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Impurity profile study of repaglinide☆

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Abstract

Three unknown impurities and a byproduct in repaglinide bulk drug at levels below 0.1% (ranging from 0.05 to 0.1%) were detected by a simple isocratic reversed-phase high performance liquid chromatography (HPLC) method. These impurities were isolated from crude sample of repaglinide using reversed-phase preparative high performance liquid chromatography. Based on the spectroscopic data (IR, NMR and MS) the structures of these impurities (I, II and IV) and byproduct (III) were characterised as 4-carboxymethyl-2-ethoxy-benzoic acid (I), 4-cyclohexylaminocarbamoyl-methyl-2-ethoxy-benzoic acid (II), 1-cyclohexyl-3-[3-methyl-1-(2-piperidin-1-yl-phenyl)-butyl]-urea (IV) and 1,3-dicyclohexyl urea (III), respectively. Their synthesis and formation is discussed.
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1. Introduction

Repaglinide, (S)-2-ethoxy-4-[2-[[3-methyl-1-[2-(1-piperidinyl)phenyl]butyl]amino]-2-oxoethyl] benzoic acid, is a new carbomoxylmethyl benzoic acid derivative exerts its effects by binding to a site on the plasma membrane of beta cells, thereby closing the ATP-sensitive potassium channels [1,2]. Repaglinide, which is administered orally at meal-times, having short duration of action, reduces the fasting glucose concentrations in patients with type-2 diabetes mellitus [3,4].

During the analysis of different batches of repaglinide, four unknown impurities were detected whose area percentage ranged from 0.05 to 0.1%. A thorough study has been undertaken to isolate and characterise these impurities by spec-

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troscopic techniques. Keeping in view the stringent purity requirements from the regulatory authorities that all the impurities $\geq 0.1\%$ must be identified and characterised, this paper not only describes the isolation and characterisation of four impurities that are present at a level of $\leq 0.1\%$ in the bulk drug of repaglinide but also explains the formation of these impurities.

2. Experimental

2.1. Samples

The investigated samples of repaglinide bulk material (B.No. Rep-Pharma) and crude samples (B.No. Rep-Crude) were obtained from Dr. Reddy's Laboratories Ltd. Bulk Actives-III, Hyderabad, India.

2.2. High performance liquid chromatography (analytical)

A Waters Model Alliance 2690 Separation module equipped with a Waters 996 photo diode array UV detector was used. An in-house LC method was developed for the analysis of repaglinide and its intermediates, where a C18 column (Hichrom-RPB, 250 × 4.6 mm i.d., Hichrom Ltd., UK) with a mobile phase consisting of a mixture of 0.01 M KH_2PO_4 and acetonitrile in the ratio of 50:50 (v/v) (pH 3.5) was used with UV detection at 200 nm at a flow rate of 1.0 ml/min for the resolution of all impurities. The data was recorded using Waters Millennium 32 software. This LC method was able to detect these impurities which ranged from 0.05 to 0.1% in the presence of parent compound.

2.3. High performance liquid chromatography (preparative)

A Shimadzu preparative HPLC equipped with LC-8A pump, SCL-8A System controller, SPD-6AV UV-VIS detector, FCV-100B Fraction collector and Rheodyne Injector Model 7725i with 2.0 ml loop was used for isolating the impurities. The data was collected and processed using Shimadzu CR7A chromatopak integrator. A 250 × 10 mm i.d. column packed with 5 μm Hichrom-C18 (Hichrom Ltd., UK) was employed for the separation. The mobile phase consisted of 0.01 M KH_2PO_4 and acetonitrile in the ratio of 50:50 (v/v), (pH 3.5). The flow rate was set at 2.0 ml/min. Detection was carried out at 200 nm.

2.4. Mass spectrometry

Mass spectra were obtained using an HP5989 mass spectrometer with an electron energy set to 70 eV. The samples were introduced via the particle beam inlet using a LC pump (HP 1050 series) and a manual injector (Rheodyne model 7125i). The source manifold and quadrupole temperatures were maintained at 250 and 100 °C, respectively.

2.5. NMR spectroscopy

NMR measurements were performed on a 200 MHz instrument Varian Gemini 2000 model (both for ^1H and ^{13}C) at 25 °C in $CDCl_3$ and DMSO-d_6.

The ^1H chemical shift values were reported on the δ scale in ppm, relative to TMS ($\delta = 0.00$) and the ^{13}C chemical shift values were reported relative to $CDCl_3$ ($\delta = 77.0$ ppm) for impurities II and IV and DMSO-d_6 ($\delta = 39.5$ ppm) for impurities I and III as internal standards, respectively.

2.6. FT-IR spectroscopy

The IR spectra for impurity I, II, III and IV were recorded in the solid state as KBr dispersion using Perkin Elmer 1650 FT IR spectrophotometer.

2.7. Melting point determination

Melting points of all the impurities were determined on a Buchi digital melting point instrument Model .535.

2.8. Synthesis of repaglinide and impurities

Repaglinide and the impurities were synthesised as per the scheme shown in Fig. 1.

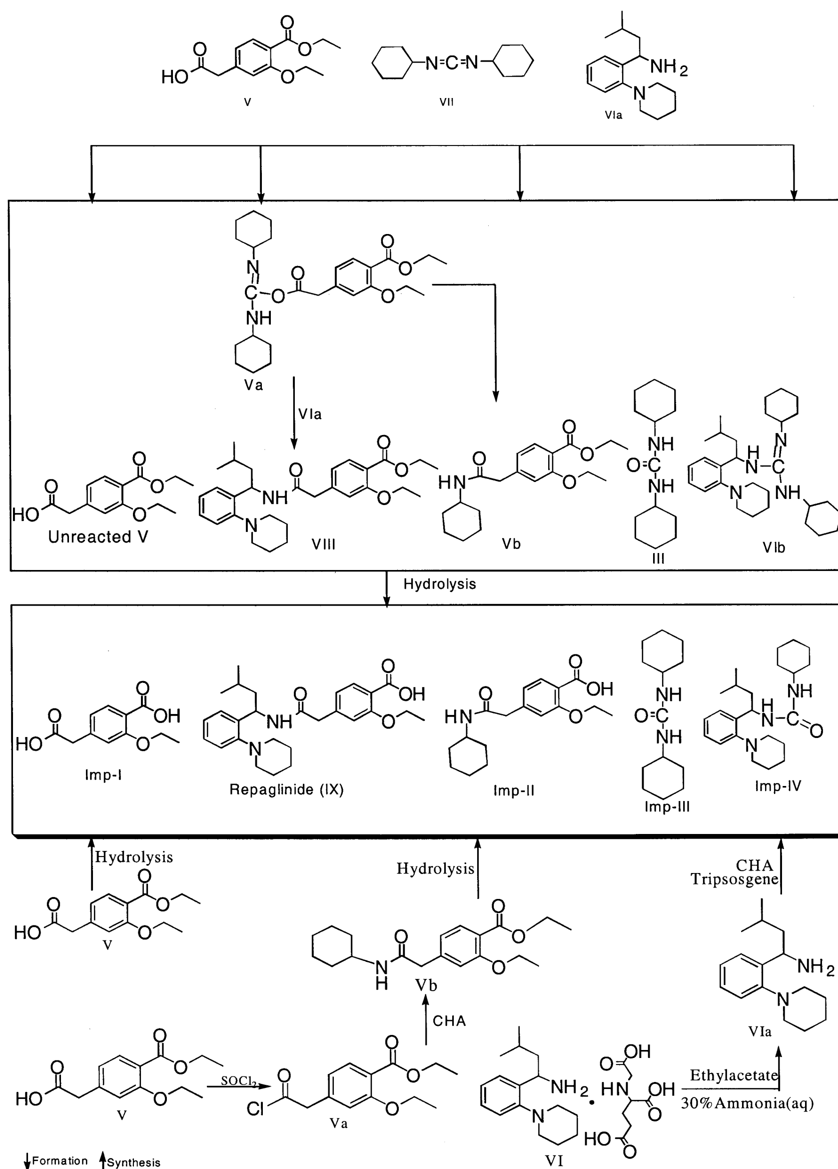

Fig. 1. Scheme for the synthesis and formation of repaglinide and impurities.

3. Results and discussions

3.1. Detection of impurities I, II, III and IV

A typical analytical LC chromatogram of a production batch of repaglinide bulk drug recorded using the LC method as described is shown in Fig. 2a. The target impurities under study are marked as Imp-I, Imp-II, Imp-III and Imp-IV. These impurities were isolated from the crude sample of repaglinide by preparative LC for spectroscopic studies.

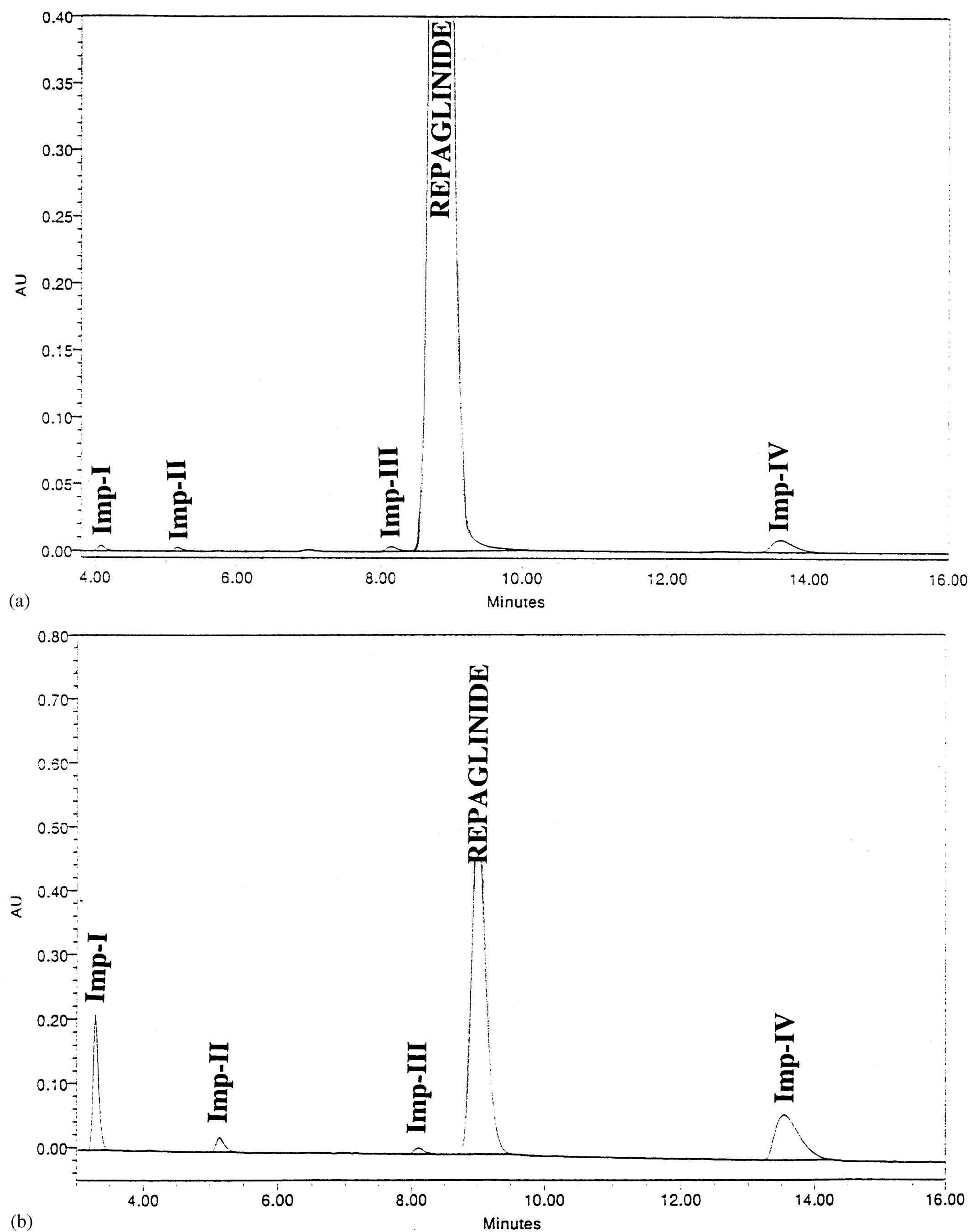

Fig. 2. (a) HPLC chromatogram of repaglinide bulk drug. (b) HPLC chromatogram of repaglinide bulk drug spiked with impurities.

3.2. Isolation of the impurities by preparative HPLC

A simple reversed-phase solvent system discussed under Section 2.3 was used for isolating these impurities. The retention times of repaglinide and impurities I, II, III and IV are 24–26, 10–12, 13–15, 17–19 and 30–32 min, respectively. Collected fractions of these impurities were pooled together and kept in the refrigerator. All the fractions of impurities isolated were concentrated under high vacuum on a Buchi Rotavapor Model R124 to strip off the organic solvent. The remaining aqueous layer was subjected to solvent–solvent extraction with chloroform to extract the compounds into the organic layer. The chloroform fractions ware pooled together and concentrated on Rotavapor under vacuum. The chromatographic purity of these impurities I, II, III and IV was tested separately before and after concentration in analytical mode (Section 2.2) and found to be 97.5, 99.2, 98.6 and 98.1%, respectively. The isolated solids obtained from concentrated fractions of impurities were used to generate spectral data. The details of the elucidation of structures of these impurities and their formation is presented in the following sections.

3.3. Structural elucidation of impurity I

The mass spectrum of impurity I exhibited a molecular ion at *m/z* 224 atomic mass units (amu). A signal at δ 12.4 ppm in the ^{1}H NMR spectrum, integrating for two protons was found to be exchangeable nature. A peak at 3251 cm^{-1} in the IR spectrum suggest that these two protons could be due to hydroxyl groups. IR peaks at 1734, and 1694 cm^{-1} and the ^{13}C signals at δ 167.6 and 172.6 ppm clearly indicates the presence of two carbonyl groups. Based on these preliminary spectral information the molecular formula of the impurity I could be $C_{11}H_{12}O_5$ with six double bond equivalents (DBE). This formula matched well with the observed molecular ion of 224 in the EI spectra.

The position of methylene group which yielded a singlet in the ^{1}H NMR spectrum was fixed based on the 1D-nOe experimental data. Based on these data, the structure of the impurity I has been characterised as 4-carboxymethyl-2-ethoxy-benzoic acid.

3.4. Structural elucidation of impurity II

The spectral data of impurity II was similar to that of impurity I. The mass spectrum of impurity II exhibited a molecular ion peak at *m/z* 305 amu which was 99 amu more than that of impurity I. The odd molecular ion of impurity II indicated the possible presence of odd number of nitrogen atoms. The ^{13}C NMR spectrum displayed signals due to 17 carbons and the DEPT spectrum displayed five negative signals due to the presence of seven methylene groups and five positive signals, one due to methyl and the rest three due to methine groups. The signals in the ^{1}H NMR spectrum at δ 5.5 and 12.4 ppm, correspond to one proton each, were found to be exchangeable in nature. One broad peak at 3387 cm^{-1} and a sharp peak at 3290 cm^{-1} indicate the presence of one hydroxyl and one amino group. The FT-IR spectrum also exhibited characteristic stretching bands at 1732 cm^{-1} due to carbonyl group and 1648 and 1544 cm^{-1} (amide band I and II, respectively) indicating the presence of an amide group in impurity II. The signals in the ^{13}C spectrum at δ 165.5 and 168.7 ppm clearly indicate the presence of two carbonyl groups. Out of these two, one carbonyl group is in the form of amide linkage. Further, the peaks at 1228 and 1176 cm^{-1} in the FT-IR spectrum are indicative of an aryl alkyl ether functionality. Based on this preliminary spectral data the molecular formula of impurity II could be $C_{17}H_{23}NO_4$ with seven DBE. This molecular formula matched well with the molecular ion observed at 305 amu in the EI spectra.

The signals in the aromatic region corresponding to three protons in the ^{1}H NMR spectrum and three aromatic methine signals in the ^{13}C spectrum indicated that impurity II has a tri-substituted aromatic system. Further, the ^{1}H NMR spectrum displayed a characteristic triplet and a quartet at δ 1.3 and 4.0 ppm, respectively, indicating the presence of ethoxy group. A singlet due to the presence of other methylene group was also observed in the ^{1}H NMR spectrum at δ 3.6 ppm.

The presence of signals in aliphatic region in the ^{1}H NMR spectrum indicated the incorporation of an aliphatic moiety in impurity II. This observation indicated the conversion of one of the hydroxyl groups in impurity I to amide which was attached to an aliphatic moiety in impurity II.

The ^{13}C spectrum of impurity II showed four additional signals when compared with the ^{13}C spectrum of impurity I. The DEPT spectrum of impurity II revealed the presence of three additional negative signals equal to five methylene groups and one additional positive signal due to methine group at δ 48.5 which were absent in that DEPT spectrum of impurity I. This observation indicated that the aliphatic moiety was a cyclohexyl ring attached to amide functional group.

Based on these data the structure of impurity II has been characterised as 4-cyclohexylaminocarbamoylmethyl-2-ethoxy-benzoic acid.

3.5. *Structural elucidation of impurity III*

Impurity III was found to be 1,3-dicyclohexyl urea (DCU) based on the spectral data.

3.6. *Structural elucidation of impurity IV*

The mass spectrum of impurity IV exhibited molecular ion at *m/z* 371 amu. The odd molecular ion indicated the presence of odd number of nitrogen atoms in impurity IV. The ^{13}C NMR spectrum displayed signals due to 23 carbons. The DEPT spectrum showed eight negative signals due to the presence of 11 methylene groups and nine positive signals, two signals due to methyl and the rest due to methine groups. The signals in the ^{1}H NMR spectrum at δ 4.4 and 6.6 ppm integrating for one proton each were found to be exchangeable in nature. A sharp peak at 3250 cm^{-1} indicated the presence of an amino group in impurity IV. The FT-IR spectrum also exhibited characteristic stretching bands at 1732 cm^{-1} due to a carbonyl group and 1624 and 1563 cm^{-1} (amide band I and II, respectively) indicating the presence of an amide group in impurity IV. The ^{13}C NMR spectrum displayed signals due to three quaternary carbons out of which one was due to the carbonyl group at δ 157.4 ppm. The signal at δ

Table 1
FT-IR, mass spectral and melting range data of impurities I, II, III and IV

Serial number	Compound	IR (KBr)	MS data	Melting point (°C)
1	Impurity I	3251 (O–H stretching), 1734 and 1694 (C=O stretching), 1526 (aromatic C=C stretching), 1219 and 1180 (C–O stretching)	*m/z* (EI; rel. int., %) 207 (26), 191 (32), 178 (90), 150 (33), 134 (12), 105 (100), M+ 224 (22)	139–141
2	Impurity-II	3287 (NH stretching), 2932 (aromatic C–H stretching), 2854 (aliphatic C–H stretching), 1732 (C=O stretching), 1648 and 1544 (amide stretching), 1228 and 1176 (C–O stretching)	*m/z* (EI; rel. int., %) 288 (6), 180 (64), 162 (100), 134 (21), M+ 305 (12)	135–138
3	Impurity-III	3328 (NH stretching), 2851 (aliphatic C–H stretching), 1752 (C=O stretching), 1628, 1572 (amide stretching), 1243 (C–O stretching).	*m/z* (EI; rel. int., %) 143 (59), 99 (100), M+ 224 (46)	221–224
4	Impurity-IV	3252 (NH stretching), 2932 (aromatic C–H stretching), 2853 (aliphatic C–H stretching), 1732 (C=O stretching), 1624 and 1563 (amide stretching), 1220 (C–O stretching)	*m/z* (EI; rel. int., %) 328 (18), 245 (94), 228 (100), 203 (25), 186 (56), 172 (90), 160 (25), 144 (25), 130 (54), 118 (31), 106 (30), M+ 371 (16)	199–203

54.9 ppm in the ^{13}C NMR and DEPT spectra due to two methylene groups indicates that these two methylene groups are attached to nitrogen. Further, the exchangeable protons are due to the presence of two NH groups.

Based on these preliminary data the corresponding molecular formula of impurity IV could be $C_{23}H_{37}N_3O$ with seven DBE.

The ^{1}H NMR spectrum of impurity IV showed signals integrating for four protons in the aromatic region indicated the presence of di-substituted aromatic system. This observation along with the additional quaternary carbon signal in the ^{13}C NMR spectrum and additional methylene and methine signals in the DEPT spectrum of impurity IV clearly indicated the possible presence of a cyclohexylamino carbonyl group. Based on these data, the structure of impurity IV was characterised as 1-cyclohexyl-3-[3-methyl-1-(2-piperidin-1-yl-phenyl)-butyl]-urea.

The spectral data of isolated impurities and impurities obtained by synthesis are in good agreement.

The synthetic standards of impurities I, II, III and IV were co-injected on LC with repaglinide and the area percentage at retention times 3, 5, 8 and 13 min were enhanced and the LC chromatogram is shown in Fig. 2b.

The FT-IR, mass spectral and melting range data for impurities I, II, III and IV are shown in Table 1. The structures of impurities I, II, III and IV are shown in Fig. 1.

3.7. Formation of impurities

The condensation of V and VIa in the presence of coupling reagent DCC (VII) yields repaglinide ester (VIII) through an O-acyl derivative (Va). The ester (VIII) upon hydrolysis yields repaglinide (IX). The inter conversion of O-acyl derivative (Va) may form an N-acyl derivative (Vb) which on further hydrolysis yields impurity II.

The likely mechanism for the formation of impurity IV can be explained in terms of coupling between VIa and VII to yield VIb which on hydrolysis yields impurity IV. The presence of unreacted V in repaglinide ester (VIII) may undergo hydrolysis during the reaction which leads to the formation of impurity I. The impurity III may be due to the presence of traces of a by product from DCC (VII) in the coupling reaction.

The scheme for the formation of impurities is shown in Fig. 1.

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