7,38 (*m; 2*H; H_{Ar}); 7.09 (*t; J* = 8.0 Hz; 1H; H_{Ar}); 7.05 (s; 4H; H_{Ar}); 6.88 (*q; J* = 5.5 Hz; 1H; H23); 5.48

(*d*; J = 16.0 Hz; H14a); 5.36 (*d*; J = 16.0 Hz; H14b); 4,58 – 4,64 (m; 1H;CH); 4.62 (*qd*; *J* = 7.5, 1.0 Hz 1H; H32); 1.85 – 1.92 (*m*; 2H; CH_2); 1.65 – 1.73 (*m*; 2H; CH_2); 1.46 (*d; J* = 5.5 Hz; 3H; H24); 1.23 – 1.47 (*m*; 6H; CH_2); 1.30 (*t; J* = 7.5 Hz; 3H; H33) (Figure S11). ^{13}C-NMR (Acetone-d_6, 125 MHz), δ (ppm): 165.6 (C1); 164.7 (C22); 154.8 (C15); 153.3 (C25); 142.5, 140.8, 140.0, 131.5, 131.1, 130.8, 130.2, 129.8, 128.4, 127.7, 127.6, 124.2, 123.6, 121.5, 114.8, 114.0 (aromatic carbons); 92.9 (C23); 77.9 (C26); 48.7 (C32); 45.7 (C14); 32.0, 31.9, 25.8, 24.1 (methylene); 19.8 (C24); 14.1 (C33) (Figure S12).

In other words, impurity D could be generated from impurity F (0.2 g) in acetone (2 mL) and concentrated hydrochloric acid (20 µL), as suggested by Havlíček et al. (2009)[21]. The reaction occurred at 75 ℃ in 6 hours, which is twice the duration of our method. Although this process obtained a yield of 68.8%, higher than our routine (44%), the product purity was not reported. According to Mohan et al. (2009), impurity D could be obtained from impurity B by mixing 40 g of impurity B with *N*,*N*-dimethylformamide (250 mL), potassium carbonate (19 g), and ethyl iodide (16 g)[20]. The reaction conducted at 70–75 ℃ in 2.5 hours produced impurities D and C simultaneously but with low purity (40% and 30%, respectively), compared to our products (98.5% for impurity D).

3.1.4. Impurity F

The alkylation of CDC under mild base activation created a white solid with an HPLC purity of 98.2%, despite the relatively low yield (40%). The product eluted by *n*-hexane–acetone (1:1) displayed a *Rf* value of 0.79.

The FT-IR spectrum of the synthesized impurity F exhibited strong similarity with that of the reference standard (Figure S13). FT-IR (cm^{-1}): 1712–1744 (C=O). 1593–1616 (aromatic C–C), 1527–1545 (N–O), 3007 (aromatic C–H), 2858–2984 (aliphatic C–H). HRMS (ESI) *m/z* calculated for $C_{35}H_{39}N_6O_6$ ($[M+H]^+$) = 639.2926, found 639.2905 (Figure S14). ^{1}H-NMR (Acetone-d_6, 500 MHz), δ (ppm): 7.80 (*dd; J* = 7.5, 1.5 Hz; 1H; H_{Ar}); 7.69 (*dd; J* = 8.0, 1.0 Hz; 1H; H_{Ar}); 7.57 (*td; J* = 7.5, 1.5 Hz; 1H; H_{Ar}); 7.56 (*dd; J* = 8.0, 1.0 Hz; 1H; H_{Ar}); 7.50 (*td; J* = 7.5, 1.5 Hz; 1H; H_{Ar}); 7.42 (*dd; J* = 7.5, 1.5 Hz; 1H; H_{Ar}); 7.20 (*t; J* = 7.5 Hz; 1H; H_{Ar}); 7.05 (*d, J* = 8.5 Hz; 2H; H_{Ar}); 7.00 (*d; J* = 8.0 Hz; 2H; H_{Ar}); 6.94 (*q; J* = 5.5 Hz; 1H; H23); 5.65 (*d; J* = 16.0 Hz; H14a); 5.60 (*d; J* = 16.0 Hz; H14b); 4.68 (*q; J* = 7.0 Hz; 2H; H32); 4.61 (*m*; 1H; H26); 4.46 (*q; J* = 7.5 Hz; 2H; H34); 1.83 – 1.93 (*m*; 2H; CH_2); 1.64 – 1.73 (*m*; 2H; CH_2); 1.52 (*d; J* = 5.5 Hz; 3H; H24); 1.48 (*t; J* = 7.0 Hz; 3H; H33); 1.22 – 1.52 (*m*; 2H; CH_2); 1.29 (*t; J* = 7.0 Hz; 3H; H35) (Figure S15). ^{13}C-NMR (Acetone-d_6, 125 MHz), δ (ppm): 165.6 (C22); 164.9 (C1); 159.8 (C25); 153.4 (C15); 143.4, 142.5, 141.0, 137.0, 132.8, 131.5, 131.1, 130.8, 130.3, 128.4, 127.7, 127.5, 124.4, 123.4, 121.6, 115.7 (aromatic carbons); 92.9 (C23); 77.8 (C26); 67.6 (C32); 48.7 (C14); 47.4 (C34); 32.0, 25.8, 24.2 (methylene); 19.8 (C24); 14.9 (C33); 14.7 (C35) (Figure S16).

Havlíček et al. (2009) also synthesized impurity F from candesartan cilexetil (6.1 g). The procedure used simple starting materials (potassium carbonate 2.5 g, acetone 150 mL) but required a more complex reactive reagent (iodomethane) and multiple reaction steps, which require a long, careful purification to obtain the pure compound[21]. Mohan et al. (2009) prepared impurity F from candesartan cilexetil via a similar procedure to impurity D. While this process was simple and rapid, the purity of the resulting product was not reported[18].

3.1.5. Impurity G

The synthesis of impurity G through alkaline hydrolysis of CDC achieved an excellent yield (95%) and a highly pure product (99%), which was crystallized as a white solid having an *Rf* value of 0.35 on TLC using DCM – methanol – acetic acid (180:20:1).

The FT-IR spectrum of the synthesized impurity G showed strong similarity with that of the reference standard (Figure S17). FT-IR (cm^{-1}): 1705 (C=O), 1548–1616 (aromatic C–C), 1010–1221 (C–N), 3034–3065 (aromatic C–H), 2835–2986 (aliphatic C–H). HRMS (ESI) *m/z* calculated for $C_{24}H_{21}N_6O_3$ ($[M-H]^-$) = 439.1524, found 439.1543 (Figure S18). ^{1}H-NMR (MeOD, 500 MHz), δ (ppm): 7.63 – 7.68 (*m*; 4H; H_{Ar}); 7.55 (*dd; J* = 7.5, 1.0 Hz; 1H; H_{Ar}); 7.51 (*dd; J* = 8.0, 1.0 Hz; 1H; H_{Ar}); 7.22 (*t; J* = 8.0 Hz; 1H; H_{Ar}); 7.03 (*d; J* = 8.5 Hz; 2H; H_{Ar}); 7.00 (*d; J* = 8.5 Hz; 2H; H_{Ar}); 5.71 (*s*; 2H; H14); 4.62 (*q; J* = 7.0 Hz; 2H; H23); 1.48 (*t; J* = 7.0 Hz; 3H; H24) (Figure S19). ^{13}C-NMR (MeOD, 125 MHz), δ (ppm): 171.3 (C22); 169.5 (C1); 160.1 (C15); 143.0, 142.5, 139.7, 138.5, 132.5, 131.9, 131.6, 130.3, 129.0, 128.1, 125.5, 124.3, 122.3, 122.2, 118.2 (aromatic carbons); 68.3 (C23); 48.9 (C14); 14.8 (C24) (Figure S20).

3.1.6. Overall diagram of the synthesis of candesartan-related impurities and discussion

A complete synthetic network of candesartan impurities (A, B, C, D, E, F and G) is presented in Figure 1. These optimized routes provide a practical, scalable, and efficient approach to prepare candesartan impurity standards for pharmaceutical quality control.

The hydrolytic pathways of CDC were influenced by acidic or basic medium. There was no reaction at room temperature in the dilute HCl solution

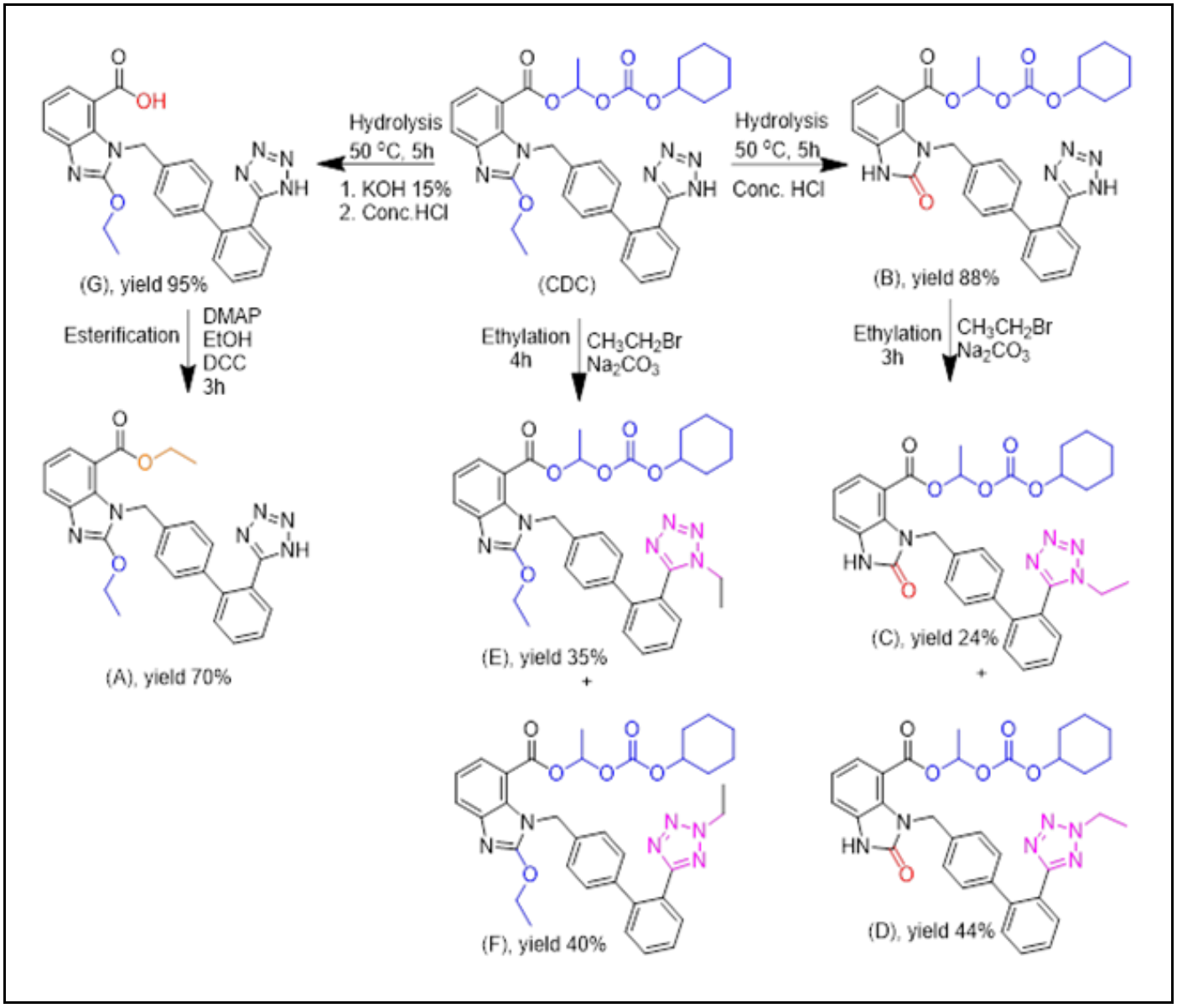

Figure 1. Synthesis of impurities A, B, C, D, E, F, G from candesartan cilexetil.

(25% concentration). In concentrated HCl (37% concentration), the ester moiety of CDC remained stable, whereas the carbamate group was selectively hydrolyzed to create impurity B. In contrast, under basic conditions (KOH 15%), the ester group was converted to the carboxylate anion -COO^- and the tetrazole NH group was deprotonated to form N^-. Consequently, in the next step, HCl was used to transfer these groups to -COOH and -NH, respectively, to obtain impurity G.

Ethylation reactions occurred via selective deprotonation of the tetrazole ring using sodium carbonate, enabling nucleophilic substitution with ethyl bromide. CDC was used as a substrate to form impurities E and F, while parallel reactions from impurity B produced impurities C and D.

Impurities C and E were also generated during a similar ethylation process of impurities D and F, respectively. However, they were not isolated as reference standards because they are not required for regulatory impurity profiling.

A comparison with literature methods (Table 2) shows that impurities B and G could be obtained in >88% and >95% yield, respectively, using simplified one-step hydrolysis. Impurities A, D, and F are more sensitive to reaction conditions, with yields limited by competing isomer formation. The developed method uses fewer hazardous reagents and milder conditions, while the products have higher purities than previously reported syntheses.

The reaction yield in the synthesis of impurities C, D, E, and F was relatively low in our study and Mohan et al. (2009)[18], primarily due to the formation of isomeric products. To enhance the yield, attention should be given to reaction conditions, such as catalysts, reaction solvents, and reactant concentrations.

Table 2. Comparison of the synthetic methods of impurities of candesartan.

Impurity	Synthesis methods		Synthesis methods	Chromatographic purity		Synthesis yield	
	Other studies	This study with references		Other studies	This study	Other studies	This study
A	Multi-step and time-consuming synthesis using uncommon intermediates[17]	Simple process (Steglich esterification)[20] at 0 °C, 20 °C in 3h from available material (CDC)	Column chromatography	97%	98.5%	-	70%
B	Acid hydrolysis of CDC at room temperature overnight[18]	Acid hydrolysis of CDC with concentrated acid at 50 °C in 5h	Washing with cold water	99%	99.0%	-	88%
C **D**	Ethylation of Imp B in 3-6 h[18, 21]	Imp B ethylation using EtBr/Na_2CO_3 (2/2.5) in acetone at 50–55 °C in 3 h	Column chromatography	D: 99%	D: 98.5%	● C: 30% ● D: 40%	● C: 24.12% ● D: 43.92%
E **F**	Alkylation of CDC in 3h[13]	Synthesis from CDC using EtBr/KI (1:5) in 2h	Column chromatography	-	F: 98.2%	-	● E: 35% ● F: 40%
G	Not mentioned	Alkaline hydrolysis of CDC[14] at 55–65 °C in 5h	Acidification, washing	-	99.0%	-	95%

The reaction ratio is a crucial part to optimize the reaction time. By adjusting this ratio, the overall reaction yield would be improved in a shorter synthesis time.

Since impurity G is rapidly synthesized via hydrolysis under simple conditions, affording a high yield of 95%, it could be used to synthesize impurity A in a single step. This method is economically feasible and scalable, although it generates minor by-products (the obtained yield of only 70%). In comparison with the study reported by Raman et al. (2011)[17], this synthetic procedure of impurity A was simpler, thereby requiring fewer reactions and a shorter reaction time. Additionally, the purity of impurity A reached 98.5%, which is higher than that reported in other references. Following the mechanism of hydrolysis in an alkaline medium described in the publication of Khurana et al. (2004)[19], we designed the synthesis of impurity G to achieve a high yield (95%). The reaction temperature was also optimized to reduce the time required for synthesizing impurity B[18].

Overall, the developed synthetic strategy enables efficient preparation of candesartan impurity reference standards using readily available starting materials and mild reaction conditions. Compared with previously reported methods, the present approach

Table 3. Results of HPLC validation for candesartan impurities A, B, D, F, G.

Parameters	Imp A	Imp B	Imp D	Imp F	Imp G
System suitability RT min (RSD%)	7.05 (0.19)	10.10 (0.26)	16.94 (0.21)	23.98 (0.16)	4.01 (0.20)
Area (RSD%)	1210.75 (0.17)	980.23 (0.19)	791.71 (0.29)	822.02 (0.23)	1013.89 (0.21)
Asymmetry (RSD%)	1.12 (1.26)	1.12 (1.27)	1.06 (0.29)	1.05 (0.43)	1.25 (1.51)
Theory plates (RSD%)	10674 (0.69)	10674 (0.69)	39180 (0.61)	65781 (1.17)	6940 (0.54)
Linear regression	y = 55.031x + 13.108	y = 46.54x + 9.5064	y = 39.794x + 0.1042	y = 37.735x + 5.1606	y = 50.395x + 11.227
R^2	0.9983	0.9988	0.9980	0.9988	0.9988
Concentration range	5 – 30 (µg/mL)				
LOD (µg/mL)	1.56	1.27	1.56	1.30	1.19
LOQ (µg/mL)	4.73	3.85	4.72	3.95	3.60
Intra-day precision (RSD%), n=6	98.46 (0.31)	99.54 (0.37)	98.45 (0.40)	98.20 (0.21)	99.37 (0.50)
Inter-day precision (RSD%), n=12	98.49 (0.51)	99.31 (0.48)	98.72 (0.52)	98.52 (0.45)	99.19 (0.47)
Accuracy (%) n=9	98.19–101.19	98.25–101.81	98.15–101.80	98.22–101.89	98.0–101.78
Content (%)	98.5	99.3	98.7	98.5	99.2

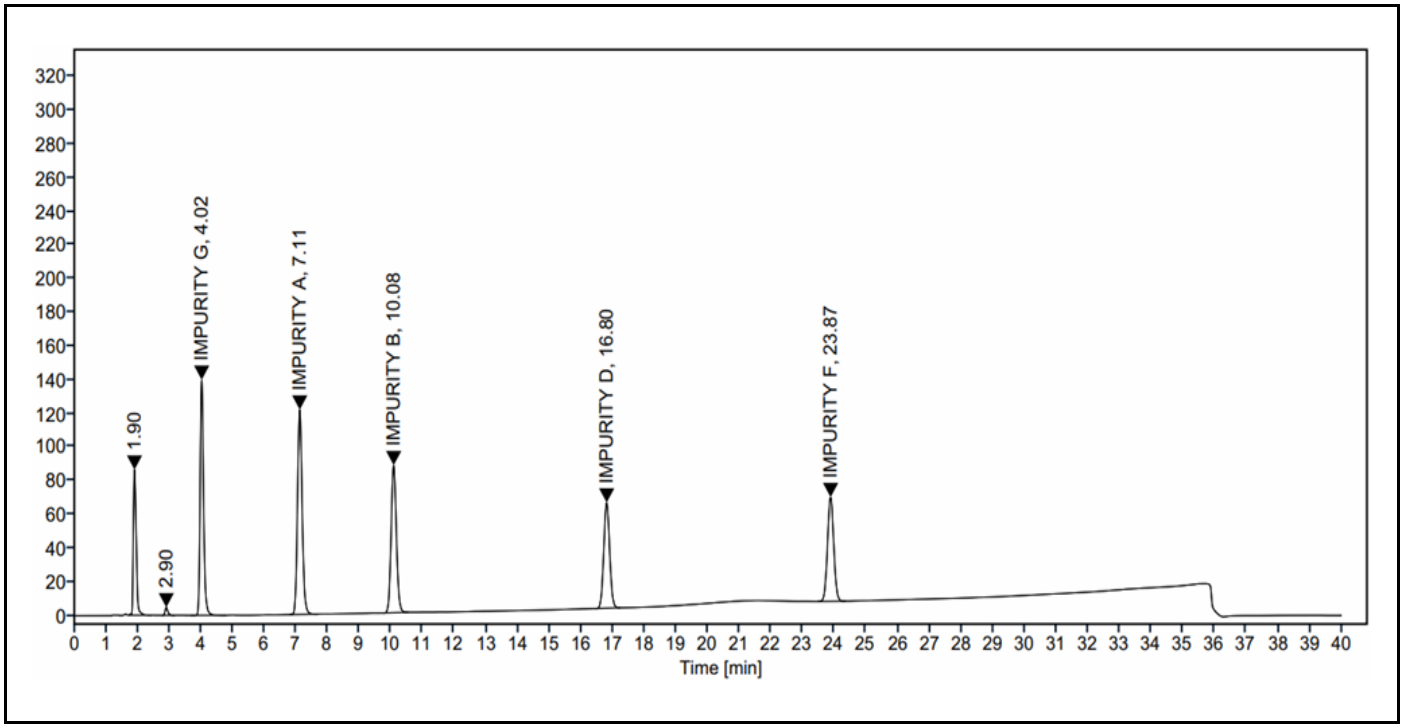

Figure 2. Chromatogram of Imp A, B, D, F, G standard mixture.

provides improved practicality, reduced synthesis complexity, and high chromatographic purity. These characteristics make the proposed workflow highly suitable for routine pharmaceutical impurity standard production.

3.2. HPLC method validation

The results of method validation are summarized in Table 3. System suitability was confirmed by six replicate injections of solution impurities A, B, D, F, and G of candesartan with a concentration of 20 µg/mL.

The chromatogram of the standard mixture is shown in Figure 2. The coefficient of variation (CVs) of peak areas from six repeated injections of samples were below 2.0%, the asymmetry factor (As) was between 0.8 and 1.5, and the number of theoretical plates (N) was over 2000. The chromatogram of the mobile phase shows no interference at the retention times of impurities A, B, D, F, and G, indicating that this method is specific for their determination. Comprehensive validation proved the high sensitivity, accuracy, inter- and intra-day precision, selectivity, and suitability for use in quality control laboratories. Linearity, LOD and limit of quantitation (LOQ) are

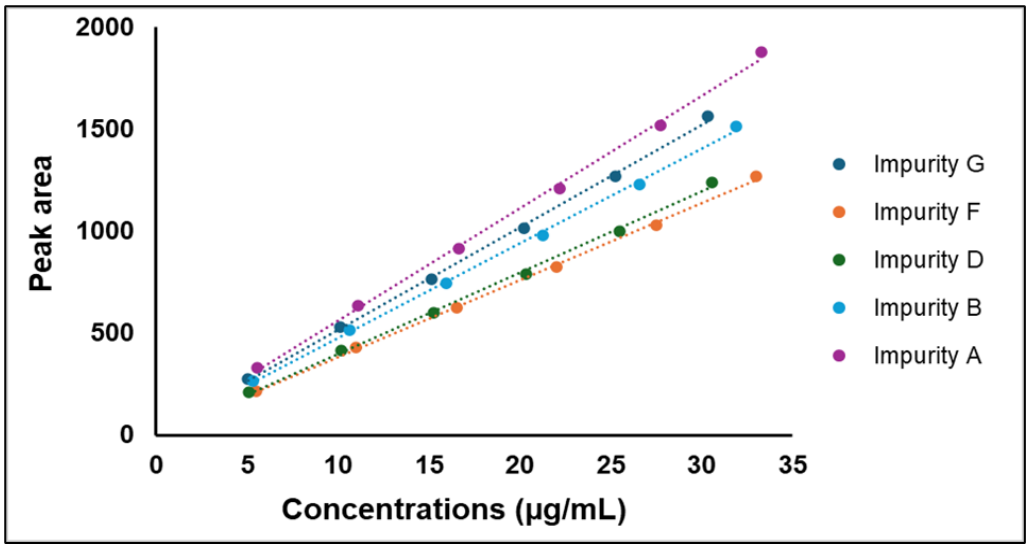

Figure 3. Linearity of candesartan impurity A, B, D, F, G.

Table 4. Measurement results for the homogeneity check.

Sample number	Imp A	Imp B	Imp D	Imp F	Imp G
1	98.40	99.08	98.63	98.23	99.22
2	98.64	99.07	98.51	98.42	99.08
3	98.50	99.18	98.51	98.26	99.08
4	98.61	99.18	98.71	98.14	99.30
5	98.53	99.21	98.57	98.19	99.00
6	98.51	99.26	98.55	98.33	99.00
7	98.59	99.25	98.54	98.12	99.13
8	98.55	99.23	98.41	98.16	98.96
9	98.51	99.12	98.54	98.16	99.03
10	98.63	99.21	98.47	98.07	99.17
Average	98.55	99.18	98.54	98.21	99.10
σ	0.01	0.00	0.01	0.01	0.01
SD	0.07	0.07	0.08	0.10	0.11
RSD%	0.07	0.07	0.08	0.11	0.11

presented in Figure 3. The C18 chromatographic column (150 x 4.6 mm; 5 µm) employed in our study is widely used in medium- to large-scale laboratories. Although the column was different from the column specified in USP 2025, C18 (150 x 3.9 mm; 4 µm), the chromatographic conditions were fully validated and comply with all requirements of ICH guidelines[22]. Therefore, the method is highly applicable in drug quality control in the national market.

3.3. Establishment of impurity reference standard

3.3.1. Homogeneity test

For a homogeneity check, 10 out of 100 vials of candesartan imp A, B, D, F, and G, packaged in nitrogen-filled dry glove boxes, were randomly selected, and the content of each sample was determined[20]. The data are shown in Table 4.

3.3.2. Assigned value

The assigned value was determined after checking the suitability system of three laboratories (Table 5, 6). The result showed that the values of x* and s* did not change from one iteration to the third modified data.

The convergence is assumed, and the assigned value with the uncertainty is in Table 7, in which

$$U_x = \frac{1.25 s^*}{\sqrt{p}}$$

It can be concluded that impurities A, B, D, F, and G candesartan are eligible for registration of national standards with their purity. The standard vials were labelled and attached to the test certification. Long-term storage of standard vials was at 2 – 8 °C and protected from the light.

4. CONCLUSIONS

This study successfully developed efficient, reproducible, and scalable synthetic routes for five major candesartan cilexetil impurities (A, B, D, F, and G).

All synthesized impurities were structurally confirmed by NMR and HRMS and subsequently established as reference standards following ISO and ICH requirements. High yields were achieved for impurities B (88%) and G (95%), while impurities A, D, and F were obtained with acceptable yields and excellent chromatographic purity (>98%). The validated RP-HPLC method demonstrated excellent specificity, accuracy, precision, and sensitivity for impurity quantification. Homogeneity testing and interlaboratory value assignment confirmed the suitability of these materials as pharmaceutical reference standards, with certified purities ranging from 98.2% to 99.1%. The developed approach provides a practical and sustainable solution for producing impurity reference standards, supporting pharmaceutical quality control, regulatory compliance, and stability studies of candesartan-containing products. This methodology can be used as the framework applied to other pharmaceutical impurities to strengthen analytical capability in pharmaceutical quality assurance.

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Table 5. The parameters of SST from three laboratories for the assay of candesartan impurities A, B, D, F, G.

	Imp A			Imp B			Imp D			Imp F			Imp G		
	Lab 1	Lab 2	Lab 3	Lab 1	Lab 2	Lab 3	Lab 1	Lab 2	Lab 3	Lab 1	Lab 2	Lab 3	Lab 1	Lab 2	Lab 3
1	98.54	98.51	98.56	99.25	99.10	99.16	98.41	98.68	98.72	98.23	98.28	98.29	99.19	99.04	99.05
2	98.57	98.57	98.60	99.04	99.02	99.12	98.62	98.57	98.61	98.30	98.23	98.32	99.04	99.17	99.13
3	98.44	98.57	98.60	98.91	99.16	99.19	98.47	98.57	98.49	98.19	98.20	98.18	99.12	99.11	99.10
4	98.55	98.63	98.46	98.95	99.10	98.92	98.57	98.37	98.55	98.16	98.09	98.01	99.04	99.10	99.05
5	98.47	98.59	98.59	99.07	98.87	98.95	98.50	98.53	98.46	98.26	98.23	98.10	99.07	99.17	99.07
6	98.43	98.51	98.47	99.22	99.07	99.03	98.67	98.75	98.67	98.19	98.17	98.18	99.11	99.10	99.07
Average	98.499	98.562	98.545	99.07	99.05	99.06	98.54	98.58	98.58	98.22	98.20	98.18	99.10	99.12	99.08
σ	0.003	0.002	0.004	0.02	0.01	0.01	0.01	0.02	0.01	0.00	0.00	0.01	0.00	0.00	0.00
SD	0.059	0.046	0.064	0.14	0.10	0.11	0.10	0.13	0.10	0.05	0.07	0.12	0.06	0.05	0.03
N	7999	8057	8026	9455	9419	9448	39563	39525	41857	63879	60314	60121	7380	7006	7352
As: 0.8 – 1.5	1.123	1.127	1.116	1.063	1.066	1.063	1.062	1.064	1.064	1.071	1.070	1.068	1.043	1.357	1.346
RSD ≤ 2	0.06	0.05	0.06	0.14	0.10	0.11	0.10	0.13	0.10	0.05	0.07	0.12	0.06	0.05	0.03

Lab 1: Reference Substances Department, Physical Instrument Department, Institute of Drug Quality Control, HCMC.
Lab 2: Cosmetic Testing Department, Institute of drug Quality Control HCMC.
Lab 3: Oriental Medicine and Pharmaceutical Testing Department, Institute of drug Quality Control HCMC.

Table 6. Determination of the assigned value of candesartan impurities A, B, D, F, G by three laboratories, together with values for the expanded uncertainties (U) of the results as reported by the laboratory.

	Imp A				Imp B				Imp D				Imp F				Imp G			
Iteration	x^*_0	x^*_1	x^*_2	x^*_3	x^*_0	x^*_1	x^*_2	x^*_3	x^*_0	x^*_1	x^*_2	x^*_3	x^*_0	x^*_1	x^*_2	x^*_3	x^*_0	x^*_1	x^*_2	x^*_3
$x^* - \delta$	98.46	98.44	98.44	98.44	98.88	98.87	98.87	98.87	98.37	98.39	98.39	98.39	98.12	98.11	98.11	98.11	99.03	99.02	99.02	99.02
$x^* + \delta$	98.65	98.63	98.63	98.63	99.27	99.25	99.25	99.25	98.76	98.75	98.75	98.75	98.28	98.30	98.30	98.30	99.17	99.17	99.17	99.17
New x^*	98.55	98.54	98.54	98.54	99.07	99.06	99.06	99.06	98.57	98.57	98.57	98.57	98.20	98.20	98.20	98.20	99.10	99.10	99.10	99.10
New s^*	0.06	0.06	0.06	0.06	0.13	0.13	0.13	0.13	0.13	0.12	0.12	0.12	0.05	0.06	0.06	0.06	0.05	0.05	0.05	0.05

Table 7. The assigned value with the uncertainty of candesartan impurities (A, B, D, F, G).

	Imp A	Imp B	Imp D	Imp F	Imp G
Assigned value	98.5%	99.1%	98.6%	98.2%	99.1%
U_x (k=2)	0.018	0.038	0.035	0.018	0.035

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Author contribution

Nguyen Thi Huynh Trang: methodology, investigation, formal analysis. Vo Thi Kim Khuyen: formal analysis, visualization, writing – original draft, writing – review and editing. Dam To Uyen: writing – original draft, investigation. Nguyen Ai Vy: writing – original draft, investigation. Pham Van Son: resources, validation, methodology. Truong Ngoc Tuyen: supervision, validation, methodology, writing – review and editing. Nguyen Duc Tuan: project administration, conceptualization, methodology, data curation, supervision, resources, writing – review and editing.

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