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Identification, Synthesis, and Strategy For Minimization of Potential Impurities Observed In Raltegravir Potassium Drug Substance

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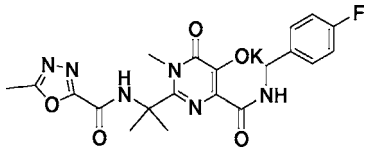
Supporting Information

ABSTRACT: Multiple sources of anticipated degradation and process impurities of raltegravir potassium drug substance observed during the laboratory optimization and later during its bulk synthesis are described in this article. The impurities were monitored by UPLC, and their structures are tentatively assigned on the basis of fragmentation patterns in LC−MS and NMR spectroscopy. Most of the impurities are synthesized, and their assigned constitutions were confirmed by co-injection in UPLC. In addition to the formation, synthesis, and characterization, strategy for minimizing these impurities to the level accepted by ICH is also described. We feel that our study will be helpful to the generic industry for obtaining chemically pure raltegravir potassium.

INTRODUCTION

Raltegravir potassium (MK-0518), belongs to a new class of compounds discovered for development as inhibitors of HIV



Raltegravir potassium (MK-0518)

integrase, an enzyme catalyzing the integration of viral DNA into the host genomic DNA, thus preventing further virus replication.[1−3] It was approved on October 12, 2007, and is marketed as ISENTRESS as a complementary agent to existing anti-retroviral therapies.

The primary objectives of process chemical research are the development of efficient, scalable, and safe reproducible synthetic routes to drug candidates within the developmental space and acting as a framework for commercial production in order to meet the requirement of various regulatory agencies. Therefore, assessment and control of the impurities in a drug substance and drug product are important aspects of drug development for the development team to obtain various marketing approvals. It is extremely challenging for an organic chemist to identify the impurities which are formed in very small quantities in a drug substance and wearisome if the product is nonpharmacopeial. Thus, in our study we described the formation, identification, synthesis, and characterization of impurities found in the preparation of raltegravir potassium. This study will help a synthetic organic chemist to understand the potential impurities in raltegravir potassium synthesis and thereby obtain the pure compound.

To ensure that desired drug metabolism, safety and clinical studies are not jeopardized by inconsistent purity or impurities having potential harmful toxicological properties, when raltegravir was synthesized by a commercial route (Scheme 1),[4a−c] many impurities were appearing in the drug substance. As regulatory guidelines promulgated by the International Conference on Harmonization (ICH)[5] dictate rigorous identification of impurities at levels of 0.1%, we were required to identify a total of nine impurities appearing in the sample of the drug substance. The literature reveals few impurities formed due to degradation, incomplete reaction, or side reactions.[6] However, no synthetic details have been reported. In this context, the present study describes identification, synthesis, characterization, and strategy for controlling these impurities.

RESULTS AND DISCUSSION

The first part of this article elaborates the identification and possible pathways for the formation of impurities, while in the second part, strategies we adapted for minimizing these impurities to the level accepted by ICH are described.

Being a popular approach for impurity identification, LC−MS was used to identify impurites in raltegravir potassium by which we observed molecular weights for impurities **A**−**I** (Table 1). These structures were later confirmed by NMR, mass, and UPLC co-injection.

Impurities **A** and **B** are the hydrolysis products of the raltegravir precursors **9** and **14**. However, the possibility of disconnection of the oxadiazole amide bond as shown in Scheme 2 cannot be ruled out. During our study we realized that the impurities listed in Table 1, cannot be removed from raltegravir potassium due to solubility issues; therefore, we decided to remove these impurities from raltegravir as the impurity profile of raltegravir remains unchanged when it is converted to its potassium salt **1**.

The final step of raltegravir involves the amide bond formation in which amine **9** and oxadiazole carbonyl chloride **14** are reacted in the presence of *N*-methylmorpholine to give pivaloyl protected raltegravir (Scheme 3). The reaction mixture was directly treated with an aqueous base such as ammonia, methyl amine, or potassium hydroxide to hydrolyze the pivaloyl

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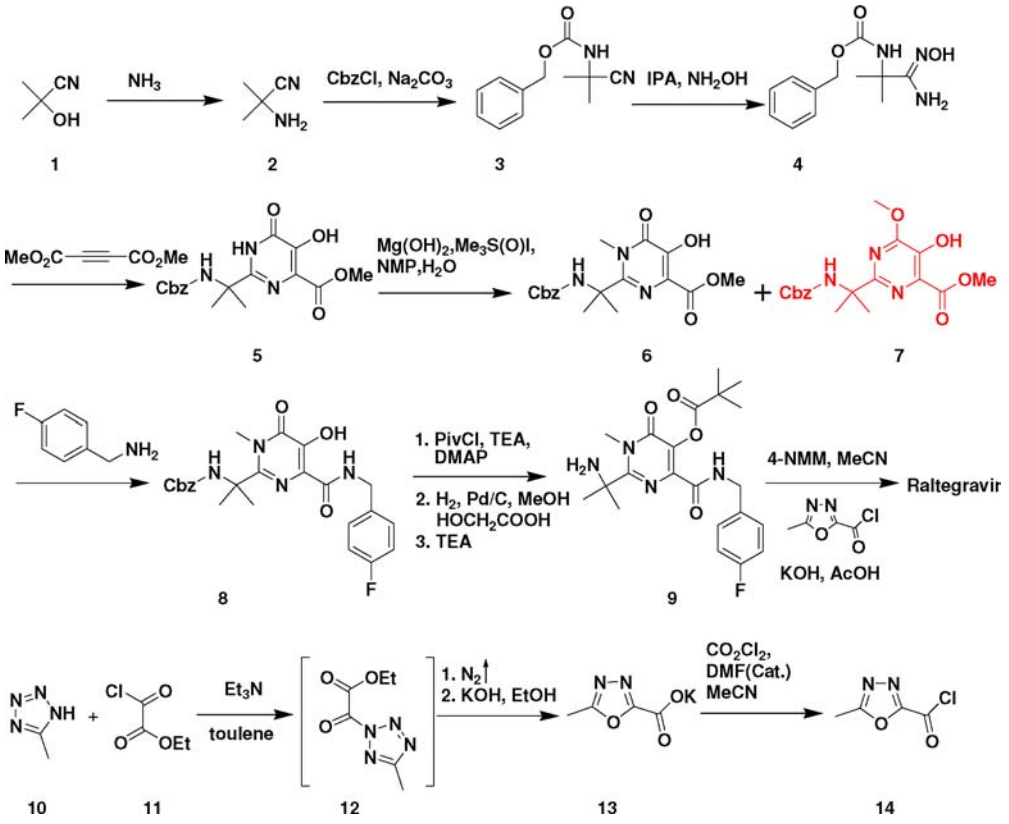
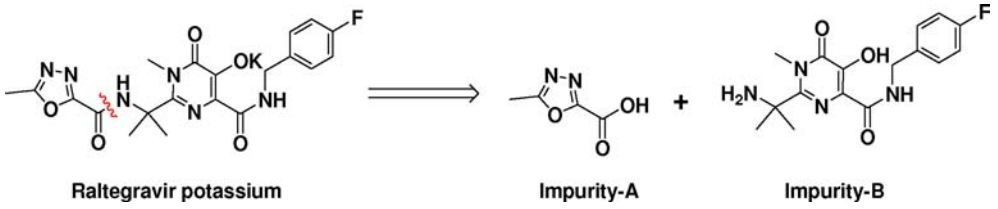
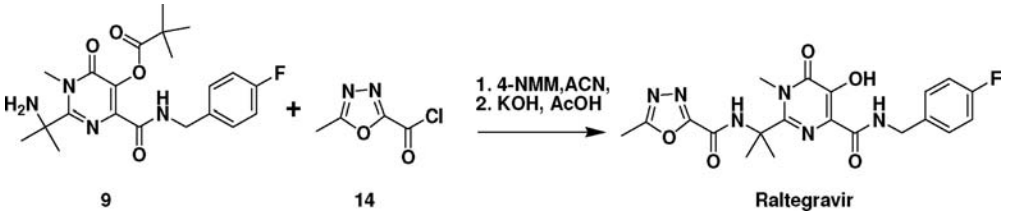
Scheme 1. Synthesis of raltegravir



Scheme 2. Amide disconnection



Scheme 3. Final step of raltegravir



ester. Acidification provides the desired product, raltegravir contaminated with impurities listed in Table 1.

Plausible Pathways. The process impurities **A**, **B**, and **H** are observed in about 0.7−2.0%. Impurity **H** may be due to the carryover of byproduct **7** to the final step. Impurity **G** may be forming due to the presence of residual ethyl oxalic acid in **13**, which was also converted to acid chloride and reacted with **9** during the reaction of **9** and **14** in about 0.5−1.2%. Further, it was difficult to remove the impurity **G** from raltegravir potassium, without significant loss in yield. Impurities **C**, **D**, **E**, and **F** are the degradation products, and **I** is the process impurity. The degradation pathway for impurity **C** due to hydrolysis of the oxadiazole moiety is shown in Scheme 4.

Impurity **G** could be the source for impurity **D** and forms when ammonia is used for quenching excess oxalyl chloride.[4]

Impurities **E** and **F** are the desfluoro analogues of raltegravir and may be forming during −Cbz removal of **8** under acidic conditions where as impurity **I** is forming due to condensation of amine **9** with oxalyl chloride during the final step of raltegravir in about 0.5%. This is a very crucial impurity and due to solubility issues is extremely difficult to remove.

Synthesis and Control. Impurities **A** and **B** are the degradation products, while impurity **C** is the hydrolysis product of raltegravir and was synthesized by stirring raltegravir with acetonitrile and aqueous KOH. The pure product was isolated by column chromatography. The structure was

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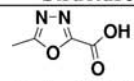
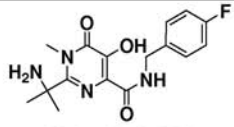
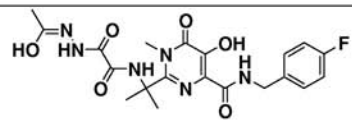
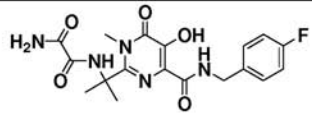
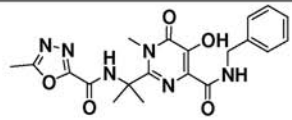
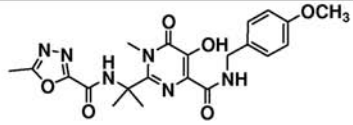
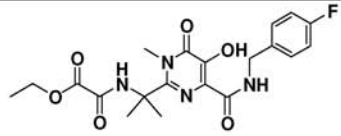
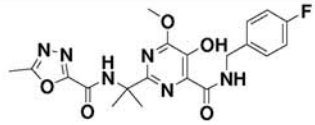
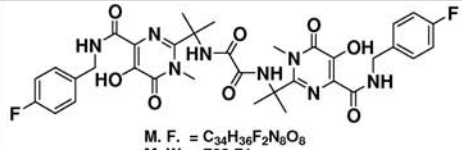
Table 1

Sr	Name	Structure	Relative Retention Time [a]
1)	Impurity-A:	 M. F. = $C_4H_4N_2O_3$ M. W. = 128.09	0.07
2)	Impurity-B:	 M. F. = $C_{16}H_{19}FN_4O_3$ M. W. = 334.35	0.15
3)	Impurity-C:	 M. F. = $C_{20}H_{23}FN_6O_6$ M. W. = 462.44	0.74
4)	Impurity-D:	 M. F. = $C_{18}H_{20}FN_5O_5$ M. W. = 405.38	0.86
5)	Impurity-E:	 M. F. = $C_{20}H_{22}N_6O_5$ M. W. = 426.43	0.95
6)	Impurity-F:	 M. F. = $C_{21}H_{24}N_6O_6$ M. W. = 456.45	0.97
7)	Impurity-G:	 M. F. = $C_{20}H_{23}FN_4O_6$ M. W. = 434.42	1.07
8)	Impurity-H:	 M. F. = $C_{20}H_{21}FN_6O_5$ M. W. = 444.42	1.25
9)	Impurity-I:	 M. F. = $C_{34}H_{36}F_2N_8O_8$ M. W. = 722.71	1.33

[a]Waters Acquity UPLC; column: Acquity CSH (2.1 mm × 100 mm), 1.7 μ; flow rate: 0.4 mL/min, λ: 210 nm; injection vol: 3.0 μL; mobile phase-A: 1 mL perchloric acid in 1000 mL water; mobile phase-B: ACN/water (65:35); Run time: 25 min; Raltegravir relative retention time: about 13.38 min.

confirmed by proton NMR, supported by ^{13}C NMR and DEPT-135 experiment, observing two sets of carbonyl due to four amide groups.

A synthetic protocol for impurities **D** and **G** is shown in Scheme 5. Impurity **G** can be synthesized by the process disclosed by Benedetta et al.[7] in which amine **15** was reacted with ethyl oxalyl chloride (**11**). Impurity **D** was synthesized

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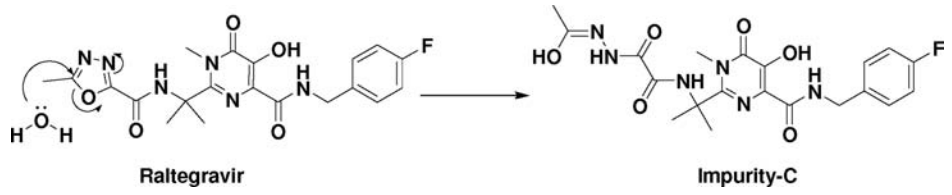
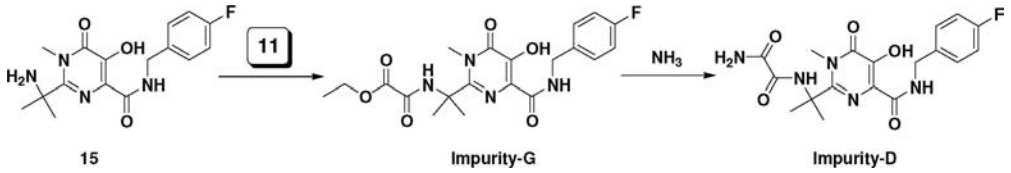
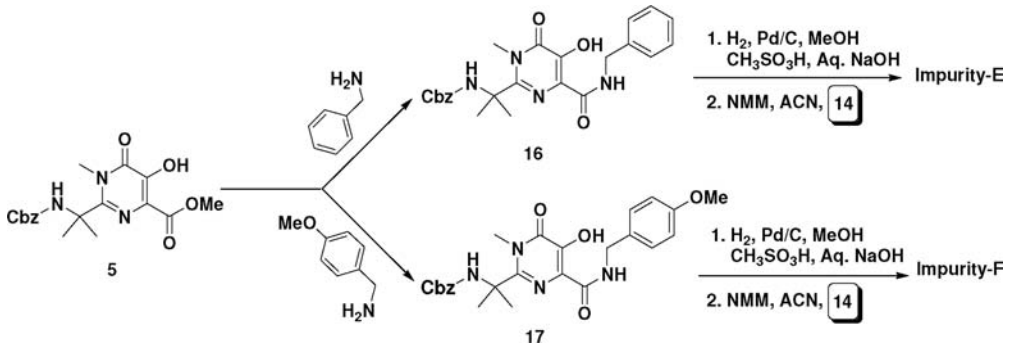
Scheme 4. Formation of impurity C



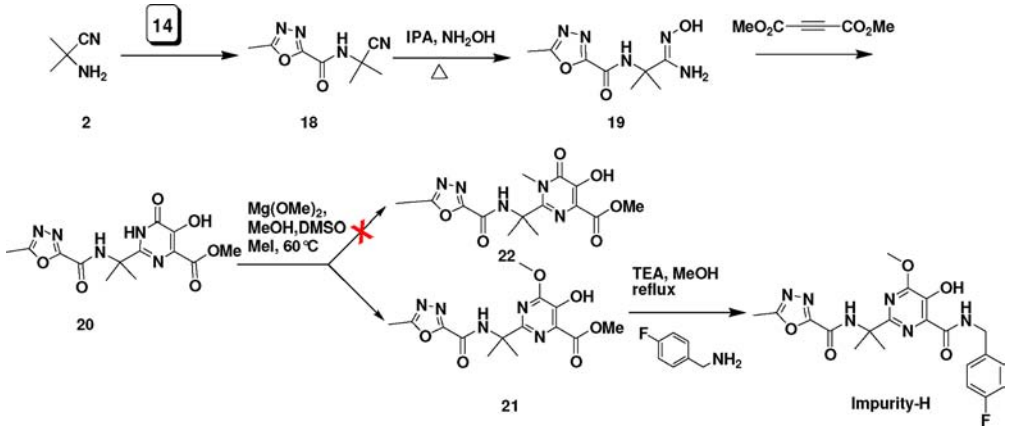
Scheme 5. Synthesis of impurities D and G



Scheme 6. Synthesis of impurities E and F



Scheme 7. Synthesis of impurity H



from impurity **G** by passing ammonia gas in a pressure reactor and methanol as the solvent almost quantitatively.

Impurities **E** and **F** are synthesized using the synthesis protocol of raltegravir (Scheme 1), except the 4-fluorobenzyl amine was replaced with 4-methoxybenzyl amine and benzyl amine, respectively (Scheme 6) in good yields and purity.

The probability of anticipated impurities due to positional isomers of 4-fluorobenzyl amine such as 2-fluorobenzyl amine and 3-fluorobenzyl amine was ruled out after evaluating the synthetic route of 4-fluorobenzyl amine.

Impurity **H** is another potential process impurity observed in raltegravir drug substance. After careful scrutiny of reported routes, we envisaged that balancing the steric factors of the 4-pyrimidone moiety may lead to selective O-methylation. Therefore, impurity **H** was synthesized exclusively by the route disclosed in Scheme 7.

Modification of a literature procedure provided an efficient process for the oxadiazole fragment **20** with high efficiency and was robust and simple to perform with a direct crystallization to afford the product **20** with good purity and yield. Formation of compound **18** was confirmed by observing the IR signals due to the nitrile group at 2243 cm^{-1} and the amide carbonyl at 1693 cm^{-1}. The structure was supported by mass (M + 1; 195.1) and proton NMR. Compound **18** was almost quantitatively converted to amidoxime **19**. Disappearance of IR signals due to the nitrile group and appearance of two IR signals at 3491 cm^{-1} and 3456 cm^{-1} due to primary amine, supported by mass (M + 1; 228.2) and proton NMR confirmed the structure of

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Scheme 8. Synthesis of impurity I:

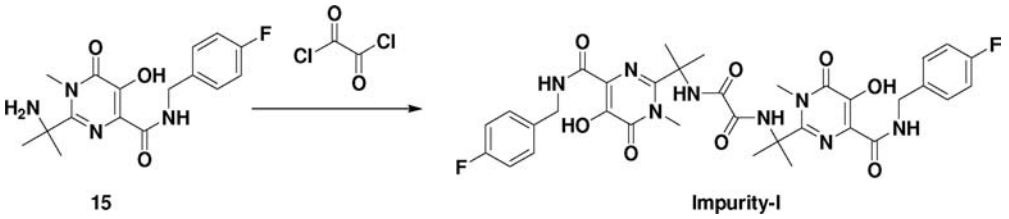
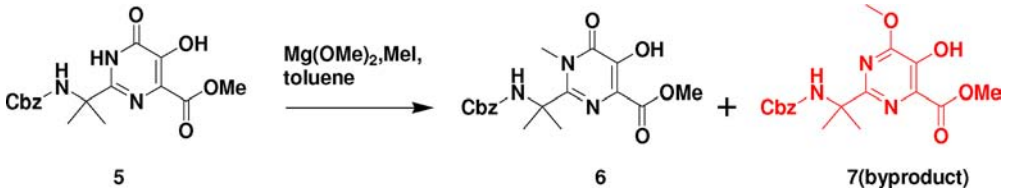


Table 2. Optimization of reaction condition[a]

sr	solvent	mole ratio of **13**	mole ratio of $C_2O_2Cl_2$	reaction time (h)	mole ratio of **9**	purity		yield (%)[b]
						imp-**I**	raltegravir	
1	THF	1.0	1.2	2	1.0	0.81	92.48	78
2	NMP	1.0	1.2	2	1.0	1.23	97.22	65
3	ACN	1.0	1.2	2	1.0	1.51	95.33	80
4	ACN	1.0	1.2	4	1.0	0.54	96.37	81
5	ACN	1.0	1.0	4	1.0	0.37	96.55	79
6	ACN	1.0	0.9	4	1.0	0.18	96.72	78
7	ACN	1.0	0.9	4	0.9	0.10	97.95	88
8	ACN	1.0	0.9	4	0.8	0.07	98.55	92
9	ACN	1.0	0.9	2	0.8	0.17	98.11	91
10	ACN	1.0	0.9	6	0.8	0.07	98.58	92

[a]All reactions performed at 0−5 °C, and *N*-methylmorpholine as the base. [b]Yields calculated based on **9**.

Scheme 9



19. Michael addition of amidoxime **19** to DMAD followed by thermal rearrangement furnished the synthesis of key **20**. The structure was confirmed by proton NMR and mass spectroscopy. After obtaining the key hydroxypyrimidinone **20**, methylation of **20** was attempted using $Mg(OCH_3)_2/CH_3I$ in DMSO solvent.[4] Unexpectedly, methylation of **20** gave a clean desired *O*-methyl product **21**. Two methyl signals appearing at δ 3.97 and δ 3.88 due to ester and −OMe group supported by mass spectra (M + 1; 338.2) confirmed the structure. UPLC and NMR analysis revealed that product **22** was formed in this reaction albeit in minor quantities. Compound **21** was then reacted with 4-fluorobenzyl amine to furnish the synthesis of impurity **H**. This impurity can be controlled by repeated crystallization of **6** from toluene. This impurity is highly soluble in toluene; it can even be removed in the final stage by crystallization of raltegravir from toluene or methanol.

Impurity **I**, another critical impurity, was synthesized by the reaction of **15** with 0.5 equivalent of oxalyl chloride in acetonitrile at lower temperature. A pure sample of impurity **I** was obtained by crystallization from acetonitrile (Scheme 8).

The structures of all these impurities were confirmed by proton NMR and mass spectroscopy, whereas relative retention times were established using UPLC.

Control. During our study, we observed that among all impurities, impurity **I** is the most critical impurity and it can be controlled to the level 0.1% during the final step of condensation between oxadiazole carbonyl chloride **14** and amine **9**. This was achieved by controlling the mole ratios of oxalyl chloride and oxadiazole potassium salt **13**, in such a way that the excess oxalyl chloride should not be available in the reaction media. After detailed study we observed that the reaction time for the reaction of **13** with oxalyl chloride to form **14** and mole ratio of **14** with respect to **9** are the important aspects to reduce the formation of impurity **I**. Table 2 summarizes some valuable experiments.

The above study indicates that entry 8 of Table 2 was the best experiment in this series and acetonitrile was the best solvent for this reaction. Entry 8 of Table 2 also shows that sufficient time should be given for the conversion of **13** to **14**. Additionally the mole ratio of **9** is also important in minimizing the critical impurity **I** to the level acceptable to ICH. When product is obtained by the conditions reported in entry 8 of Table 2 and purified from methanol, all impurities were reduced to below 0.1%, and all organic volatile impurities (OVI) were found well within ICH criteria, particularly acetonitrile was found below 5 ppm by GC-HS.

Impurities **A** and **B** are controlled by selecting the mole ratios given in entry 8 of Table 2. We observed that in the reaction performed using 0.8 equiv of **9**, the unreacted oxadiazole moiety, i.e. impurity **A**, being highly water soluble, gets removed during workup.

Impurity **C** is the degradation product, and it forms up to 0.2% during alkaline hydrolysis of pivaloyl raltegravir to raltegravir; this impurity gets removed in methanol purification.

Impurities **D** and **G** are the byproducts and appear in raltegravir due to carryover of ethyl oxalic acid during the

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synthesis of **14**. Therefore **13** was purified using 3 volumes of methanol at ambient temperature to remove the residual ethyl oxalic acid.

Impurities **E** and **F** are controlled by performing −Cbz removal at 10−15 °C using 10% dry Pd/C. Impurity **H** is the process impurity, and during process optimization we observed that, if the % of **7** is below 5% in **6**, then this impurity does not appear in raltegravir. Therefore, **6** was purified using refluxing toluene to lower the level of byproduct **7** below 5% (Scheme 9).

CONCLUSION

For the better understanding of the synthetic pathway of an active pharmaceutical ingredient (API) it is necessary to identify all the impurities formed/anticipated. In this regard, we have synthesized and characterized all potential impurities of raltegravir potassium. In addition to this we have also demonstrated the strategy for minimization of these impurities to the level accepted by the International Conference on Harmonization (ICH).

EXPERIMENTAL SECTION

All materials were purchased from commercial suppliers. Unless specified otherwise, all reagents and solvents were used as supplied by manufacturers. Melting points were determined by open air capillary with an X-6 melting point apparatus, Beijing Tech instrument Co. Ltd., and are uncorrected. Varian ^{1}H NMR spectra (400 MHz) and ^{13}CNMR spectra (100 MHz) were recorded in $CDCl_3$, DMSO-d_6, and mass spectra were determined on API-2000LCMS mass spectrometer, Applied Biosciences.

Synthesis of Impurity C. To a suspension of raltegravir (20 g, 0.045 mol) in acetonitrile (100 mL) was added 50% aqueous KOH solution (10 mL), and the reaction mixture was stirred at ambient temperature for 4−5 h. The solid was filtered and purified using flash chromatography to give impurity **C** (10 g, 99.44% purity) in 50% yield.

The compound was purified using The Reveleris Flash system on 330 g of prepacked 40 μ silica gel cartridge supplied by Grace-USA, the mobile phase was $CHCl_3$/methanol (9:1).

Mp: 292−296 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.19 (s, 1H), 10.38 (s, 1H), 9.85 (s, 1H), 9.41 (s, 1H), 9.06 (t, 1H), 7.36−7.4 (m, 2H), 7.15−7.19 (m, 2H), 4.51 (d, 2H, J = 6.32 Hz), 3.42 (s, 3H), 1.86 (s, 3H), 1.70 (s, 6H); IR 3273, 3007, 2947, 1728, 1670, 1354, 1223 cm^{-1}; MS (CI): calcd For $C_{20}H_{23}FN_6O_6$ (M + H)/z: 463.44, found: (M + H)/z: 463.1.

Synthesis of Impurity D. Compound **15**[4] (15 g, 0.044 mol) was added to a solution of triethyl amine (13.5 g, 0.134 mol) in dichloromethane (150 mL) under nitrogen atmosphere. The reaction mixture was cooled to 0−5 °C, and ethyl oxalyl chloride (9.3 g, 0.067 mol) was added dropwise. The conversion was monitored by TLC ($CHCl_3$/MeOH, 9:1); after TLC showed disappearance of **15**, 1 N HCl (100 mL) was added, and the organic layer was separated. The organic layer was washed with water, dried over sodium sulfate, and evaporated under reduced pressure to give a syrupy mass, which was treated with methanolic ammonia at 2−3 kg/cm^2 ammonia pressure in an autoclave for 5−6 h. After completion of the reaction, methanol was evaporated under reduced pressure to give impurity **D** (17 g, 97.04% purity) in 93% yield

Mp: 243−247 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.73 (br s, 1H), 9.08 (br s, 1H), 7.99 (br s, 1 H), 7.77 (s, 1H), 7.32−7.35 (m, 2H), 7.11−7.15 (m, 2H), 6.83 (br s, 1H), 4.46 (d, 2H, J = 5.64 Hz), 3.37 (s, 3H), 1.64 (s, 6H); IR 3667, 1682, 1346, 1223 cm^{-1}; MS (CI): calcd For $C_{18}H_{20}FN_5O_5$ (M + H)/z: 406.3, found: (M + H)/z: 406.3.

Synthesis of Impurity E. To a slurry of hydroxypyrimidone **6**[4] (45 g, 0.119 mol) and methanol (315 mL) was added benzyl amine (19.26 g, 0.179 mol) over 30 min. The resultant solution was aged at 60−65 °C for 4−5 h. The conversion was monitored by TLC ($CHCl_3$/MeOH, 9:1). After TLC showed the disappearance of **6**, the reaction mixture was cooled to 50−55 °C, and acetic acid (13.5 mL) was added. Water (360 mL) was added, and the precipitated solid was stirred at 25−35 °C for 30−45 min and filtered; the cake was washed with 1:1 of methanol/water (2 × 50 mL) and dried at 40 °C under reduced pressure to afford **16** (50 g, 98.42% purity) in 93% yield.

Mp; 178−183 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.94 (br s, 2H), 7.78 (br s, 1H), 7.39−7.26 (m, 10H), 5.00 (s, 2H), 4.60 (d, 2H), 3.64 (s, 3H), 1.66 (s, 6H); IR 3312, 1716, 1678, 1593, 1249 cm^{-1}; MS (CI): calcd For $C_{24}H_{26}N_4O_5$ (M + H)/z: 451.4, found: (M + H)/z: 451.2.

To a clean autoclave was charged compound **16** (49 g, 0.108 mol) in a mixture of 10% Pd/C (0.98 g), methanesulfonic acid (10.79 g, 0.114 mol), and methanol (392 mL). The content was hydrogenated at 50−55 °C for 2−3 h at 2 kg/cm^2 hydrogen pressure. The conversion was monitored by TLC ($CHCl_3$/MeOH, 9:1). After completion, the reaction mixture was cooled to 25−35 °C, and hydrogen was replaced with nitrogen. The Pd/C was removed by Celite filtration, and to the filtrate was added 1 N NaOH to adjust the pH 7−8. The precipitated amine was filtered and dried to yield 2-(2-aminopropan-2-yl)-*N*-benzyl-1,6-dihydro-5-hydroxy-1-methyl-6-oxopyrimidine-4-carboxamide (amine) (28 g, 98.22% purity) in 82% yield.

Mp: 182−185 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70 (br s, 1H), 7.34−7.21 (m, 5H), 6.23 (s, 1H), 4.50 (d, 2H), 3.76 (s, 3H), 3.33 (br s, 2H), 1.53 (s, 6H); IR 3036, 1620, 1572, 1533, 1305 cm^{-1}; MS (CI): calcd For $C_{16}H_{20}N_4O_3$ (M + H)/z: 317.3, found: (M + H)/z: 317.2.

A slurry of oxadiazole K salt **13**[4] (25.41 g, 0.153 mol) in acetonitrile (198 mL) and DMF (0.3 mL) was cooled to 0−5 °C, and oxalyl chloride (18.12 g, 0.142 mol) was added, keeping the internal temperature 0−5 °C under inert atmosphere. The slurry was aged for 1−2 h at 0−5 °C. A slurry of amine (22 g, 0.069 mol) and THF (594 mL) was cooled to 0−5 °C, and NMM (16.87 g, 0.167 mol)was added. The acetonitrile slurry of **14** was added slowly maintaining the reaction temlperature 0−5 °C. The slurry was maintained for 1 h, and 25% aqueous ammonia (13.2 mL) was added to adjust the pH 8−9. The reaction mixture was acidified to pH 2−3 by 2 HCl and evaporated under reduced pressure to give a syrupy mass. A mixture of isopropanol (154 mL) and water (528 mL) was added, and the precipitated solid was filtered and dried under vacuum to yield impurity **E** (13 g, 98.56% purity) in 46% yield.

Mp: 132−135 °C; ^{1}H NMR (400 MHz, DMSO-d^6) δ 12.24 (s, 1H), 8.85 (s, 1H), 9.06 (t, 1H), 7.24−7.35 (m, 5H), 4.54 (d, 2H, J = 6.44 Hz), 3.49 (s, 3H), 2.56 (s, 3H), 1.75 (s, 6H); IR 3385, 3244, 3059 and 2940, 1688 and 1674, 1605 and 1555, 1360 cm^{-1}; MS (CI): calcd For $C_{20}H_{22}N_6O_5$ (M + H)/z: 427.43, found: (M + H)/z: 427.2.

Synthesis of Impurity F. To a slurry of hydroxypyrimidone **6** (45 g, 0.119 mol) and methanol (315 mL) was

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added 4-methoxybenzyl amine (24.66 g, 0.179 mol) over 30 min. The resultant solution was aged at 60−65 °C for 4−5 h. The conversion was monitored by TLC ($CHCl_3$/MeOH, 9:1). After TLC showed disappearance of **6**, the reaction mixture was cooled to 50−55 °C, and acetic acid (13.5 mL) was added. Water (360 mL) was added, and the precipitated solid was stirred at 25−35 °C for 30−45 min and filtered; the cake was washed with a 1:1 mixture of methanol and water (2 × 50 mL) and dried at 40 °C under reduced pressure to afford **17** (53.3 g, 98.02% purity) in 93% yield.

Mp: 182−185 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.87 (br s, 2H), 7.70 (br s, 1H), 7.27−7.24 (m, 7H), 6.90−6.88 (d, 2H), 5.08 (s, 2H), 4.53 (d, 2H), 3.81 (s, 3H), 3.64 (s, 3H), 1.65 (s, 6H); IR 3362, 3331, 1712, 1676, 1535, 1251 cm^{-1}; MS (CI): calcd For $C_{25}H_{28}N_4O_6$ (M + H)/z: 481.5, found: (M + H)/z: 481.3.

To a clean autoclave compound **17** (53 g, 0.110 mol) was charged methanesulfonic acid (11.12 g, 0.115 mol) and methanol (424 mL) in a mixture of 10% Pd/C (1.06 g). The content was hydrogenated at 50−55 °C for 2−3 h at 2 kg/cm^2 hydrogen pressure. The conversion was monitored by TLC ($CHCl_3$/MeOH, 9:1). After completion, the reaction mixture was cooled to 25−35 °C, and hydrogen was replaced with nitrogen. The Pd/C was removed by Celite filtration, and to the filtrate was added 1 N NaOH to adjust the pH 7−8. The precipitated amine was filtered and dried to yield *N*-(4-methoxybenzyl)-2-(2-aminopropan-2-yl)-1,6-dihydro-5-hydroxy-1-methyl-6-oxopyrimidine-4-carboxamide (amine) (31 g, 98.62% purity) in 81% yield.

Mp: 190−194 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.50 (br s, 1H), 7.24−7.22 (d, 2H), 6.89−6.87 (d, 2H), 6.16 (s, 1H), 4.43 (d, 2H), 3.84 (s, 3H), 3.72 (s, 3H), 3.32 (br s, 2H), 1.52 (s, 6H); IR 3489, 3271, 1693, 1539, 1516, 1309, 1244 cm^{-1}; MS (CI): calcd For $C_{17}H_{22}N_4O_4$ (M + H)/z: 347.3, found: (M + H)/z: 347.2.

A slurry of oxadiazole K salt **13** (29.21 g, 0.176 mol) in acetonitrile (252 mL) and DMF (1.12 mL) was cooled to 0−5 °C, and oxalyl chloride (20.82 g, 0.164 mol) added, keeping the internal temperature 0−5 °C under inert atmosphere. The slurry was aged for 1−2 h at 0−5 °C. A slurry of amine (28 g, 0.08 mol) and THF (756 mL) was cooled to 0−5 °C, and NMM (19.39 g, 0.191 mol) was added. The acetonitrile slurry of **14** was added slowly, maintaining the reaction temperature 0−5 °C. The slurry was maintained for 1 h, and 25% aqueous ammonia (16.8 mL) was added to adjust the pH 8−9. The reaction mass was acidified to pH 2−3 by 2 N HCl (126 mL). The reaction mixture was distilled under reduced pressure, and a mixture of isopropanol (196 mL) and water (755 mL) was added. The precipitated solid was filtered and dried under vacuum to yield impurity **F** (25 g, 98.14% purity) in 67% yield.

Mp: 280−287 °C; ^{1}H NMR (400 MHz, DMSO-d^6) δ 11.56 (t, 1H), 9.79 (br s, 1H), 7.21 (d, 2H, J = 8.64), 6.86 (d, 2H, J = 8.64), 4.38 (d, 2H, J = 5.8), 3.72 (s, 3H), 3.39 (s, 3H), 2.56 (s, 3H), 1.70 (s, 6H); IR 2999, 1686, 1636 and 1570, 1350, 1032 cm^{-1}; MS (CI): calcd For $C_{21}H_{24}N_6O_6$ (M − H)/z: 455.45, found: (M − H)/z: 455.2.

Synthesis of Impurity G. Compound **15** (15 g, 0.044 mol) was added to a solution of triethyl amine (13.5 g, 0.134 mol) in dichloromethane (150 mL) under nitrogen atmosphere. The reaction mixture was cooled to 0−5 °C, and ethyl oxalyl chloride (9.3 g, 0.067 mol) was added dropwise. The conversion was monitored by TLC ($CHCl_3$/MeOH, 9:1), after TLC showed disappearance of **15**; 1 N HCl (100 mL) was added, and the organic layer was separated. The organic layer was washed with water, dried over sodium sulfate, and evaporated under reduced pressure to give impurity **G** (17 g, 99.05% purity) in 89% yield.

Mp: 176−180 °C; ^{1}H NMR (400 MHz, DMSO-d^6) δ 12.18 (br s, 1H), 9.46 (s, 1H), 9.05 (t, 1H), 7.37−7.40 (m, 2H), 7.14−7.19 (m, 2H), 4.50 (d, 2H), 4.20−4.25 (q, 2H), 3.45 (s, 3H), 1.68 (s, 6H), 1.26 (t, 3H); IR 2999, 1686, 1636 and 1570, 1350, 1032 cm^{-1}; MS (CI): calcd For $C_{20}H_{23}FN_4O_6$ (M + H)/z: 435.42, found: (M + H)/z: 435.2.

Synthesis of Impurity H. To a suspension of **13** (10 g, 0.060 mol) and DMF (0.5 mL) in DCM (50 mL) was added oxalyl chloride (9.1 g, 0.0723 mol) dropwise at 0−5 °C. *N*-Methylmorpholine (8.52 g) was introduced while maintaining the temperature between 0 and 5 °C. A solution of aminonitrile **2** (7.6 g, 0.0904 mol) in DCM (10 mL) was added dropwise at 0−5 °C. The reaction was monitored by TLC ($CHCl_3$: MeOH, 9:1). After completion of the reaction, salts were filtered, and the filtrate was completely evaporated under reduced pressure. To the resulting residue was added isopropanol (10 mL), and the mixture was again evaporated under reduced pressure. The residue was triturated with isopropanol (20 mL) to precipitate the solid which was filtered and dried under vacuum to give a pale-yellow compound **18** (8.3 g, 71%). Purity by HPLC > 99%, mp 142−145 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (br s, 1 H), 2.64 (s, 3 H), 1.84 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.7, 158.4, 152.8, 119.9, 47.4, 27.1, 11.4; MS (CI): Calcd for $C_8H_{10}N_4O_2$ m/z 195.1 (M + 1). Found: m/z 195.1.

[HPLC analysis; Phenomenex Gemini (4.6 mm × 150 mm) 3 μ, column; buffer: 0.01 M Na_2HPO_4 pH 3.0 by orthophosphoric acid, eluent: (A) Buffer: ACN (85:15), (B) ACN/MeOH/H_2O (55:10:35); flow rate: 1.5 mL/min; detector: 210 nm]

A mixture of the amide **18** (8 g, 0.0412 mol) and isopropanol (20 mL) was warmed to 60 °C, and then 50% (w/w) aqueous solution of hydroxylamine (3.3 mL, 0.0495 mol) was added over 25 min. The solution was heated at 60 °C for 30 min and cooled to 0−5 °C; heptane (20 mL) was added over 30 min. The resultant slurry was filtered, and the cake was washed with heptane (8 mL). The residue was dried at 45−50 °C under vacuum to afford compound **19** (9.2 g, 98%). Purity by HPLC > 97%, mp 175−178 °C; ^{1}H NMR (400 MHz, DMSO-d^6) δ 9.36 (br s, 1H), 8.75 (s, 1H), 7.43 (br s, 1 H), 5.58 (s, 2 H), 2.55 (s, 3 H), 1.55 (s, 6 H); ^{13}C NMR (100 MHz, DMSO-d^6) δ 165.6, 158.9, 155.1, 151.7, 54.9, 24.9, 10.7; MS (CI): Calcd for $C_8H_{13}N_5O_3$ m/z: 228.22 (M + H). Found: m/z: 228.1.

[HPLC analysis; Phenomenex Gemini (4.6 mm × 150 mm) 3 μ, column; buffer: 0.01 M Na_2HPO_4 pH 3.0 by orthophosphoric acid, eluent: (A) buffer/ACN (85:15), B) ACN/MeOH/H_2O (55:10:35); flow rate: 1.5 mL/min; detector: 210 nm]

A slurry of the amidoxime derivative **19** (9 g, 0.0396 mol) and methanol (63 mL) was cooled to 15−25 °C. DMAD (6.5 g, 0.0455 mol) was added over 30 min, maintaining the batch temperature between 15 and 25 °C. The resultant solution was aged at 25 °C for 2−3 h. The solution was concentrated under reduced pressure and solvent switched, by feed and bleed, at constant volume to xylenes (27 mL). The final batch temperature was maintained below 70 °C during the solvent switch. The mixture was heated to 125 °C for 2 h, raised to 135 °C for 6 h, and cooled to 60 °C. Methanol (7.7 mL) was added and stirred for 1 h, and the MTBE (28 mL) was introduced. The solution was cooled to 0−5 °C for 2 h, filtered, washed

with 9:1 of MTBE/methanol (2 × 16 mL), and dried to afford **20** (9 g, 66%). Purity by HPLC >97%, mp 234−237 °C; ^{1}H NMR (400 MHz, DMSO-d^6) δ 12.74 (s, 1 H), 10.35 (s, 1 H), 9.12 (s, 1 H), 3.81 (s, 3 H), 2.58 (s, 3 H), 1.59 (s, 6 H); ^{13}C NMR (100 MHz, DMSO-d^6) δ 166.6, 166.1, 160.2, 159.2, 153.2, 152.9, 145.6, 128.3, 56.6, 52.9, 26.2, 11.3; MS (CI): Calcd for $C_{13}H_{15}N_5O_6$ m/z: 338.29 (M + H). Found: m/z: 338.2.

[HPLC analysis: Phenomenex Gemini (4.6 mm × 150 mm) 3 μ, column; buffer: 0.01 M Na_2HPO_4 pH 3.0 by orthophosphoric acid, eluent: (A) buffer/ACN (85:15), (B) ACN/MeOH/H_2O (55:10:35); flow rate: 1.5 mL/min; detector: 210 nm]

To a mixture of hydroxypyrimidinone **20** (8.5 g, 0.025 mol) and DMSO (68 mL) was added a solution of $Mg(OMe)_2$ (4.35 g, 0.0504 mol) in MeOH (57 mL). Excess MeOH was then evaporated under vacuum at 40−45 °C. After cooling to ambient temperature, MeI (14.24 g, 0.1 mol) was added dropwise, and the mixture was stirred at 20−25 °C for 2 h, then at 60 ± 5 °C for 5 h and cooled to 20−25 °C. HCl (2 M, 85 mL) was added followed by 5 wt % sodium bisulfite (10 mL). Water (170 mL) was added over 40 min, and the slurry was stirred for 40 min; the resultant slurry cooled to 0−5 °C. The crystalline product was collected by filtration and washed with water (85 L). The product was dried under vacuum to give **21** (8 g, 98.5% purity) in 91% yield.

To a slurry of hydroxypyrimidone **21** (7 g, 0.019 mol) and methanol (50 mL) was added 4-fluorobenzyl amine (3.73 g, 0.029 mol) over 30 min. The resultant solution was aged at 60−65 °C for 4−5 h. The conversion was monitored by TLC ($CHCl_3$/MeOH, 9:1). After TLC showed the disappearance of **21**, the reaction mixture was cooled to 50−55 °C, and acetic acid (2 mL) was added. Water (77 mL) was added, and the precipitated solid was stirred at 25−35 °C for 30 to 45 min and filtered, the cake was washed with 1:1 of methanol/water (2 × 50 mL) and dried at 40 °C under reduced pressure to give impurity **H** (7 g, in 83% yield). Impurity **H** (7 g, 0.015 mol) was suspended in methanol (70 mL), and potassium *tert*-butoxide (1.76 g, 0.015 mol) was added at 0−5 °C; the reaction mixture was stirred for 2 h, filtered, and dried to give pure potassium salt of impurity **H** (7 g, 99.12% purity) 92% yield.

Mp: 277−281 °C; ^{1}H NMR (400 MHz, DMSO-d) δ 12.19 (br s, 1H), 9.78 (br s, 1H), 7.32−7.36 (m, 2H), 7.10−7.14 (m, 2H), 4.48 (d, 2H), 3.82 (s, 3H), 2.59 (s, 3H), 1.70 (s, 6H); IR 3323, 2992 and 2943, 1703, 1643 and 1556, 1348, 1223 cm^{-1}; MS (CI): calcd For $C_{20}H_{20}FKN_6O_5$ (M − k)/z: 445.1, (M + H)/z: 483.51, found: (M − k)/z: 445.1, (M + H)/z: 483.1

Synthesis of Impurity I. To a solution of oxalyl chloride (1.9 g, 0.014 mol) and *N*-methylmorpholine (1.7 g, 0.016 mol) in acetonitrile (100 mL) was added **15** (10 g, 0.029 mol) lot-wise over 30 min. The resultant solution was aged at ambient temperature for 8−10 h. The conversion was monitored by TLC ($CHCl_3$/MeOH, 9:1). After TLC showed the disappearance of **15**, the reaction mixture was cooled to 0−5 °C, and water (100 mL) was added; the precipitated solid was stirred at 25−35 °C for 30−45 min and filtered; the cake was washed with acetonitrile (2 × 50 mL) and dried at 40 °C under reduced pressure to give impurity **I** (15 g, 99.48% purity) in 72% yield.

Mp: 315−318 °C; ^{1}H NMR (400 MHz, DMSO-d^6) δ 12.11 (br s, 2H), 11.04 (br s, 2H), 9.21 (s, 2H), 7.35−7.31 (m, 4H), 7.14−7.10 (m, 4H), 4.45 (d, 4H), 3.35 (s, 6H), 1.64 (s, 12H); IR 3323, 2992 and 2943, 1703, 1643 and 1556, 1348, 1223 cm^{-1}; MS (CI): calcd For $C_{34}H_{36}F_2N_8O_8$ (M + H)/z: 723.71, found: (M + H)/z: 723.3

■ ASSOCIATED CONTENT

Supporting Information

Additional characterization data of **15**−**21** and impurities **C**−**I**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Pace, P.; Spieser, S.; Summa, V. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3865−3869.

(2) Croxtall, D. J.; Williamson, K, A.; Perry, C. M. *Drugs* **2008**, *68*, 131−138.

(3) Summa, V.; Petrocchi, A.; Bonelli, F.; Crescenzi, B.; Donghi, M.; Ferrara, M.; Fiore, F.; Gardelli, C.; Gonzalez Paz, O.; Hazuda, D. J.; Jones, P.; Kinzel, O.; Laufer, R.; Monteagudo, E.; Muraglia, E.; Nizi, E.; Orvieto, F.; Pace, P.; Pescatore, G.; Scarpelli, R.; Stillmock, K.; Witmer, M. V.; Rowley, M. J. *J. Med. Chem.* **2008**, *51*, 5843−5855.

(4) (a) Crescenzi, Benedetta, C.; Cristina, G.; Ester, M.; Federica, O.; Paola, P.; Giovanna, P.; Alessia, P.; Marco, P.; Michael, R.; Rita, S.; Summa, V.; Emanuela, N. U. S. Patent 7,169,780. 2007. (b) Belyk, K. M.; Morrison, H. G.; Jones, P.; Summa, V.; Cooper, V. B.; Mahajan, A. J.; Kumke, D. J.; Tung, H.; Wai, L.; Pruzinsky, V.; Pye, P.; Angelaud, R.; Mancheno, D.U. S. Patent 20060122205. 2006. (c) Humphrey, G. R.; Pye, P. J.; Zhong, Y. Li.; Angelaud, R.; Askin, D.; Belyk, K. M.; Maligres, P. E.; Mancheno, D. E.; Miller, R. A.; Reamer, R. A.; Weissman, S. A. *Org. Process Res. Dev.* **2011**, *15*, 73−83.

(5) *ICH Q3A Impurities in New Drug Substances*, R2; International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH): Geneva, Switzerland, October 2006; http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3A_R2/Step4/Q3A_R2__Guideline.pdf.

(6) *Quality Guidelines*; International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH): Geneva, Switzerland, http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html.

(7) Novak, T. J.; Grinberg, N.; Hartman, B.; Marcinko, S.; DiMichele, L.; Mao, B. *J. Pharm. Biomed. Anal.* **2010**, *51*, 78−83.

(8) Benedetta, C.; Cristina, G.; Ester, M.; Emanuela, N.; Federica, O; Paola, P.; Giovanna, P.; Alessia, P.; Marco, P.; Michael, R.; Rita, S.; Summa V. World Patent WO/03/035077, 2003.