Reaction of irbesartan with nitrous acid produces irbesartan oxime derivatives, rather than *N*-nitrosoirbesartan

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ABSTRACT

In October 2021, an India-based pharmaceutical company withdrew a number of batches of irbesartan and irbesartan hydrochlorothiazide finished products from the US market, due to the presence of unacceptable level of the "probably carcinogenic" nitrosamine "*N*-nitrosoirbesartan". Nevertheless, there is no revealing of the exact structure of this so called "*N*-nitrosoirbesartan". In the current study, we performed a set of 10 reactions of irbesartan with nitrous acid under various conditions and found no trace of this "*N*-nitrosoirbesartan" by a sensitive and accurate LC-MS method with limit of detection (LOD) of 30 ppb. With the use of LC-PDA/UV-high resolution MS[n] as well as 1D/2D NMR, the reaction products formed are found to be the two isomeric oxime derivatives of irbesartan, with the *Z*-isomer as the predominant product. Furthermore, no trace of this "*N*-nitrosoirbesartan" can be detected in representative commercial batches of irbesartan. Despite of the fact that *in silico* evaluation suggests that the two irbesartan oximes may be controlled as regular impurities, analysis of

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representative irbesartan commercial batches by the LC-MS method indicates that the oximes are not detected (LOD: 30 ppb).

1. INTRODUCTION

Irbesartan (Fig. 1), with its IUPAC name as 2-butyl-3-[[4-[2-(2H-tetrazol-5-yl)-phenyl]phenyl]methyl]-1,3-diazaspiro[4.4]non-1-en-4-one, is a potent and selective angiotensin II subtype 1 receptor antagonist or blocker. Hence it belongs to the family of ARB (angiotensin receptor blocker) drugs, which are also known as sartan drugs. Irbesartan is clinically indicated for treatment of hypertension, including patients with type 2 diabetes mellitus and nephropathy.[1] In mid-2018, *N*-nitrosodimethylamine (NDMA), an *N*-nitrosoamine impurity with probable carcinogenicity to human,[2] was found in another sartan drug, valsartan, which caused voluntary recalls of impacted valsartan products from the market.[3] Since that time, NDMA, as well as other *N*-nitrosoamine impurities, have been found in other categories of drugs beyond sartans, e.g., ranitidine and metformin drug products.[4] Regulatory agencies have since issued various guidance and regulatory documents to mitigate and reduce the potential risk associated with *N*-nitrosoamine impurities,[5] which are among the "cohort of concert" genotoxic impurities as defined in ICH M7.[6]

Most recently in October 2021, an India-based pharmaceutical company withdrew a number of batches of irbesartan and irbesartan hydrochlorothiazide finished products from the US market, due to the potential presence of unacceptable level of "*N*-nitrosoirbesartan".[7] Nevertheless, there is no revealing of the exact structure of

this so called "*N*-nitrosoirbesartan". Search of literature did not show any structure corresponding to "*N*-nitrosoirbesartan". On the other hand, a few websites for companies engaged in supplying pharmaceutical impurities listed the structure of "*N*-nitrosoirbesartan" as the one with the nitroso group attached to the tetrazole moiety of irbesartan (Fig. 1); nevertheless, no detailed structure characterization can be provided.[8] Under this background, we set out to determine if "*N*-nitrosoirbesartan" can be produced by reaction between irbesartan and nitrous acid (formed *in situ* between a nitrite salt and acid) and if yes, what its exact structure would be.

Through a systematic study in which various reaction conditions have been tried, we did isolate two products from the reaction of irbesartan and nitrous acid, with their molecular formulas consistent with that of "*N*-nitrosoirbesartan". Nevertheless, the two products are not "*N*-nitrosoirbesartan", but rather are irbesartan oximes (Fig. 1), which are isomeric to "*N*-nitrosoirbesartan". In this paper, we describe the details of the experimental design, isolation of the two products, their structural elucidation by LC-high resolution MS^n (n=1, 2), and 1D and 2D NMR. We also performed genotoxic risk assessment using the software CASE Ultra, developed by MultiCASE, and found that they can be classified as Class 5 impurities, indicating that they may be controlled as regular impurities.

2. RESULTS AND DISCUSSION

2.1. Original goal of the study for the reaction of irbesartan with nitrous acid

The news of the product recall by the India-based pharmaceutical company due to

the potential occurrence of "*N*-nitrosoirbesartan" in its irbesartan formulated products[7] prompted us to look into the possibility of if this "*N*-nitrosoirbesartan" could be present in our irbesartan product, despite of its being a remote possibility based on the risk assessment of our process chemistry.

2.2 Product distribution in the reaction of irbesartan with nitrous acid

In the first reaction of irbesartan with nitrous acid (generated *in situ* from sodium nitrite and HCl), 3.00 g irbesartan (7.00 mmol) was dissolved in 10 mL NMP. To this solution was added 0.300 g $NaNO_2$ (4.35 mmol) followed by addition of 2.0 mL concentrated hydrochloric acid (24 mmol) and 1.0 mL water. The reaction solution was allowed to stir at 40 ± 2°C for 18 h. LC-UV/PDA-high resolution MS^n (n=1, 2) analysis of the reaction solution indicated that two product peaks eluted at 22.6 and 27.0 min (Fig. 2), with their peak area percentages at 0.81% and 42.85%, respectively. It was found that the UV spectra of the 22.6 min and 27.0 min products are quite similar to that of irbesartan with three UV absorption peaks near 225, 255 and 280 nm, indicating that these three compounds have similar chromophores (Fig. S1; Supporting information).

2.3 High resolution mass spectrometric analysis of the two products formed in the reaction of irbesartan with nitrous acid

The accurate MS spectra of the minor 22.6 min product and major 27.0 min product displayed their protonated *m/z* values at 458.2322 and 458.2326 under ESI positive mode (Fig. S2), respectively, both matching a formula of $C_{25}H_{27}N_7O_2$ within an experimental error of 10 ppm. The results indicate that the minor 22.6 min and

major 27.0 min products are isomeric toward each other. By comparing with the formula of irbesartan, $C_{25}H_{28}N_6O$, the 22.6 min and 27.0 min products have one additional NO component and less one hydrogen atom. Hence, the formula of both products from the reaction of irbesartan with nitrous acid would be consistent with that of "*N*-nitrosoirbesartan". In order to further prove the identities of both products, MS/MS fragmentation experiments were performed on irbesartan and its two products.

The MS/MS spectra (Fig. 3) of both protonated 22.6 min and 27.0 min products (*m/z* 458) and irbesartan (*m/z* 429) did not show many fragment ions but exhibited three common fragment ions at *m/z* 180, *m/z* 207 and *m/z* 235 under the collision energy of 30 eV. These three ions are the specific fragments originating from the biphenyltetrazole moiety, and their occurrence is indicative of the presence of an unmodified tetrazole moiety. Hence, the presence of these three common fragment ions suggests that the 22.6 min and 27.0 min products are very likely to have the same core structure of biphenyltetrazole as irbesartan. In other words, it does not seem to be likely that the two products are "*N*-nitrosoirbesartan".

In order to determine the exact structures of the 22.6 min and 27.0 min products, it became necessary to isolate the two degradants for 1D and 2D NMR structural elucidation.

2.4. Structural elucidation of the 22.6 min and 27.0 min products by 1D and 2D NMR

The two degradants were isolated by preparative reversed phase HPLC, the

details of which are described in the experimental section. All the ^{1}H NMR (Fig. 4) and ^{13}C NMR (Fig. 5) data of irbesartan, 22.6 min and 27.0 min products are summarized in Table 1. The ^{1}H and ^{13}C chemical shifts of irbesartan presented are consistent with those reported by Bernhart et. al.[9] Like irbesartan, the ^{13}C NMR spectra of 22.6 min and 27.0 min products both displayed 21 carbon signals, the chemical shifts of which are quite similar to those of irbesartan, except for C-22 and, to a less degree, C-17. The C-22 signals of the 22.6 min and 27.0 min products are at 152.1 and 148.5 ppm, respectively, which are both quaternary carbons. On the other hand, the C-22 signal of irbesartan is a methylene carbon ($-CH_2-$) with a chemical shift at 27.5 ppm. These results suggest that the C-22 position of irbesartan was modified during its reaction with nitrous acid. Considering the fact that nitrous acid is an electrophilic reagent by virtue of its capability of donating $^+$NO ion and that the C-22 position of irbesartan is nucleophilic in its enamine form,[10] the initial reaction between irbesartan and nitrous acid would be for the C-22 position to launch a nucleophilic attack on the NO moiety of nitrous acid to form the carbon-based NO-substituted irbesartan intermediate, which would then rearrange into the two isomeric irbesartan oxime derivatives, i.e., the 22.6 min and 27.0 min products (Scheme 1). It is quite clear that the NMR results are consistent with the C-22 substituted oxime structures, rather than the corresponding C-22 nitroso derivative. Specifically, the C-22 chemical shifts of 152.1 and 148.5 ppm are consistent with the fact that they stem from the quaternary carbons of the oxime structures proposed. Furthermore, the absence of ^{1}H signals on C-22 positions of both the 22.6 min and

27.0 min products is also supportive toward the oxime structures. The driver for favoring the oximes may be attributable to the capability for the oximes to conjugate with the 3,5-dihydro-4-oxoimidazole ring.

The 2D NMR results are also consistent with the two oxime structures assigned. For example, the HMBC correlations of H-26 (=N-O-H, δ_H 11.6 ppm, 1H, s) with C-22 (δ_C 148.6 ppm) in the 22.6 min product and H-26 (=N-O-H, δ_H 12.1 ppm, 1H, s) with C-22 (δ_C 152.1 ppm) in the 27.0 min product (Fig. 6) indicate that both products are irbesartan oximes. In order to assign the configuration for the two isomeric irbesartan oximes, NOESY experiments were performed and a cross space correlation between H-26 (N-O-H, δ_H 12.1 ppm, 1H) and H-14 (δ_H 4.98, 2H, s) was present only in the 27.0 min product, indicating that the 27.0 min product is the *Z*-oxime derivative of irbesartan (Fig. 7).

2.5 Plausible fragmentation pathways of protonated irbesartan and its two oxime derivatives

With the structures of the two oximes unequivocally determined based on the results from the above LC-PDA/UV-high resolution MS^n and 1D/2D NMR experiments, plausible fragmentation pathways of the protonated irbesartan and its two oxime derivatives are proposed in Schemes 2 and 3, respectively.

2.6 Product distributions in the reactions of irbesartan with nitrous acid under various different conditions

In the first reaction between irbesartan and nitrous acid, no trace of so called "*N*-nitrosoirbesartan" could be found by the analysis of the reaction solution with the

LC-PDA/UV-high resolution MS method (refer to Section 4.4) under the single-ion-detection mode (SIM), which had a detection limit of 60 ppb. In order to explore the possibility that "*N*-nitrosoirbesartan" might be formed under different reaction conditions, an additional 9 sets of reactions were carried out. In these experiments, nitrous acid was generated from *in situ* reaction of $NaNO_2$ with either HCl, acetic acid, or phosphate buffer. The reaction parameters that were systematically varied include 1) molar ratios of $NaNO_2$ versus that of irbesartan (from 0.62 to 3.1), 2) pH of aqueous co-solvents (from <1 to 6.8), 3) reaction time (from 6 to 18 h), 4) reaction temperature (from 10 to 40°C), and 5) organic co-solvents (NMP or Xylene-NMP). Despite of all the efforts, no trace of "*N*-nitrosoirbesartan" could be found under all the above reaction conditions: under the SIM detection mode (LOD: 30 ppb) of an LC-MS method (refer to Section 4.5), the only reaction products that could be observed were the two oxime derivatives of irbesartan, with the predominant formation of the *Z*-isomer (yields varying between ~14% to 95%). The theoretically possible "*N*-nitrosoirbesartan" would be the one where the NO group is attached to the tetrazole moiety of the biphenyl group as speculated in several websites;[8] its non-existence in the reactions between irbesartan and nitrous acid is probably due to the poor nucleophilicity of the tetrazole moiety toward the electrophilic NO moiety. The details of the reactions of irbesartan with nitrous acid are summarized in Table 2.

2.7 Analysis of irbesartan commercial batches for irbesartan oximes and "*N*-nitrosoirbesartan"

Since the report of trace amount of NDMA in valsartan in July, 2018 and the

subsequent events related to ranitidine, metformin and other medicines, regulatory agencies have issued a number of guidelines and guidance documents, in which current limits for *N*-nitrosoamines are established.[5] For example, according to the assessment report of "*Lessons learnt from presence of N-nitrosamine impurities in sartan medicines*" issued by EMA (European Medicines Agency, Procedure number: EMA/526934/2019), *N*-nitrosoamine impurities with no PDE or AI data, or cannot be justified based on QSAR relationship, the maximum exposure of these impurities is limited to 18 ng/day.[5a] On the other hand, FDA set the limit for these *N*-nitrosoamine impurities at 26.5 ng/day based on the TD_{50} data of NDEA.[5b] As the maximum dosage of irbesartan for human is 300 mg/day,[11] the presence of "*N*-nitrosoirbesartan" would only be allowed at no more than 60 ppb (18 ng/day÷300 mg/day) in irbesartan commercial batches, if the more restrictive EMA limit of 18 ng/day is adopted. Hence, the LC-MS limit test method with SIM detection mode (refer to Section 4.5; LOD: 30 ppb, with irbesartan *Z*-oxime as the model reference compound) was employed to analyze a number of representative commercial batches of irbesartan. The results revealed neither presence of "*N*-nitrosoirbesaratan" nor irbesartan oximes, which is consistent with the risk assessment for our irbesartan chemical process.

2.8 *In silico* toxicological evaluation

Based on our study as presented above, there is a remote "theoretical" possibility that oxime might be present in irbesartan. Since the oxime moiety is not an obvious alerting structure,[12] in normal situation it may be controlled as a regular impurity. Nevertheless, due to the ramification from the NDMA and subsequent events, we

performed *in silico* toxicological evaluation of the irbesartan oximes identified in this study with the toxicological assessment software CASE Ultra (version: vl.8.0.2). The QSAR module calculated the probability for the oxime impurities being Ames positive was 15.2% (negative) using the model GT1_BMUT. Moreover, the impurities were predicted to be 36.4% (negative) in the reference database of the expert rule system GT_EXPERT. According to the algorithm of CASE Ultra, the calculated probability is lower than the model's classification threshold and not within the gray zone. Furthermore, the Konsolidator module of CASE Ultra suggested that the outcome was negative for the query chemicals. Based on the overall evaluation by CASE Ultra, irbesartan oximes can be justified as Class 5 substances, i.e., they may be controlled as regular impurities.

3. CONCLUSIONS

Through the systematic study of the reactions between irbesartan and nitrous acid, the reaction products formed are found to be the *E*-oxime and *Z*-oxime derivatives of irbesartan, with the *Z*-isomer as the predominant product. No trace of so called "*N*-nitrosoirbesartan" can be detected in the reaction solutions of irbesartan with nitrous acid or in commercial batches of irbesartan. Despite of the fact that *in silico* evaluation suggests that the two irbesartan oximes may be controlled as regular impurities, analysis of representative irbesartan commercial batches by a sensitive and accurate LC-MS method indicates that the oximes are not detected.

4. EXPERIMENTAL SECTION

4.1. General. Unless otherwise noted, all solvents and reagents for reactions were used without further purification. All solvents and reagents utilized in HPLC analyses were HPLC grade. All solvents and reagents employed in LC-MS analyses were of mass purity grade. ^{1}H and ^{13}C NMR and 2D NMR spectra of the compounds were acquired on an Agilent 400 MHz spectrometer at 25°C. ^{1}H and ^{13}C resonances were assigned and confirmed by results from the following 2D NMR experiments: gCOSY, gHSQC, gHMBC and NOESY. Chemical shifts are reported in ppm (δ units) relative to the internal standard of tetramethylsilane [$(CH_3)_4Si$, TMS] or residual signal of DMSO-d_5; coupling constants are reported in hertz (Hz). Multiplicities are as follows: s=singlet, d=doublet, t=triplet, q=quartet, dd=doublet of doublets, m=multiplet. Irbesartan (CAS# 138402-11-6) was a product of Huahai Pharmaceutical. Sodium nitrite (CAS# 7632-00-0), concentrated hydrochloric acid (37%, CAS# 7647-01-0), *N*-methyl pyrrolidone (NMP, CAS# 872-50-4), disodium hydrogen phosphate (Na_2HPO_4, CAS# 7558-79-4), sodium dihydrogen phosphate (NaH_2PO_4, CAS# 7558-80-7), and xylene were sourced from commercial vendors.

4.2. Reactions of irbesartan with nitrous acid under various different conditions. In a 20 mL of round bottom flask, 3.00 g of irbesartan (7.00 mmol) and 1.50 g sodium nitrite (21.7 mmol) were added into 10 mL solvent, followed by the addition of 3 mL of concentrated hydrochloric acid (36.0 mmol) or phosphate buffer (pH=4.0 or 6.8). The resulting solution was allowed to stir at 40±2°C or 10±2°C for 6 h or 18 h. After the end of the reaction, the reaction solution was either cooled or warmed to

reach room temperature, followed by analysis with either the HPLC method (Section 4.3) or LC-PDA/UV-high resolution-MS^n method (Section 4.4).

4.3. HPLC analysis for reaction solutions of irbesartan with nitrous acid. A Thermo Scientific Dionex Ultimate 3000 HPLC system was used, which was equipped with a PDA/UV detector and an Agilent Zorbax SB-C18 column (250×4.6 mm, 5 μm). The mobile phase system consisted of A (15 mM ammonium formate aqueous solution, adjusted to pH 3.0 with formic acid) and B (acetonitrile), with the gradient varied according to the following program: 0 min (25% B), 7 min (25% B), 28 min (60% B), 35 min (60% B), 37 min (25% B) and 45 min (25% B). The analyses were performed at a flow rate of 1.0 mL/min for 45 min and a column temperature of 30℃, with an injection volume of 10 μL. The wavelength was set at 220 nm and UV spectra were collected by the PDA/UV detector with a wavelength range of 200-400 nm.

4.4. LC-PDA/UV-QTOF-MS^n (n = 1, 2) analyses. An Agilent HPLC instrument (1260 series, Agilent Technologies, USA) interfaced to a quadrupole time-of-flight (Q-TOF) mass spectrometer (6545 series, Agilent Technologies, USA) was used for the LC-PDA/UV-MS^n (n = 1, 2) analysis of samples. The chromatographic conditions were the same as those of the HPLC method described in Section 4.3. The Q-TOF mass spectrometer was operated under positive ESI mode with the following source parameters: drying gas flow 10 L/min, drying gas temperature 320℃, nebulizer pressure 60 psi, source temperature 325℃, sheath gas temperature 350℃, sheath gas flow 12 L/min, nozzle voltage 600 V, fragmentor 60 V, skimmer 65 V and capillary

voltage 4.0 kV. The acquisition range of *m/z* value was 50 – 1,000 and for the MS^2 analyses, the collision energy was set to 30 eV.

4.5. LC-MS limit test method. An Agilent HPLC instrument (1260 series, Agilent Technologies, USA) interfaced to a single quadrupole mass spectrometer (6120 series, Agilent Technologies, USA) was used for the limit test of irbesartan oximes in irbesartan samples. A Thermo Accucore^{TM} Phenyl-Hexyl column (150×4.6 mm, 2.6 μm) was used. The mobile phase system consisted of A (0.1% formic acid in water) and B (acetonitrile), with the gradient varied according to the following program: 0 min (50% B), 7 min (50% B), 7.01 min (100% B), 15 min (100% B), 15.01 min (50% B) and 20 min (50% B). The analyses were performed at a flow rate of 0.8 mL/min for 20 min and a column temperature of 25°C, with an injection column of 5 μL. The LC-MS mass spectrometer was operated under positive ESI mode with the following parameters: scan mode SIM, SIM ion (*m/z* 458), drying gas flow 10 L/min, drying gas temperature 350°C, fragmentor 130 V, nebulizer pressure 60 psi, peak width 0.1 min, gain factor 1.0, and capillary voltage 3.0 kV. The acquisition range of *m/z* value was 50-1,000 and the mass acquisition time was 4.8~6.5 min.

The irbesartan *Z*-oxime was used as the reference sample. The signal of *m/z* 458, resulting from the protonated irbesartan oximes under ESI positive SIM mode, is employed as the semi-quantitation ion in the study. The concentration of the irbesartan sample solution was 10 mg/mL, while the concentration of irbesartan *Z*-oxime in the LOD solution was 0.3 ng/mL, which was 30 ppb relative to the sample concentration. Injection of the LOD solution yielded the peak of irbesartan *Z*-oxime at 5.292 min

with S/N of 11. The concentration of irbesartan *Z*-oxime in the "system suitability solution" was 0.6 ng/mL, which was 60 ppb relative to the sample concentration. The RSD of the six replicate injections of the "system suitability solution" was 8.2% (Table S1) and the average recovery of three spiked solutions at 60 ppb was 108.1%. These results indicated that the LC-MS limit test method is sensitive with adequate precision and accuracy.

4.6 Isolation of the 22.6 min and 27.0 min products. A Shimadzu preparative high performance liquid chromatography with a UV detector was used for isolation of the 22.6 min and 27.0 min products. A YMC-Pack ODS-AQ column (250×20 mm, 5 μm) was used. The mobile phase system consisted of A (15 mM ammonium formate aqueous solution, adjusted to pH 3.0 with formic acid) and B (acetonitrile), with the gradient varied according to the following program: 0 min (25% B), 7 min (25% B), 28 min (60% B), 35 min (60% B), 37 min (25% B) and 45 min (25% B). The analyses were performed at a flow rate of 19.0 mL/min for 45 min and the column temperature was ambient. The injection volume for the 22.6 min product was 2 mL, while the injection volume for the 27.0 min product was 3 mL. The wavelength was set at 220 nm. The fractions at 22.1~22.6 min for the 22.6 min product and 26.1~27.1 min for the 27.0 min product were collected. The fractions collected were pooled and then concentrated by lyophilization; approximately 20 mg of the 22.6 min product and 190 mg of the 27.0 min product were obtained with 85% and 97% HPLC purity, respectively, for NMR structural determination.

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Notes

The authors declare no conflict financial interest.

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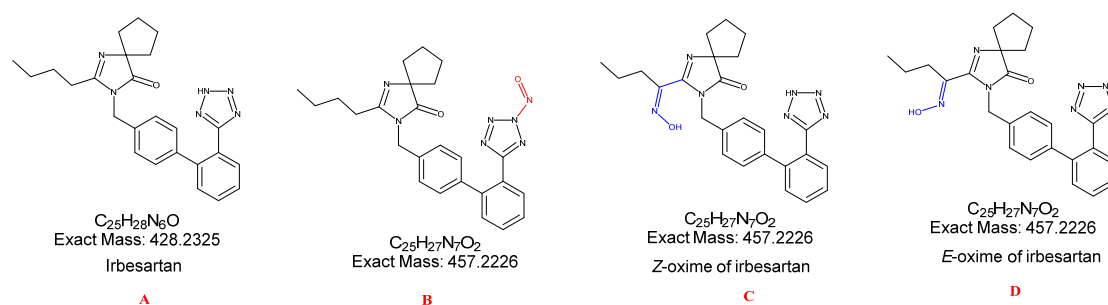
Fig. 1. Structures of irbesartan (A), the so called "*N*-nitrosoirbesartan" (B), and the two oxime derivatives of irbesartan (C and D).
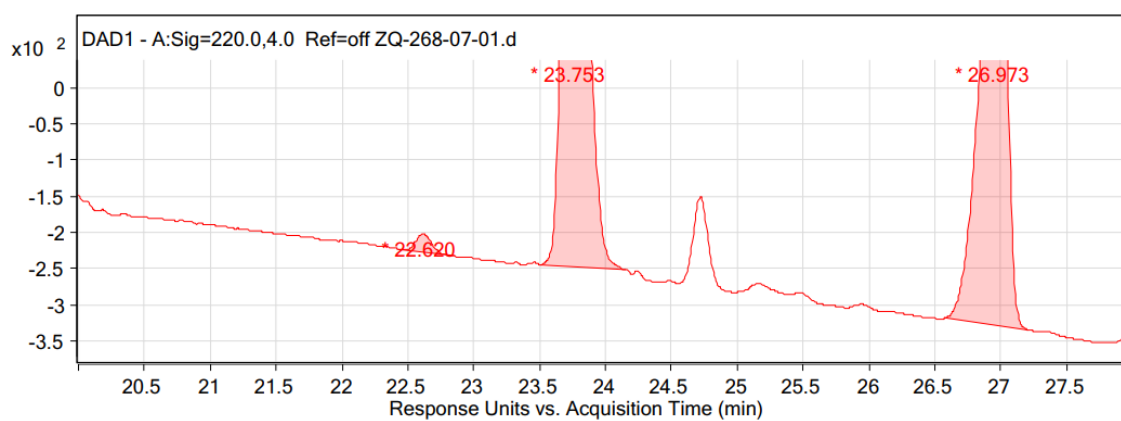

Fig. 2. HPLC chromatogram at 220 nm of the reaction solution of irebesartan and sodium nitrite in acidic condition. The two reaction products of irbesartan were observed at 22.620 min and 26.973 min.
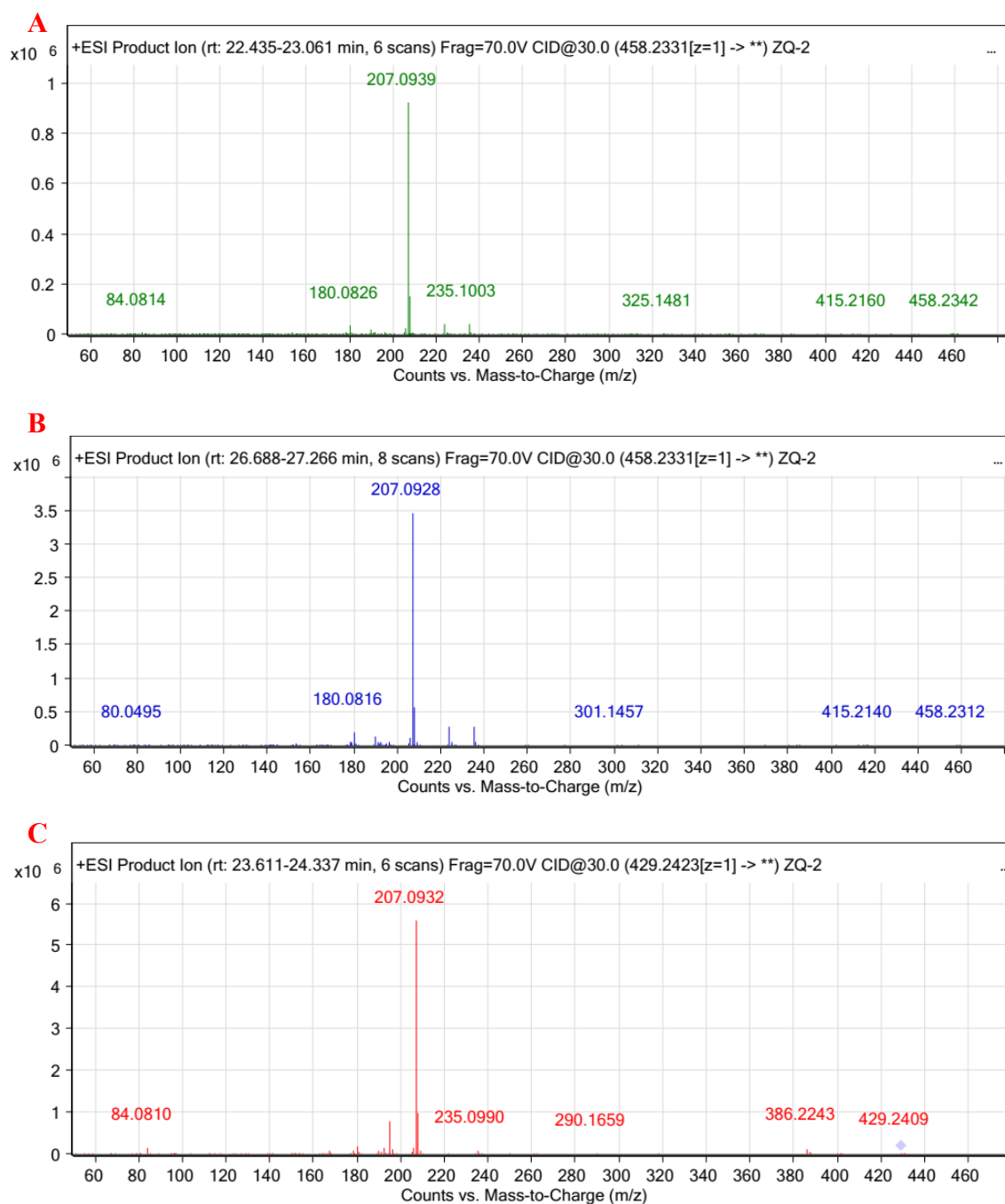
Fig. 3. LC-ESI-MS/MS spectra of the protonated ions of 22.6 min and 27.0 min products (both of the precursor ions shown at *m/z* 458, A & B) and protonated irbesartan (*m/z* 429, C) under the collision energy of 30 eV.
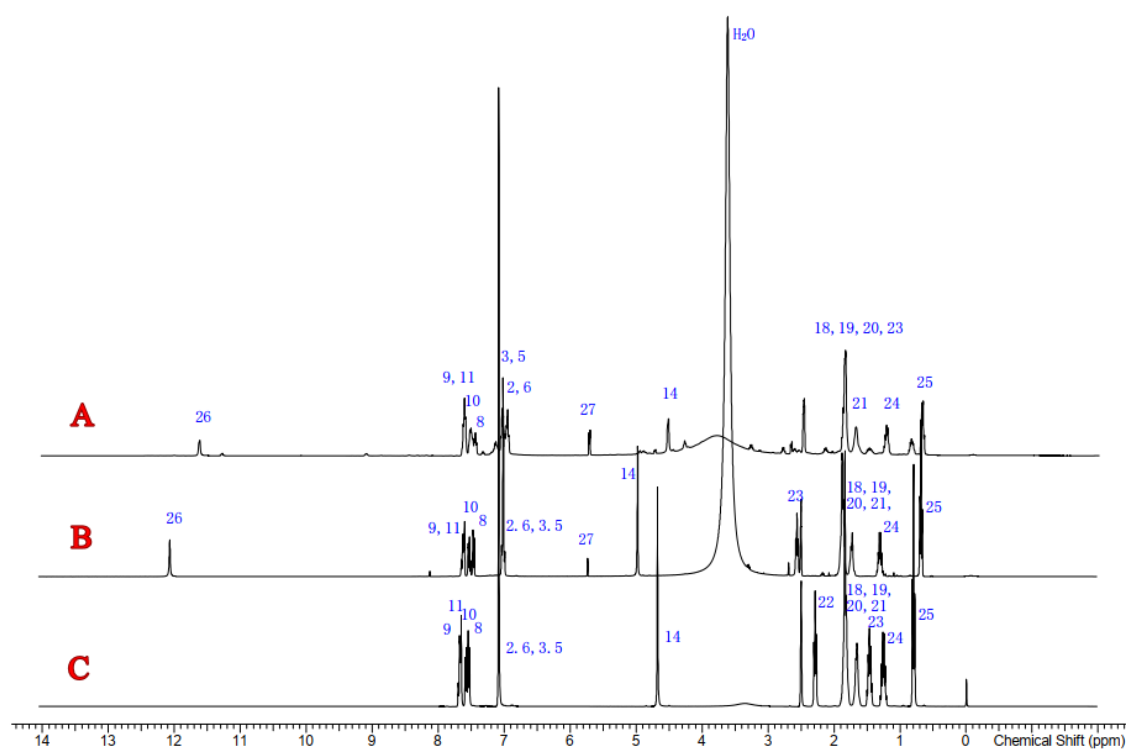
Fig. 4. Overlaid ^{1}H-NMR spectra of the 22.6 min product (A), 27.0 min product (B) and irbesartan (C).
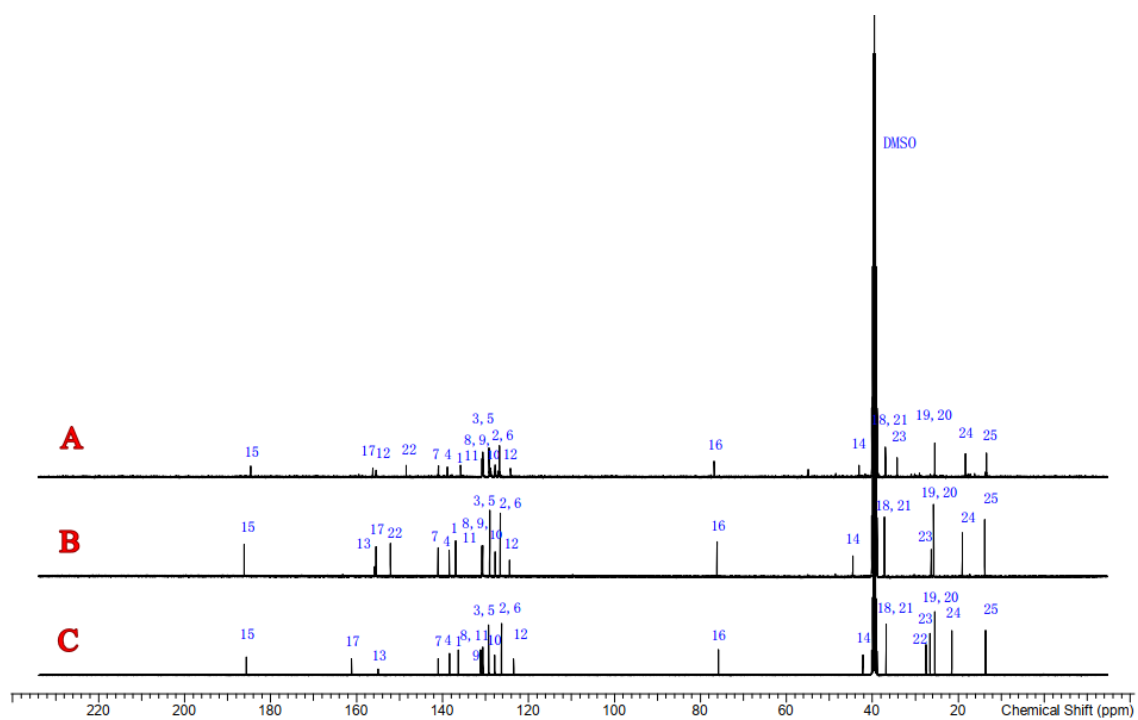

Fig. 5. Overlaid ^{13}C-NMR spectra of irbesartan *E*-oxime (22.6 min product, A), irbesartan *Z*-oxime (27.0 min product, B) and irbesartan (C).
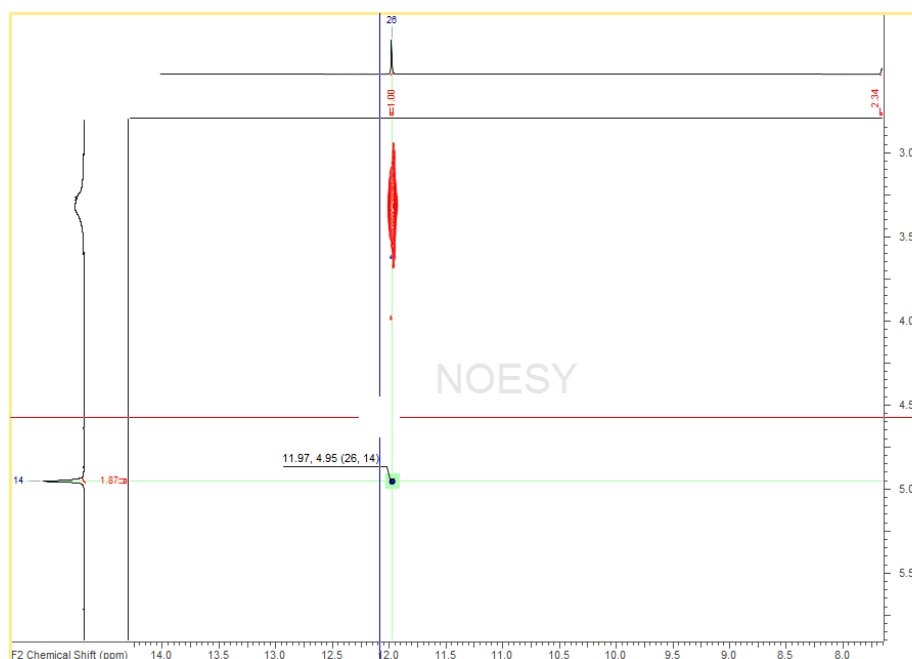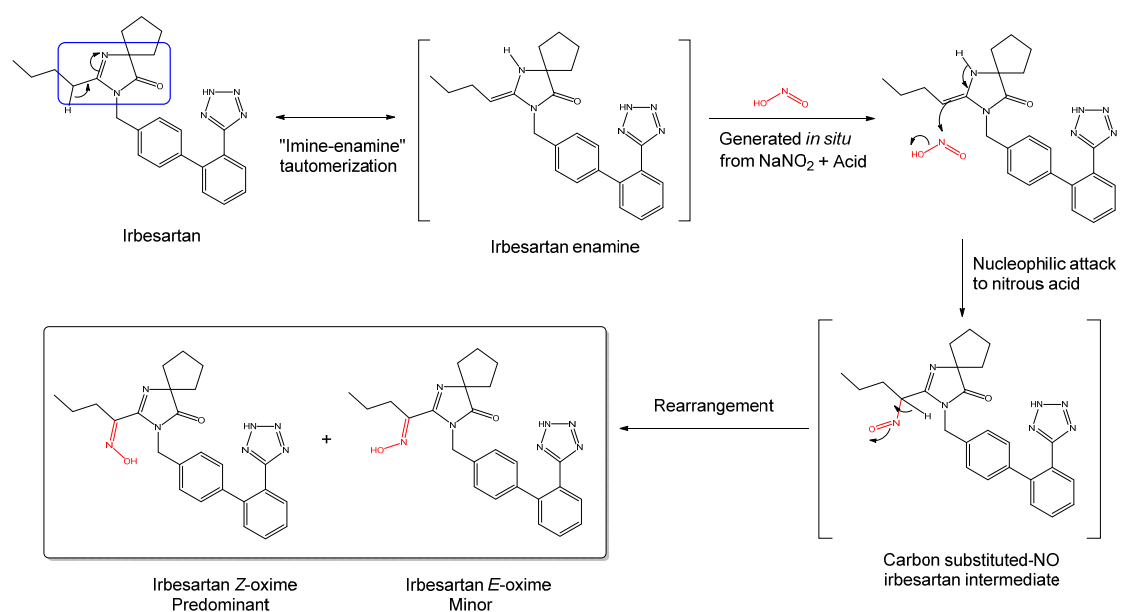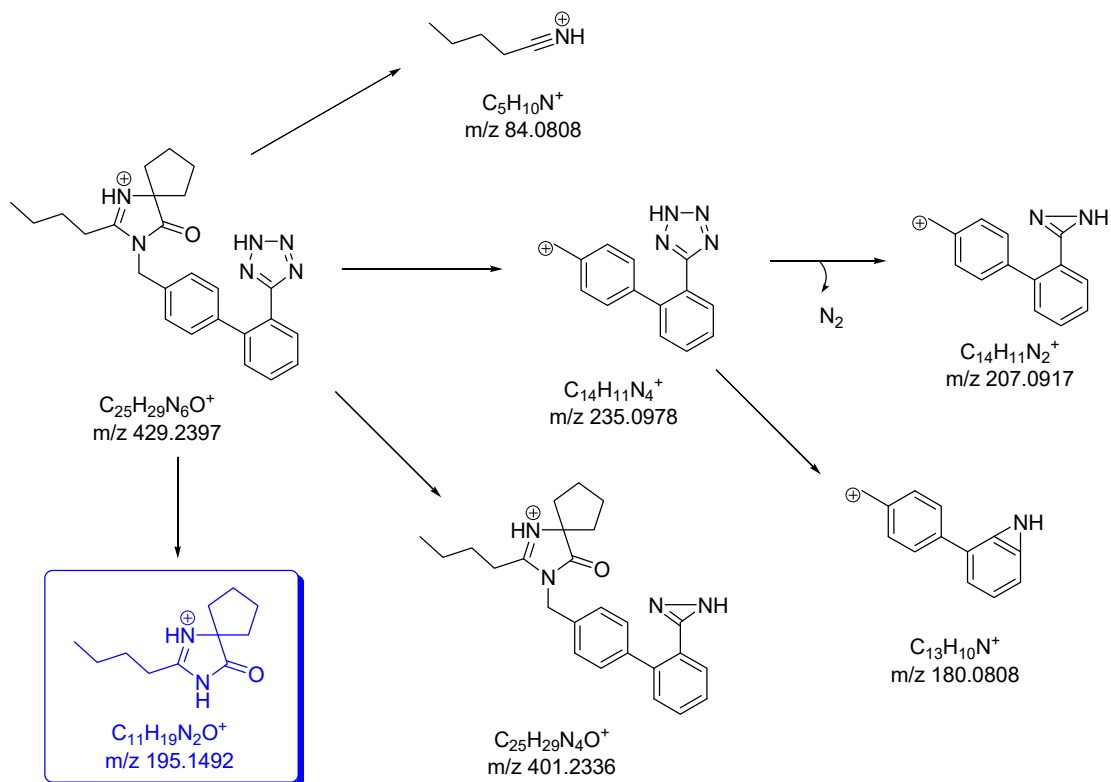
Fig. 6. A) Key HMBC correlations of irbesartan *E*-oxime (22.6 min product) and B) irbesartan *Z*-oxime (27.0 min product).
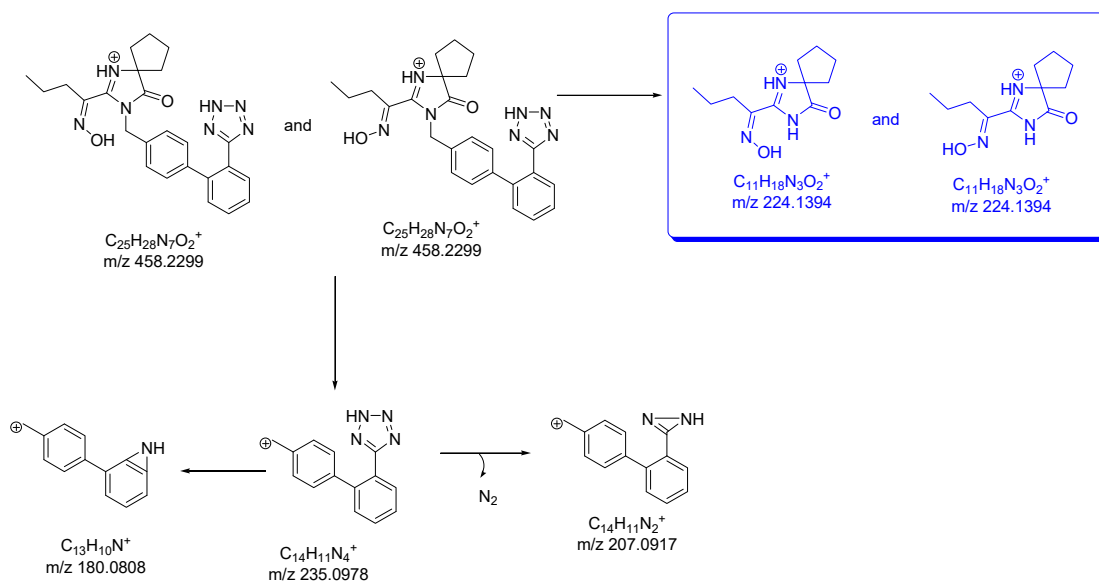
Fig. 7. Key NOESY correlation of irbesartan *Z*-oxime (27.0 min product).

Scheme 1. Proposed formation mechanism of the *E*- and *Z*-oximes of irbesartan in the reaction of irbesartan with nitrous acid.

Scheme 2. Proposed LC-MS/MS fragmentation pathway of protonated irbesartan.

Scheme 3. Proposed LC-MS/MS fragmentation pathway of protonated irbesartan oximes.

Table 1. ^{1}H and ^{13}C NMR spectroscopic data of irbesartan (DMSO-d_6), irbesartan *E*-oxime and irbesartan *Z*-oxime (DMSO-d_6, δ in ppm)

Position	Irbesartan ^{1}H shifts	Irbesartan ^{13}C shifts	Irbesartan *E*-oxime ^{1}H shifts	Irbesartan *E*-oxime ^{13}C shifts	Irbesartan *Z*-oxime ^{1}H shifts	Irbesartan *Z*-oxime ^{13}C shifts
1	-	136.3	-	135.8	-	136.9
2, 6	7.08	126.3	6.95 (2H, m)	126.7	7.01 (2H, m)	126.6
3, 5	7.08	129.3	7.03 (2H, m)	129.2	7.02 (2H, s)	129.0
4	-	138.3	-	138.9	-	138.4
7	-	141.0	-	141.0	-	141.1
8	7.54	130.6	7.44 (1H, m)	130.5	7.46 (1H, m)	130.7
9	7.68	131.1	7.60 (1H, m)	130.6	7.62 (1H, m)	130.8
10	7.57	127.9	7.51 (1H, m)	127.8	7.53 (1H, m)	127.8
11	7.66	130.6	7.60 (1H, m)	130.8	7.62 (1H, m)	130.7
12	-	123.4	-	124.2	-	124.4
13	-	155.0	-	155.5	-	155.8
14	4.67	42.2	4.51 (2H, s)	43.1	4.98 (2H, s)	44.5
15	-	185.7	-	184.6	-	186.1
16	-	75.8	-	76.8	-	76.2
17	-	161.1	-	156.2	-	155.5
18, 21	1.66, 1.82	36.8	1.66 (2H, m), 1.83 (2H, m)	37.0	1.72 (2H, d, *J*=7.83), 1.87, (2H, m)	37.2
19, 20	1.84	25.5	1.82 (4H, m)	25.5	1.88 (4H, m)	25.7
22	**2.29**	**27.5**	**-**	**148.5**	**-**	**152.1**
23	1.46	26.6	1.85 (2H, m)	34.2	2.56 (2H, br t, *J*=7.43,7.43 Hz)	26.3
24	1.25	21.5	1.20 (2H, m)	18.4	1.30 (2H, m)	19.1
25	0.80	13.7	0.66 (3H, m)	13.4	0.68 (3H, t, *J*=7.43,7.43 Hz)	13.8
NH	-	-	5.71 (1H, s)	-	-	-
N-OH	-	-	11.6 (1H, s)	-	12.07 (1H, br s)	-

Notes: 1) The numbering of the carbon skeleton in irbesartan, irbesartan *E*-oxime and irbesartan *Z*-oxime is shown in the structures below. 2) Abbreviations: N.A., not applicable; o, overlap signals; br s, broad singlet signal.
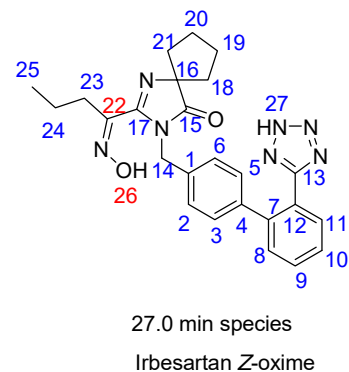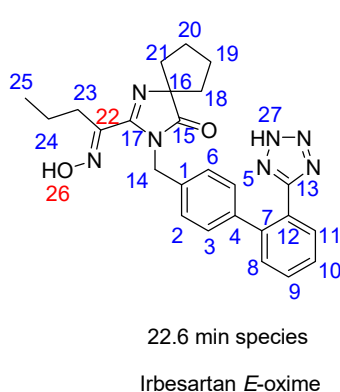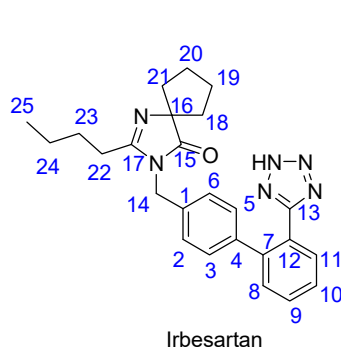

Irbesartan

22.6 min species

Irbesartan *E*-oxime

27.0 min species

Irbesartan *Z*-oxime

Table 2. Distribution of irbesartan *E*-oxime and irbesartan *Z*-oxime in reactions of irbesartan with nitrous acid under various different conditions.

Experiment	Molar ratio of NaNO$_2$	pH value	Reaction time (h)	Temperature (°C)	Solvent	Content of irbesartan *E*-oxime (%)	Content of irbesartan *Z*-oxime (%)
1	0.62 eq	<1.0	18	40±2	NMP	0.81	42.85
2	3.1 eq	<1.0	18	40±2	NMP	1.68	76.13
3	3.1 eq	<1.0	6	10±2	NMP	1.86	94.91
4	3.1 eq	<1.0	18	10±2	NMP	2.02	93.66
5	3.1 eq	<1.0	6	40±2	Xylene:NMP (7:3)	0.84	87.92
6	3.1 eq	<1.0	6	10±2	Xylene:NMP (7:3)	0.97	79.89
7	3.1 eq	4.0	6	40±2	NMP	N.D.	14.68
8	3.1 eq	4.0	18	40±2	NMP	0.72	41.55
9	3.1 eq	6.8	6	40±2	NMP	N.D.	14.45
10	3.1 eq	6.8	18	40±2	NMP	0.65	39.96