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Review article

A critical review of Roxadustat formulations, solid state studies, and analytical methodology

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ABSTRACT

This review aims to collate information about the analytical methodologies, bioanalytical methodologies, pharmaceutical formulations, solid-state studies, and the current and future market scenario for a relatively new class of drugs, Roxadustat. Roxadustat is a hypoxia-inducible factor propyl hydroxylase inhibitor that significantly increases blood hemoglobin via the action of transcriptional activator HIF. As the molecule has a promising role in stimulating erythropoiesis, it is considered an ideal therapeutic agent for patients with anemia. In the current review, an attempt has been made to compile the pharmacological, pharmacokinetic, and pharmacodynamic characteristics of Roxadustat and systematically present product development data. This drug has several polymorphs of cocrystal, co-former, and salt, which have been explained in detail in the current work. The comprehensive review summarizes all the chromatographic methods and is presented in table form. This review has extensively covered Liquid chromatography-tandem mass spectrometry methods used to analyze Roxadustat in the biological matrix. The literature needs more data on forced degradation study, impurity profiling, gas chromatography, analytical methods for assay, dissolution, and different formulation aspects of Roxadustat.

1. Introduction

Anemia is a clinical condition where the patient lacks adequate healthy red blood cells to carry acceptable oxygen levels to the body tissues [1]. Anemia can be a result of chronic kidney disease (CKD) due to impairment of multiple biological processes like irregular iron metabolism, inflammation, dysfunction of cascade pathway of synthesis of red blood cells, oxidative stress, erythropoietin (EPO) deficiency, blood loss, infection, and inadequate supply of nutrients [2]. Comparatively, EPO deficiency is the primary cause of anemia. EPO synthesis occurs in peritubular interstitial fibroblast cells located in the kidney. In CKD, renal EPO-producing cells get dysfunctional and transdifferentiate into myofibroblasts which leads to a decrease in the synthesis of EPO, thereby resulting in the progressive development of anemia. Roxadustat is a hypoxia-inducible prolyl hydroxylase inhibitor with a proven promising erythropoiesis-stimulating ability. It causes an increase in blood oxygen-carrying capacity in patients with chronic kidney disease with anemia [3–5]. Generally, the drug is administered orally (50 mg/dose) thrice a week to anemic patients. The dose can vary according

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Abbreviations

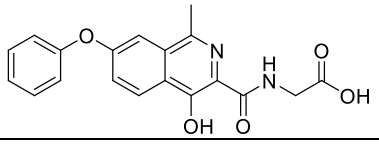
LC-MS	Liquid chromatography-mass spectrometry
API	Active pharmaceutical ingredient
CKD	Chronic kidney disease
EPO	Erythropoietin
NDD	Non-dialysis dependent
DD	Dialysis-dependent
HIF-PHD	Hypoxia-Inducible-Factor-Prolyl-Hydroxylase
CDSCO	Central Drugs Standard Control Organisation
DMFs	Drug master file
DMSO	Dimethyl sulfoxide
DSC	Differential Scanning Calorimetry
WADA	World Anti-Doping Agency
QuEChERS	Quick Easy Cheap Effective Rugged Safe

to the patient's condition, but it should not exceed 3.0 mg/kg. However, Roxadustat is currently used under the brand Evrezo in Chile, South Korea, and parts of China and Japan for treating anemia in CKD non-dialysis-dependent (NDD) and dialysis-dependent (DD) adult patients. The European Medicines Agency has accepted the application for the sale of Evrezo in European countries. Furthermore, AstraZeneca, FibroGen, and other pharmaceutical companies are aggressively trying to commercialize Roxadustat in Europe, Turkey, Russia, the Commonwealth of the Independent States, the Middle East, and South Africa [6].

There exist reports stating the misuse of Roxadustat by sports personnel owning to its erythropoietic properties apart from its use for the treatment of anemia [7]. Subsequently, a suitable quantitative and qualitative analysis method is required to identify Roxadustat and its metabolites in biological fluids. Furthermore, developing an analytical methodology for identifying impurities is necessary for every new drug candidate. Inclusive impurity profiling and regulatory requirements are essential for understanding the safety and efficacy of pharmaceutical products. Similarly, forced degradation studies are necessary to access information on drug stability under various degradation conditions. The current review presented a comprehensive overview of the status of analytical methods, pharmaceutical formulations, solid-state studies, and market scenarios related to Roxadustat. In addition, the review encompassed all the physicochemical properties of Roxadustat, which would play a pivotal role in formulation establishment.

The prevailing article further emphasized that many polymorphs, cocrystals, salts, and co-formers are listed along with the analytical results. In addition to their clinical applications, pharmacokinetic and pharmacodynamic parameters have also been considered. An overview of all the analytical methodologies, such as Chromatography and LC-MS, has been provided. To the best of our knowledge, only a few articles were published on bioanalytical methods for analyzing Roxadustat from biological matrices such as urine and plasma using chromatography. There are very few articles for the analysis of API and formulation related to Roxadustat. Further, various in vitro studies were reported in the literature involving liver microsomes, human hepatocytes, equine liver microsomes, *Cunninghamella elegans*, and S9. To give a clear perspective on the market and regulatory status of Roxadustat, we have covered all the current patent-related information.

Table 1
Physicochemical properties of Roxadustat.

Chemical Structure of Roxadustat	
IUPAC name	(4-hydroxy-1-methyl-7-phenoxyisoquinoline-3-carbonyl) glycine 2-[(4-hydroxy-1-methyl-7-phenoxyisoquinoline-3-carbonyl) amino] acetic acid
Appearance	White to Pale Green Solid
Chemical Formula	$C_{19}H_{16}N_2O_5$
Molecular Weight	352.3460
m/z:	352.1059 (100.0%), 353.1093 (20.5%), 354.1126 (2.0%), 354.1102 (1.0%)
CAS No.	808,118-40-3
Melting range	199–215 °C
Solubility	Slightly in water, 30 mg/mL DMSO, Ethanol
LogP	3.13, 1.85
pKa (Strongest Acidic)	2.75
pKa (Strongest Basic)	3.84
Index of Refraction	1.674

2. Global regulatory and intellectual property status

Roxadustat, a Hypoxia-Inducible-Factor-Prolyl-Hydroxylase (HIF-PHD) enzyme inhibitor, was developed by FibroGen in collaboration with Astellas and AstraZeneca. FibroGen holds several patents on this drug in the US and some in China. This molecule was first approved for anemic treatment in chronic kidney disease patients in China in December 2018 [8]. Japanese authorities approved its use in 2019 for treating anemic patients with chronic kidney disease who are on a dialysis cycle and in 2020 for patients without dialysis [9]. In April 2021, CDSCO granted permission to import and market Roxadustat in India to treat anemia. The European Medicines Agency approved the use of the Roxadustat drug in August 2021 [10].

3. Market status

8 API suppliers for Roxadustat are listed on the international platform, with 2 US DMFs filed. The reference price of the drug in USD/kg is $13,200. Dr. Reddy's Laboratories Ltd. is one of the API suppliers with US DMFs filed. Other API suppliers include Suzhou Biosyntech, Visit Pharmaceuticals, Mylan Inc., Sichuan Renan Pharmaceuticals, Hangzhou Longshine Biotech Co., Ltd., and Teva API Ltd [11].

4. Physicochemical properties

The physicochemical properties of Roxadustat are summarized in Table 1. The drug is a white to pale green solid and exhibits solubilities in organic solvents like DMSO (30 mg/mL) and DMF (50 mg/mL), and water (0.1 mg/mL). Roxadustat is an N-acyl glycine analog of 4-hydroxy-1-1methy-7-phenoxyisoquinoline-3-carboxylic acid. This molecule possesses relatively high permeability with low solubility. This active pharmaceutical ingredient has both acidic and basic functionalities. Therefore, Roxadustat ionization can be controlled by both acidic as well as basic solutions. The ionized form of the molecule shows much higher water solubility; however, the neutral state is lipophilic with increased membrane permeability [11]. A hydrophobic pocket in the enzyme Hypoxia-inducible factor-PHI(Propyl hydroxylase) is essential for the binding of Roxadustat [12]. Roxadustat exhibits more than one crystalline form having different physical properties. The crystalline forms of a drug can also show different stability behaviors and bioavailability.

5. Solid-state characterization

The solid-state properties of a compound are crucial in the field of pharmaceutical development. A drug molecule or compound may exist as a co-former, cocrystal, salt, or amorphous state. Each separate crystalline form has its physical, chemical, and physicochemical properties, such as dissolution rate, vapor pressure, melting point, solubility, hygroscopicity, density, stability, and particle shape. Solid forms may differ in significant ways, leading to the generation of different pharmaceutical products. As a point of formulation development and regulatory concern, it is vital to have a dosage form that meets the safety and efficacy with enhanced pharmacokinetic and dynamic properties. We have attempted to enumerate all the possible forms of compounds in our present review.

Table 2
Solid-state properties.

Compound	Form	X-ray powder diffractogram	DSC
Compound A Form A	[(4-hydroxy-l-methyl-7-phenoxy- isoquinoline-3-carbonyl)-amino] -acetic acid	8.5, 16.2, and 27.4 °2Θ ± 0.2 °2Θ	endotherm at 223 °C
Compound A Form B	[(4-hydroxy-l-methyl-7-phenoxy- isoquinoline-3-carbonyl)-amino] -acetic acid hemihydrate	4.2, 8.3, and 16.6 °2Θ ± 0.2 °2Θ	222 °C
Compound A Form C	[(4-hydroxy-l-methyl-7-phenoxy- isoquinoline-3-carbonyl)-amino] -acetic acid hexafluoropropylene-2-ol solvate	4.5, 13.7, and 16.4 °2Θ ± 0.2 °2Θ	222 °C
Compound A Form D	[(4-hydroxy-l-methyl-7-phenoxy- isoquinoline-3-carbonyl)-amino] -acetic acid DMSO: water solvate	8.4, 8.5, and 16.8 °2Θ ± 0.2 °2Θ	222 °C
Compound A sodium salt	[(4-hydroxy-l-methyl-7-phenoxy- isoquinoline-3-carbonyl)-amino] -acetic acid sodium salt	5.3, 16.0, and 21.6 °2Θ ± 0.2 °2Θ	314 °C
Compound A L-arginine salt)	[(4-hydroxy-l-methyl-7-phenoxy- isoquinoline-3-carbonyl)-amino] -acetic acid L-arginine salt	20.8, 21.8, and 25.4°2θ ± 0.2 °2Θ	210 °C
Compound A L-lysine salt	[(4-hydroxy-l-methyl-7-phenoxy- isoquinoline-3-carbonyl)-amino] -acetic acid L-lysine salt	19.8, 20.7, and 21.2°2θ ± 0.2 °2Θ	237 °C.
Compound A ethanolamine salt	[(4-hydroxy-l-methyl-7-phenoxy- isoquinoline-3-carbonyl)-amino] -acetic acid ethanolamine salt	21.8, 22.7, and 27.1°2θ ± 0.2 °2Θ	171 °C
Compound A diethanolamine salt	[(4-hydroxy-l-methyl-7-phenoxy- isoquinoline-3-carbonyl)-amino] -acetic acid diethanolamine salt	16.9, 23.7, and 25.0 °2Θ ± 0.2 °2Θ	150 °C
Compound A tromethamine salt	[(4-hydroxy-l-methyl-7-phenoxy- isoquinoline-3-carbonyl)-amino] -acetic acid tromethamine salt	10.1, 14.2, and 21.1 °2Θ ± 0.2 °2Θ	176 °C.
amorphous Compound A	[(4-hydroxy-l-methyl-7-phenoxy- isoquinoline-3-carbonyl)-amino] -acetic acid		291 °C
Compound A potassium salt	[(4-hydroxy-l-methyl-7- phenoxy-isoquinoline-3-carbonyl)-amino] -acetic acid potassium salt		291 °C

5.1. Polymorphism, microcrystal, and Co-crystal

The importance of polymorphs or cocrystals is that any new forms may offer an opportunity to improve the pharmaceutical performance of the drug. Concerning the new forms, formulation scientists can work on multiple dosage forms to achieve their objectives. Roxadustat shows different polymorphic and cocrystal forms. Table 2 shows different salt, amorphous, and crystal forms of [(4-hydroxyl-methyl-7-phenoxy -isoquinoline-3-carbonyl)-amino]-acetic acid with an analytical outcome like X-ray powder diffractogram and DSC have been mentioned [13]. Kallem et al. hold several patents on crystalline form- γ and crystalline form-δ of the Roxadustat [14]. Xinshan et al. has also described several crystalline forms, including preparation methods, i.e., forms I, II, III, IV, V, VI, and VII, in their patent [15]. Hanyue et al. have filed a patent for the microcrystalline form of Roxadustat as an anorthic system; the details include crystal X-ray diffraction results on cell parameters with a = 8.5830, b = 9.2790, c = 11.359: with angle α = 99.16(3°), β = 108.36(3°), γ = 102.16(3°), unit cell volume of 814.2(3)^3 with molecular number Z = 2 in structure cell [16]. The different preparation methods employed for microcrystal Roxadustat are presented in Table 3. Due to the poor solubility (1.71 mg/L) of Roxadustat in water, several groups have reported the cocrystal form of Roxadustat [17]. The concept of cocrystals involves solid crystals of ionic compounds in a stoichiometric ratio. The structure comprises two or more compounds to form a unique crystal. In cocrystals, one component is an active pharmaceutical ingredient, and the other is a conformer. The selection of conformers is a critical task to achieve compatibility and effective formulation. Many of the reported suitable co-formers to form the water-soluble form of Roxadustat are from the group consisting of meglumine, N, N′-dibenzylethylenediamine, ter-butylamine, diethylamine, dicyclohexylamine, ammonia salt, magnesium, calcium, potassium, lithium, iron (III) salt, iron (II),2-naphthalene sulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, 3-ethyl-l-methyl-IH-imidazole-3-ium acetate, and caffeine [18]. A cocrystal form is a good option for effective formulation due to intrinsic barriers to drug delivery, insufficient solubility, dissolution, permeability, first-pass metabolism, and drug effect on bioavailability. At the molecular level of interaction of cocrystal drugs with different compounds, hydrogen bonding is involved without the formation of salts. This is a characteristic feature of a cocrystal since Brønsted acid-base chemistry is not required [19]. There exist multiple ways for cocrystal formation via hydrates, clathrate, and solvates based on the principle of host-guest chemistry. Also, there exist several methods for the preparation of cocrystals like solid-state methods that work on the principle of mixing two or more components, a controlled atmospheric environment that involves spontaneous mixing of API and conformer; grinding; extrusion methods; hot-melt extrusion (HME); high shear wet granulation; evaporation; cooling crystallization methods; isothermal slurry preparation; cocrystal with supercritical solvents; laser irradiation; freeze-drying; and electrospray technology [20]. Jetti et al. reported the formation of Roxadustat cocrystal with D-proline by mixing Roxadustat with D-proline in the presence of a solvent, followed by isolation of the cocrystal [21]. Jinchao synthesized and characterized four cocrystals of Roxadustat, cinnamate, benzamide, proline, and niacinamide by solution crystallization method [17]. Dr. Reddy's Laboratories, India, has filed patents on the cocrystal form of RLP (Roxadustat with L-proline), RNM (Roxadustat with nicotinamide), and RU (Roxadustat with Urea) by using solution crystallization methods [13,20–28].

Table 3
Summary of microcrystal preparation.

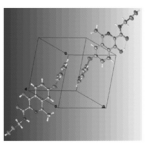
Sr.No	Preparation method	w/v of solvent	Method of dissolution	Condition	Dissolving agent	
Embodiment 1	Drug dissolved in separate organic solvent acetonitrile, dehydrated alcohol, acetone	1 g:1 mL	Ultrasonic dissolution	cool to room temperature	Hydrochloric acid or acetic acid.	
Embodiment 2	combination of any two solvents acetonitrile, dehydrated alcohol, acetone	1 g:10 mL	Ultrasonic dissolution			
Embodiment 3	combination of three kinds in acetonitrile, dehydrated alcohol, acetone	1 g:40 mL	Heating for dissolving at 30 °C			
Embodiment 4	combination of acetonitrile, dehydrated alcohol, acetone	1 g:60 mL	Heating for dissolving at 80 °C			
Embodiment 5	acetonitrile, dehydrated alcohol, acetone	1 g:50 mL	heating for dissolving at 50 °C			

Table 4
Tablet formulations with some excipients.

Roxadustat dose in tablet (mg)	Lactose (mg)	Allura Red AC aluminium lake (mg)	Soya lecithin (mg)
20	40.5	0.9	0.21
50	101.2	1.7	0.39
70	141.6	2.1	0.47
100	202.4	2.8	0.63
150	303.5	3.7	0.84

Table 5
Chromatographic methods for the analysis of the drug substance in different biological matrices.

Matrix	Objective of analysis	Stationary phase	Mobile phase	Gradient		Detection	Flow rate	Column temperature	Reference
Urine (Human)	Drug content	Zorbax SB-C8 Column 2.1 × 100mm,1.8 μm	Mobile phase A-10 mM ammonium formate and acetic acid (pH 4) Mobile B- Acetonitrile	Time	%B	Triple quadrupole mass spectrometer (Waters) Positive mode	400 μL/ min	20 °C	[31]
				10	10				
				8	55				
				8.1	100				
				8.6	10				
				10.6	10				
Urine (human)	Drug content	Zorbax XDB-C8 Column 2.1 × 150mm,5.0 μm	Mobile phase A-10 mM ammonium formate and acetic acid (pH 4) Mobile B- Acetonitrile	Time	%B	TSQ Quantum Ultra triple quadrupole mass spectrometer With positive mode	250 μL/ min		
				0	40				
				5	90				
				9.5	90				
				9.6	40				
				15	40				
Plasma and urine (human)	Drug and metabolite analysis	Biphenyl (100 × 2.1 mm, 2.7 μm	Mobile phase A-25 mM ammonium formate with 0.1% formic acid Mobile B- Acetonitrile	Time	%B	Triple quadrupole mass spectrometer	400 μL/ min	40 °C	[7]
				0	14				
				0.5	70				
				6.5	70				
				6.6	95				
				7.5	95				
				7.6	14				
				9.0	14				
Human hepatocyte	Metabolites study	Waters, BEH C18 Column 2.1 × 50mm,1.7 μm	Mobile phase A-5 mM ammonium acetate pH 3.5 Mobile B- Acetonitrile	Time	%B	Water Xevo TQ-S triple quadrupole mass spectrometer	300 μL/ min	50 °C	[33]
				0	0.5				
				0.5	5				
				6.0	50				
				8.0	50				
				10.0	70				
				12.0	95				
				14	95				
				16.5	5				
Liver microsome and fungal sample		Acquity UPLC BEH C18 Column 2.1 × 100 mm,1.7 μm	Mobile phase A-0.1% Formic acid B- methanol	Time	%B		500 μL/ min	65 °C	
				0	5				
				1.0	5				
				6.0	95				
				8.0	95				
				8.1	5				
				10.0	5				
Plasma and urine (mice)	Metabolites study	Acquity HSS T3; C18, 150mmx2.1 mm,1.8 μm	Mobile phase A-0.01% Formic acid B- Acetonitrile with 0.01% Formic acid	Time	%B	Waters synapt G2-Si QTOF MS with ESI	400 μL/ min	40 °C	[34]
				0	1				
				1.0	1				
				8.0	99				
				13	85				
				10.0	1				
Plasma (human)	Pharmacokinetics and drug-drug interaction study	C18 Column	Mobile phase A-0.1% Formic acid B- acetonitrile 0.1% Formic acid	Isocratic – A: B (45:55)					[37]
Urine	Antidoping analysis	Synchronis C18 (100 × 2.1 mm, 1.7 μm)	Mobile phase A-0.2% Formic acid B- acetonitrile 0.2% Formic acid	Time	%B	Thermo-scientific Q Exactive plus tandem mass spectrometer	500 μL/ min		[38]
				0	2				
				0.5	2				

(*continued on next page*)

Table 5 (*continued*)

Matrix	Objective of analysis	Stationary phase	Mobile phase	Gradient		Detection	Flow rate	Column temperature	Reference
				8.5	95				
				10.0	95				
				12.0	2				
Urine (human)	Simultaneous analysis of drug	Supelco AscentisR C18 (150 mm × 2.1mm × 2.7 μm)	Mobile phase A-0.1% Formic acid B- acetonitrile 0.1% Formic acid	Time	%B	QExactive focus benchtop Orbitrap-based mass spectrometer	250 μL/ min	30 °C	[39]
				0	5				
				7	65				
				8	100				
				11.0	100				
				13.0	5				
Urine (Horse)	Metabolites study	Eclipse plus C18 (4.6 × 150mm, 3.5 μm)	Mobile phase A-5Mm ammonium acetate or 0.2% formic in water B-acetonitrile	Time	%B	QExactive high-resolution accurate mass spectrometer	600 μL/ min		[40]
				0	2				
				5	95				
				8.0	95				
				8.5	50				
				13.5	50				
				14.0	50				
				15.5	2				
Liposome formulation	HPLC assay	Supelco Discovery BIO wide pore C5, 250mmx4.6 mm,5 μm	Mobile phase A-5% acetonitrile and 0.1% trifluoroacetic acid in water Mobile phase B-95% acetonitrile and 0.1% trifluoroacetic acid in water	Time	%B	UV 348 nm	1 mL/ min		[41]
				0	30				
				20	100				
				21	30				
Active pharmaceutical substance	Purity analysis	Kinetex C18 150mmX3.0 mm,2.6 μm	Mobile phase A-10 mM ammonium formate pH 6.3 Mobile Phase B-acetonitrile	Time	%B	LTQ XL Orbitrap Mass spectrometer (Thermo)	0.6 mL/ min		[42]
				0	30				
				4	30				
				18	100				
				23	100				
				25	30				
				30	30				
		Supelco- Ascentis Express C18	Mobile phase A-0.01 M pH 3.0 Phase B-Acetonitrile	Time	%B	UV 225 nm			
				0	10				
				1	10				
				10	90				
				12	90				
				13	10				
				14	10				

6. Pharmaceutical formulation aspects

Roxadustat is available in the market under the brand Evrenzo in the form of a film-coated tablets with strengths of 20 mg, 50 mg, and 100 mg. The formulation contains excipients like microcrystalline (E460) cellulose, povidone (E1201), croscarmellose (E468), lactose hydrate, and sodium magnesium stearate (E470b), as shown in Table 4. The film coating excipient was used, such as titanium oxide (E171), partially hydrolyzed polyvinyl alcohol (E1203), macrogol (E1521), Allura red AC aluminum lake (E129), Lecithin (E322), talc, ferric oxide, ferric oxide [29]. Due to its photodegradation [30] nature, Roxadustat is used along with photo stabilizing agents in the formulation, such as titanium dioxide, along with additional dye agents like Allura red AC, aluminium lake, iron oxide, iron oxide yellow, sunset yellow, sunset yellow FCF, indigotin, and indigotin, aluminium lake are used to stabilize the molecule [30].

7. Methods of analysis of Roxadustat and its metabolites

Various analytical and bioanalytical methods for analysis of Roxadustat are reported in the literature. All the available methods involving chromatographic conditions are summarized in Table 5. Notably, most of the analytical methodology for studying Roxadustat and the derivative is limited to biological samples. To our knowledge, there were only a few reported methods for analyzing API, pharmaceutical formulations, but no methods available for routing stability and degradation products. This review covered all the available bioanalytical methods for analyzing Roxadustat using various hyphenated techniques. In all the methods, the drug has been analyzed under different objectives like doping control, metabolite study, drug correlation study, and pharmacokinetics approach. In 2016, Buisson et al. [31], for the first time, published LC-MS/MS method for the analysis of Roxadustat, which was reported to be used by some athletes to increase their performances as an alternative doping agent. HIF stabilizers were listed as prohibited substances by the World Anti-Doping Agency (WADA) in 2011 [32]. As discussed earlier, HIF propyl-hydroxylase inhibitor increases erythropoiesis and RBC production, and this application is correlated with the possibility that a new erythropoiesis stimulator can be used to enhance athletic performance. Analysis was performed by collecting plasma and urine samples of an athlete before and after consumption of the Roxadustat drug. A direct identification approach considers monitoring the drug content. In this study, the authors used a sample preparation technique involving solid-phase extraction with the C18 cartridge. They achieved a low limit of detection (LLOD) of 400 pg/mL for the initial doping testing procedure [31].

Later, A doping control analysis was reported to study the drug's metabolites by performing urine analysis and analysis of plasma-derived metabolites from phase-I clinical trials using the UPLC-MS/MS technique [7]. The detection limit range was 0.05–1 ng/mL for urine and 1–5 ng/mL for plasma. Along with the metabolite study, the authors also mentioned a light-induced rearrangement product, a photo isomer of the drug Roxadustat. In order to prepare the sample, the following steps were considered: Methanolic solution of a stable isotope of the drug was added as an internal standard for urine sample. The solution was mixed using a vortex mixer and passed through HLB SPE cartridge using methanol as eluent. The plasma sample was also spiked with internal isotope standard and isopropanol was added. The vortexed sample was centrifuged, and the supernatant was collected and concentrated. The concentrate was further diluted with pH 5.0 sodium acetate buffer. Solid-phase extraction was carried out on bond Elut nexus SPE cation exchange sorbent with washing solution as water and elution solution as methanol. The sample was evaporated and reconstituted with a diluent before injection in the LC-MS system. Mass spectrometric conditions include electrospray ionization in positive mode with probe maintained at 1500 V, the source temperature was 150 °C, desolvation was carried out at a temperature of 450 °C and cone gas flow of 150 L/h. The mass values reported were M1- (*m/z* 369), M4 (*m/z* 296), M8 (*m/z* 529), and M11 (*m/z* 449) (see Table-6) corresponds to the metabolites with mention of one photo isomer (see Fig. 2). It was also reported that glucuronic acid conjugated metabolites show the longest response, up to 167 h, which is a good indication for doping analysis in the athlete. The other three metabolites can be traced for up to 24 h. All possible metabolites with the possible mechanism pathways for metabolism are mentioned in Fig. 4. Roxadustat undergoes phase-I Biotransformation to form M1-M6 metabolites, most of which occur via cytochrome P-450. M8-M10 metabolites form by glucuronidation conjugates via UDP-glucuronosyltransferases, while M11-M13 are formed by sulfation via sulfotransferases.

In another method [33], performed several *in-vitro* metabolite studies involving liver microsomes, S9 fraction, human hepatocytes, equine liver microsomes, fungus model *Cunninghamella elegance*, and *in-vivo* urine samples. They identified a total of 12 metabolites in their study using LC-MS. Further, they reported that monohydroxylated metabolite (*m/z* 369.1079), one of the metabolites, was common in 3 *in-vitro* studies, which is present in equine liver microsomes, *Cunninghamella elegans*, and human liver microsomes. On the contrary, eleven other metabolites formed due to the sulfonation, dihydroxylation, monohydroxylated, mono sulfonated, and glycosylated of Roxadustat and its photo isomer are observed in *Cunninghamella elegans*. In the urine sample, the observed metabolite was glucuronide metabolite (*m/z* 529.144). No metabolites were observed in other in-vitro studies with S9 fraction and human hepatocyte.

Saigusa et al. [34], developed different chromatographic methods with mass spectrometric analysis of Roxadustat and its novel metabolites (Rox-methyl and Rox-Gluc) in mice. The authors used principal component analysis and partial least square discrimination analysis tools for the study design. For the first time in literature, using a mouse model, the G-met protocol was applied to detect unknown metabolites in doping. The authors also included a pharmacokinetic analysis that has not been published in earlier studies.

In another approach, a bioanalytical method was developed for drug-drug interaction studies involving lanthanum carbonate and Roxadustat. Lanthanum carbonate acts as a phosphate binder and is recommended for hyperphosphatemia in patients with kidney disease. It has been reported that lanthanum carbonate reduces the bioavailability of some drugs, like ciprofloxacin [35] and levothyroxine [36]. To check a similar effect on Roxadustat, the pharmacokinetic study was carried out in healthy, non-elderly adult males [37]. Pharmacokinetic parameters like C_{max}, AUC, and T_{max} were studied using UPLC/MS/MS techniques. No drug-drug interaction was reported when the drug was given separately and along with lanthanum carbonate.

Table 6
Metabolites of roxadustat.

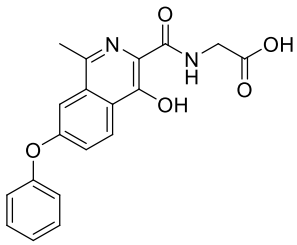
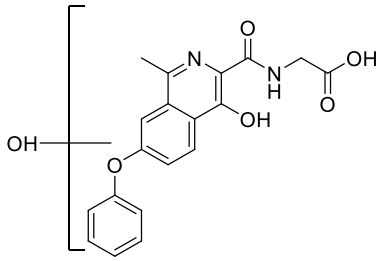
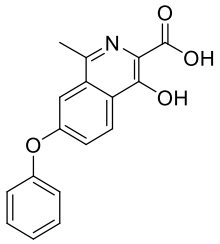
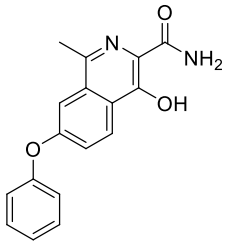
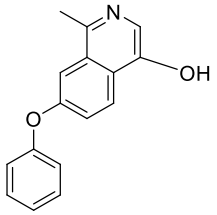
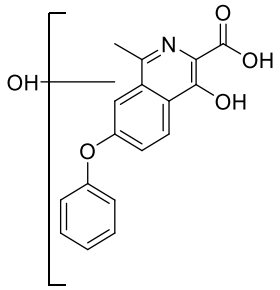
Name	RT	*m/z* $[M+H]^+$	Chemical Structure
M (Roxadustat)	11.04	353.1125	
M1	8.82	369.1075	
M2	7.53	369.1074	
M3	9.82	369.1075	
M4	8.03	296.0911	
M5	11.05	295.1074	
M6	11.82	252.1016	
M7	10.99	312.0860	

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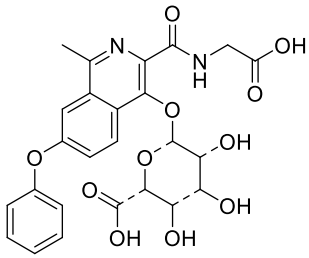
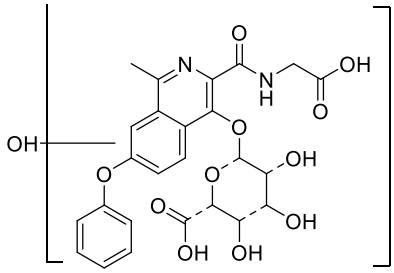
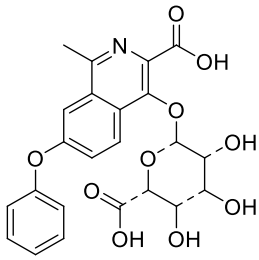
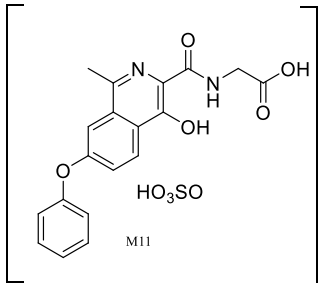
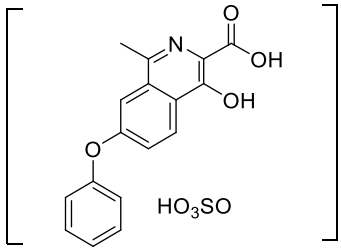
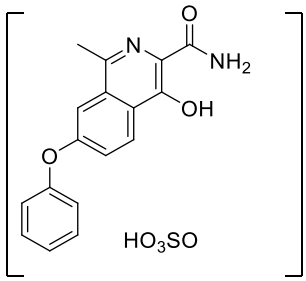
Table 6 (*continued*)

Name	RT	*m/z* $[M+H]^+$	Chemical Structure
M8	7.56	529.1446	
M9	7.15	545.1397	
M10	8.38	472.1231	
M11	7.74	449.0636	
M12	7.33	390.0293	

(*continued on next page*)

Table 6 (*continued*)

Name	RT	*m/z* $[M+H]^+$	Chemical Structure
M13	8.34	389.0451	

In the sixth method by Kim et al. [38], UPLC/MS/MS was developed as a simple and cost-effective method for anti-doping analysis. The researchers used the novel extraction technique, QuEChERS, to screen for anti-doping for a human urine sample. After several trials, it was observed that the double extraction methodology with 1% formic acid in acetonitrile provided the highest recovery for accurate analysis. The researchers employed Mass spectrometric analysis with positive and negative modes with the capillary temperature set at 300 °C and spray voltage as 4000 V for positive and 3500 V for negative. The retention time was found as 6.3 min.

In another method [39], developed a single analytical LCMS technique for the determination of 9 HIF propyl-hydroxylase inhibitors, which are good references for anti-doping analysis of Roxadustat, vadadustat, molidustat, desidustat, daprodustat, FG2216, IOX2, IOX4, and JNJ-42041935. In this method, the retention time for Roxadustat was observed as 10.79 min.

In 2021 [40], identified drug metabolites in horse urine using LC with HR-MS and found 13 metabolites. Phase I biotransformation converts the parent molecule into a more polar form by oxidation, reduction, or hydrolysis mechanism. The authors found seven

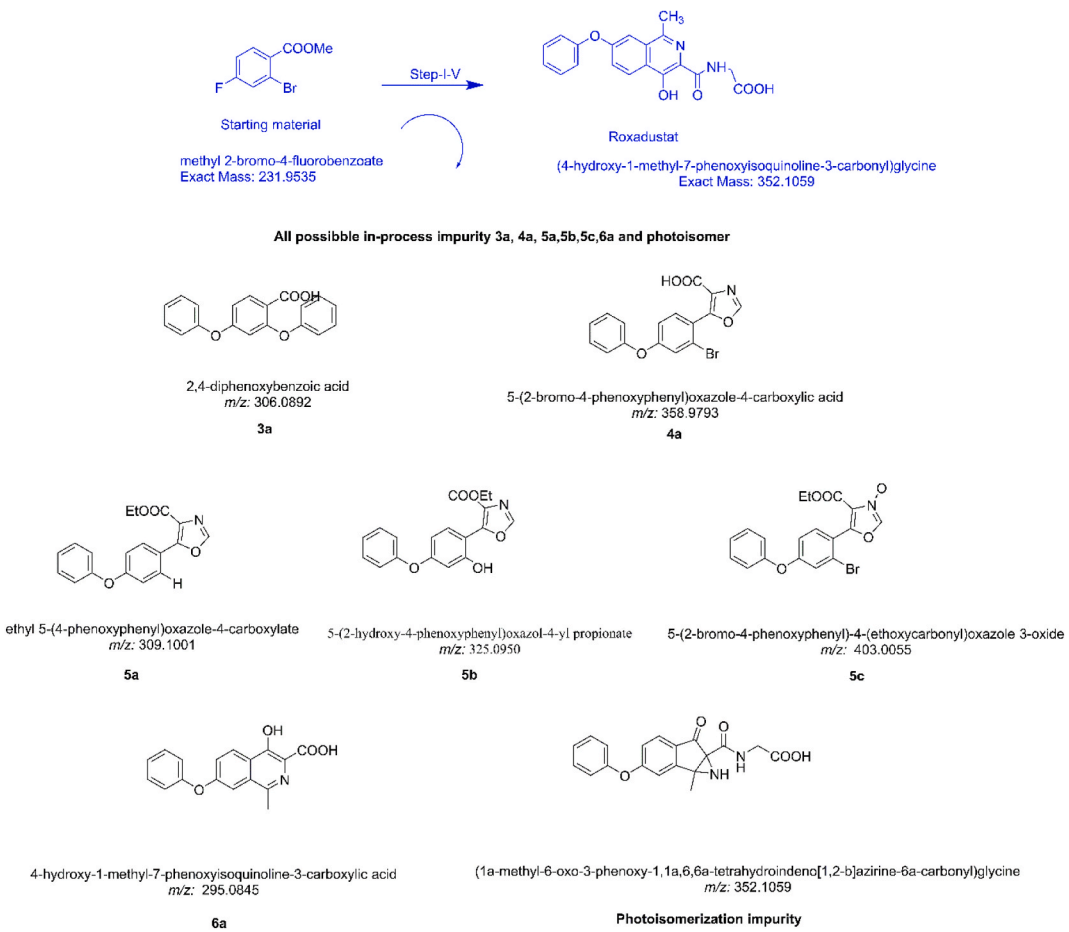


Fig. 1. In-process impurities during the synthesis of Roxadustat.

metabolites in phase I. In phase II, the authors detected one metabolite and five conjugated metabolites in phase I. All the metabolites were detected in positive mode mass spectrometry. Similar to earlier reports, the major metabolite was hydroxylated in phase I and glucuronic acid conjugated in phase II. The metabolites were hydroxylated, dealkylated, hydrolyzed, glucuronic acid conjugated, and sulfonic acid conjugated. Metabolites with *m/z* are mentioned in Table 6. This analytical technique can be an essential method for doping analysis in sports.

For all the above methods, the mass spectrometric parameters used were capillary voltage range from 1.5 to 4.0 kV for positive mode and −2.5-4.5 kV for negative mode, cone voltage between 10 and 40 V, desolvation temperature between 320 and 550 °C, nebulization gas flow 1000 L/h, and collision energy between 15 and 30eV.

8. Analytical methodology for API, process impurities, and formulation

In our literature search, we found only a few research articles supporting chromatographic analysis for liposomes as novel formulations [41] and details on impurities generated during five-step synthesis [42]. As it is known that liposome formulation demonstrates potential effects with fewer side effects, efficient dosing, and targeted delivery, Cheng-bang Jian et al. developed Roxadustat-loaded liposomes and studied drug loading efficiency along with liposome assay using HPLC.

Considering scalable synthesis, Pisa et al. synthesized Roxadustat and all the possible in-process impurities in the laboratory and monitored purity by HPLC. All the potential in-process impurities are mentioned in Fig. 1. In Fig. 2, we have suggested the possible route for generating isomeric photo impurities.

In the present review, we have extended our topic of study by including the information related to substances and in-process impurities from different suppliers like Aozeal certified standards [43], Clearsynth [44], TLC Pharmaceutical standards [45], and Synzeal research [46], which have been mentioned in their official websites. It is hypothesized that the present review will serve as a reference for researchers to conduct future analytical studies like impurity profiling and stability studies for the characterization of unknown impurities (Table 7).

8.1. Other methods

Roxadustat pKa parameter has been analyzed by two analytical techniques potentiometric pH-metric titration and Spectrophotometric-UV method. Meloun et al. described the spectrophotometric analysis method as more sensitive among the methods, with a detection range between 10^{-5} to 10^{-6} M concentration [47]. A GC-MS method is also described in the literature to study the effect of Roxadustat on retinopathy treatment under hypoxia conditions. The authors studied the role of the serine metabolic pathway in the disease and the behaviour of Roxadustat [48]. Roxadustat was analyzed using NMR, XRPD, IR, and DSC in one research paper detailing synthetic process development [49]. A literature search was conducted to investigate the analytical methodologies and formulation aspects. A great deal of scope was identified for developing analytical methods for assays, dissolution tests, and related substance tests. No single analytical method exists for impurity profiling, process-related impurity, and stability tests. Even hyphenated techniques like GC are not mentioned anywhere in the literature for solvent analysis.

9. Pharmacology

Mechanism of action: Roxadustat is hypoxia-inducible factor propyl hydroxylase (HIF-PH) enzyme inhibitor, which regulates the degradation of transcription factors in HIF and enhances endogenous erythropoietin production through erythropoietin gene expression, which causes an increase in RBC production, and is helpful to maintain the haemoglobin level in patients with chronic kidney diseases and anaemia (Fig. 3) [50]. In the reported study, the drug also showed an increase in iron bioavailability by suppressing hepcidin levels in the body [51].

9.1. Pharmacokinetics

Roxadustat showed rapid absorption when studied in dialysis patients. Pharmacokinetic parameters like the apparent volume of distribution after oral dose administration revealed 22–57 L, apparent renal clearance of 0.030–0.026 L/h, and apparent clearance of 1.2–2.65 L/h in healthy volunteers. The elimination half-life was 9.6–16 h. Plasma binding was 99%, and the fraction eliminated by hemodialysis was 2.34% [52]. The maximum plasma concentration (C_{max}) was achieved between 2.0 and 3.5 h after drug administration. In a clinical trial, patients were studied for the treatment of efficacy of Roxadustat. Evaluation parameters considered the amount of endogenous erythropoietin, iron content, iron-binding capacity, transferrin saturation, and hepcidin level [51].

Fig. 2. Schematic representation of a pathway related to Photo isomeric impurity.

Table 7
Related substance and possible In-process Impurity of Roxadustat.

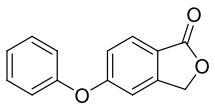
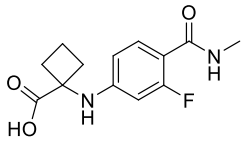
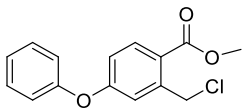
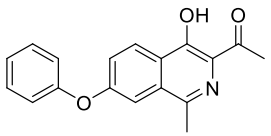
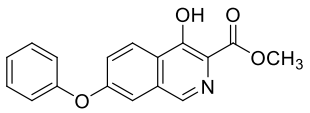
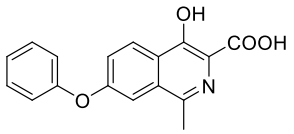
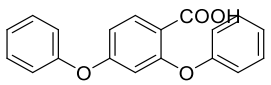
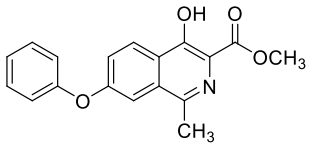
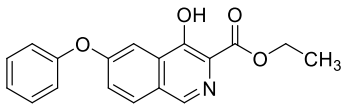
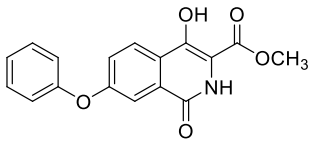
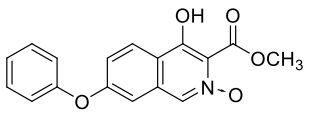
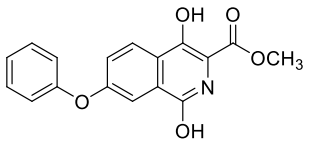
Compound name	Exact mass (Da)	Chemical Structure
5-phenoxyisobenzofuran-1(3H)-one Chemical Formula:	226.0630	
1-((3-fluoro-4-(methylcarbamoyl)phenyl)amino)cyclobutane-1-carboxylic acid Chemical Formula: C13H15FN2O3	266.1067	
methyl 2-(chloromethyl)-4-phenoxybenzoate Chemical Formula: C15H13ClO3	276.0553	
1-(4-hydroxy-1-methyl-7-phenoxyisoquinolin-3-yl)ethan-1-one Chemical Formula: C18H15NO3	293.1052	
methyl 4-hydroxy-7-phenoxyisoquinoline-3-carboxylate Chemical Formula: C17H13NO4	295.0845	
4-hydroxy-1-methyl-7-phenoxyisoquinoline-3-carboxylic acid	295.0845	
2,4-diphenoxybenzoic acid	306.0892	
methyl 4-hydroxy-1-methyl-7-phenoxyisoquinoline-3-carboxylate	309.1001	
ethyl 4-hydroxy-6-phenoxyisoquinoline-3-carboxylate	309.1001	
methyl 4-hydroxy-1-oxo-7-phenoxy-1,2-dihydroisoquinoline-3-carboxylate	311.0794	
4-hydroxy-3-(methoxycarbonyl)-7-phenoxyisoquinoline 2-oxide	311.0794	
methyl 1,4-dihydroxy-7-phenoxyisoquinoline-3-carboxylate	311.0794	

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Table 7 (*continued*)

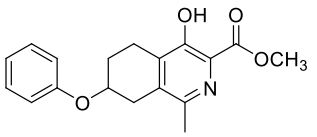
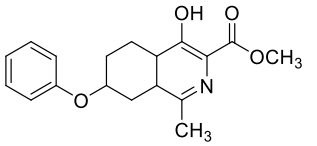
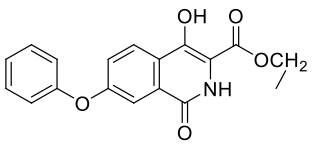
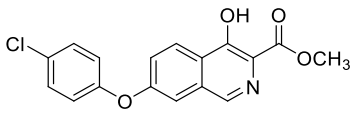
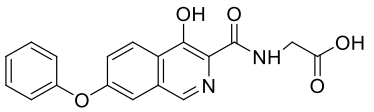
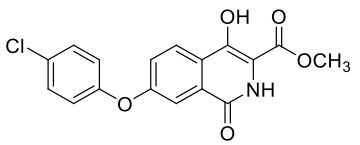
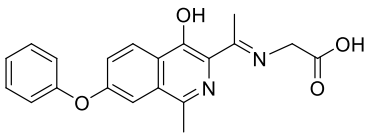
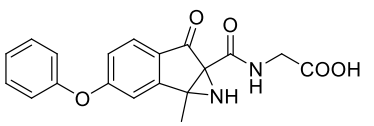
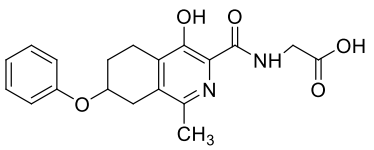
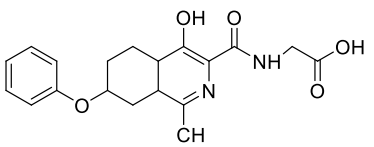
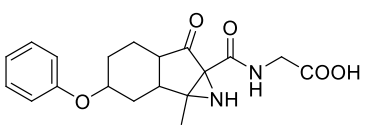
Compound name	Exact mass (Da)	Chemical Structure
methyl 4-hydroxy-1-methyl-7-phenoxy-5,6,7,8-tetrahydroisoquinoline-3-carboxylate	313.1314	
methyl 4-hydroxy-1-methyl-7-phenoxy-4a,5,6,7,8,8a-hexahydroisoquinoline-3-carboxylate	315.1471	
ethyl 4-hydroxy-1-oxo-7-phenoxy-1,2-dihydroisoquinoline-3-carboxylate	325.0950	
methyl 7-(4-chlorophenoxy)-4-hydroxyisoquinoline-3-carboxylate	329.0455	
(4-hydroxy-7-phenoxyisoquinoline-3-carbonyl)glycine	338.0903	
methyl 7-(4-chlorophenoxy)-4-hydroxy-1-oxo-1,2-dihydroisoquinoline-3-carboxylate	345.0404	
(E)-2-((1-(4-hydroxy-1-methyl-7-phenoxyisoquinolin-3-yl)ethylidene)amino) acetic acid	350.1267	
(1a-methyl-6-oxo-3-phenoxy-1,1a,6,6a-tetrahydroindeno [1,2-b]azirine-6a-carbonyl)glycine	352.1059	
(4-hydroxy-1-methyl-7-phenoxy-5,6,7,8-tetrahydroisoquinoline-3-carbonyl) glycine	356.1372	
(4-hydroxy-1-methyl-7-phenoxy-4a,5,6,7,8,8a-hexahydroisoquinoline-3-carbonyl)glycine	358.1529	
(1a-methyl-6-oxo-3-phenoxydecahydroindeno [1,2-b]azirine-6a-carbonyl) glycine	358.1529	

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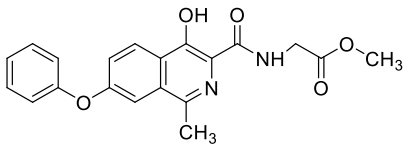
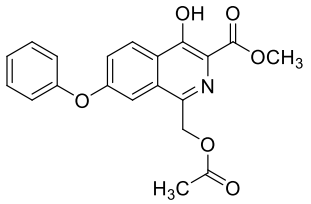
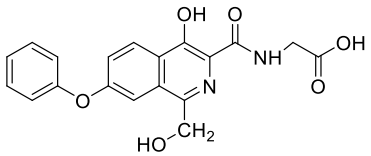
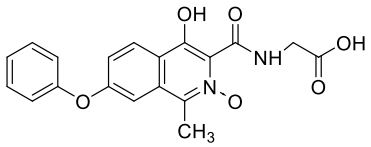
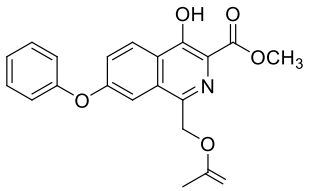
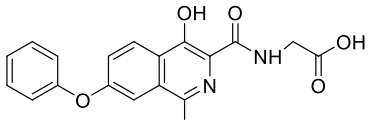
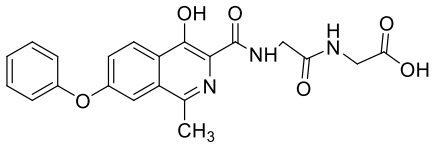
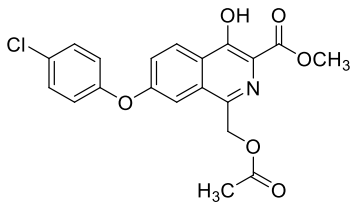
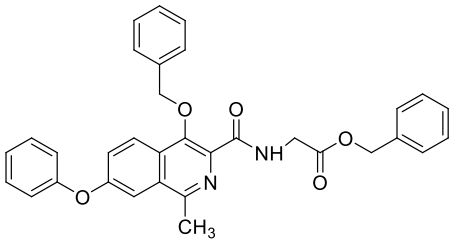
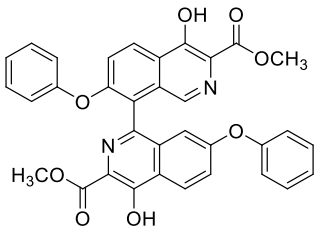
Table 7 (*continued*)

Compound name	Exact mass (Da)	Chemical Structure
methyl (4-hydroxy-1-methyl-7-phenoxyisoquinoline-3-carbonyl)glycinate	366.1216	
methyl 1-(acetoxymethyl)-4-hydroxy-7-phenoxyisoquinoline-3-carboxylate	367.1056	
(4-hydroxy-1-(hydroxymethyl)-7-phenoxyisoquinoline-3-carbonyl)glycine	368.1008	
3-((carboxymethyl)carbamoyl)-4-hydroxy-1-methyl-7-phenoxyisoquinoline 2-oxide	368.1008	
methyl 1-(acetoxymethyl)-4-hydroxy-7-phenoxyisoquinoline-3-carboxylate	367.1056	
(1-chloro-4-hydroxy-7-phenoxyisoquinoline-3-carbonyl)glycine	372.0513	
(4-hydroxy-1-methyl-7-phenoxyisoquinoline-3-carbonyl)glycylglycine	409.1274	
methyl 1-(acetoxymethyl)-7-(4-chlorophenoxy)-4-hydroxyisoquinoline-3-carboxylate	401.0666	

(*continued on next page*)

Table 7 (*continued*)

Compound name	Exact mass (Da)	Chemical Structure
benzyl (4-(benzyloxy)-1-methyl-7-phenoxyisoquinoline-3-carbonyl)glycinate	532.1998	
dimethyl 4,4′-dihydroxy-7,7′-diphenoxy-[1,8′-biisoquinoline]-3,3′-dicarboxylate	588.1533	

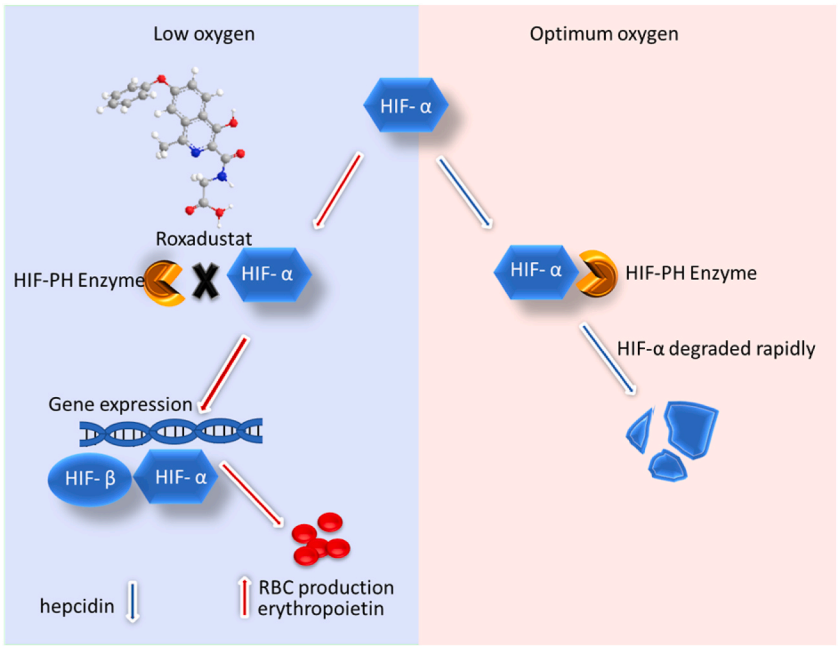


Fig. 3. Mechanism of action of Roxadustat.

10. Clinical trial details

The drug Roxadustat was investigated in clinical trials in a variety of pathophysiological conditions, including anemic patients, anemic patients with chronic kidney disease (CKD), CKD patients on dialysis, CKD patients without dialysis, chemotherapy-induced anemics, healthy patients, and end-stage renal disease patients.). Different study goals involved investigating dosing efficacy, safety, and tolerability aspects, as well as pharmacokinetics, and multiple-dose studies. A few studies investigated Roxadustat's effect compared to other treatments, such as erythropoietin recombinant and darbepoetin alfa. In two clinical studies, Roxadustat was studied as a drug-drug interaction with warfarin and Rosiglitazone. In one of the clinical studies, a pediatric formulation was studied in which the bioavailability of Roxadustat after a single dose of an azo dye-free formulation was assessed. The interventions were considered drugs and were compared with placebo, erythropoietin, recombinant human erythropoietin, epoetin alfa, darbepoetin alfa, and iron in all studies. In one of the articles from J. Barratt et al. he investigated Roxadustat versus erythropoiesis-stimulating agents

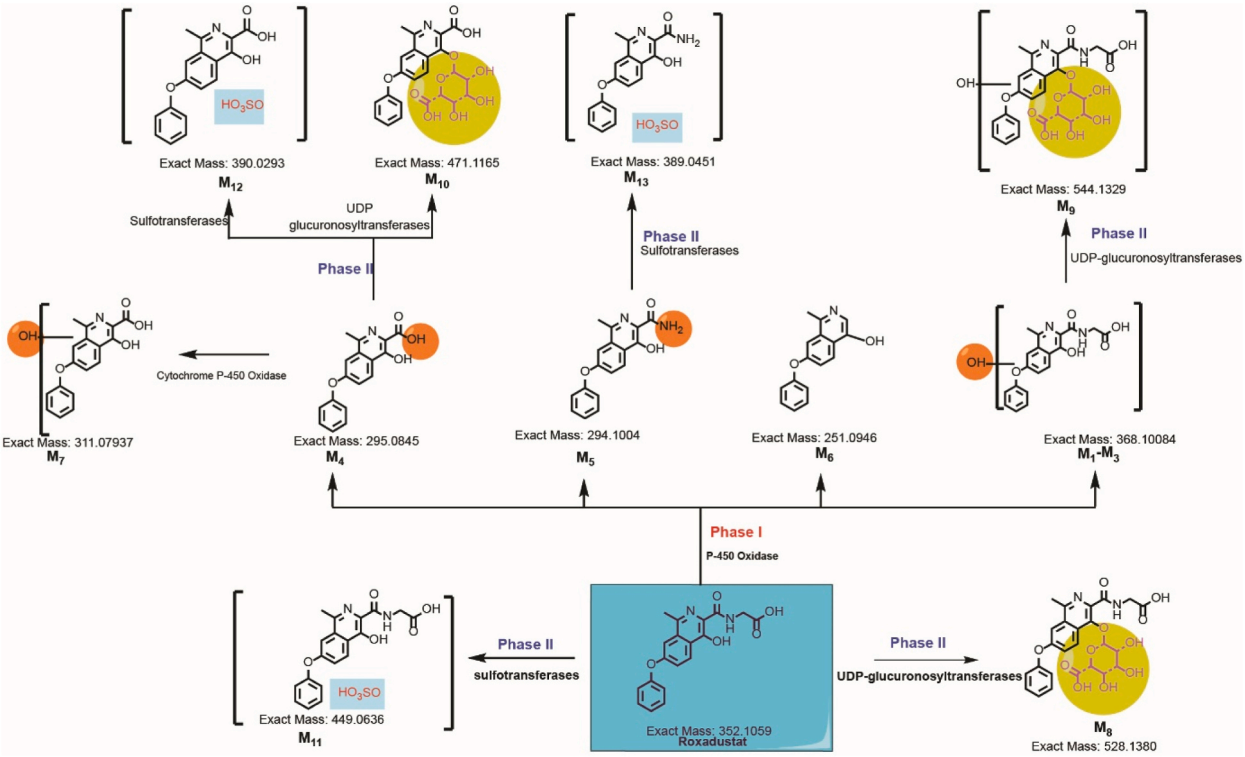


Fig. 4. Possible Mechanistic pathway of Metabolism.

(ESAs) in dialysis-dependent patients in a phase 3 clinical study, and it was found Roxadustat shows non-inferiors effects as compared to ESA in dialysis-dependent chronic kidney diseases patients [53]. He also highlighted cardiovascular safety aspects in his study. In another study, the same efficacy and cardiovascular safety were asses for Roxadustat in a patient with non-dialysis-dependent CKD [54]. All 38 completed clinical trials are listed in Table 8 with all essential details, including NCT Number, Interventions, Conditions, and Sponsor [55].

11. Summary & future perspective

As Roxadustat is a relatively new therapeutic molecule, the information related to Roxadustat's formulations manufacturers and API providers, and the country where the molecule is approved for clinical usage is the first time covered in this review. In addition, this review provides the reader with a complete picture of physicochemical properties, formulation details, etc. The information presented in this review shows a strong need for developing precise analytical methods to analyze available cocrystals and polymorphs of the drug apart from their forced degradation products. Given the increasing misuse of Roxadustat among sports personnel, more bioanalytical methods to analyze the drug and its metabolites are required from various biofluids such as blood, plasma, and urine matrix using hyphenated LC-MS/MS techniques. Much more can be done to develop formulations and study the impurity profiles of drugs and formulations with the additional scope for drug-excipient interaction studies.

12. Conclusion

This review provides comprehensive information regarding the drug Roxadustat in terms of drug developments. It provides guidelines for developing new formulations in the future. As a drug with a promising function in numerous anaemic conditions, it offers fresh hope for the creation of novel formulations. From the very beginning, such as the supplier, to the very end, such as the product patentee information, the review offers us all the information. While multiple conformers and co-crystal characteristics exist, it was discovered that solid state investigation also has a scope of work. The author discussed every bioanalysis technique that can potentially be utilised for QC departments for monitoring. Ultimately, this review includes all facts on a single platform.

Credit author statement

Rupali Mahajan: Writing original draft, writing review. **Amit Asthana**: Supervision, writing and editing Supervision, Writing - review & editing. **Samanthula Gananadhamu** and **Saurabh Srivastava**: Review, suggestion and editing.

Table 8
Summary of all the 38 completed clinical trials related Roxadustat.

Sr No.	NCT Number	Interventions	Study Title	Conditions	Sponsor:
1	NCT04076943	Drug: Roxadustat	Evaluation of Efficacy and Safety of Roxadustat for the Treatment of Chemotherapy Induced Anemia Has Results	Chemotherapy Induced Anemia	• FibroGen•AstraZeneca • Astellas Pharma Inc
2	NCT04454879	Drug: Roxadustat	Different Doses of Roxadustat Treatment for Anemia in Peritoneal Dialysis Patients	• Renal Anemia	• FibroGen•AstraZeneca • Astellas Pharma Inc
3	NCT04484857	Drug: Roxadustat	Study of Roxadustat Conversion in Participants Receiving Stable ESA or as Initial Anemia Treatment in Hemodialysis Participants	• Anemia Associated With End Stage Renal Disease	FibroGen • AstraZeneca
4	NCT04410198	Drug: Roxadustat	Study of Roxadustat Conversion in Participants Receiving Stabe Erythropoiesis-Stimulating Agent (ESA) or as Initial Anemia Treatment in Chronic Dialysis Participants	• Anemia Associated With End Stage Renal Disease (ESRD)	• FibroGen • AstraZeneca
5	NCT04655027	Drug: Roxadustat Drug: rHuEPO	A Study to Investigate the Effect of Roxadustat Versus Recombinant Human Erythropoietin (rHuEPO) on Oral Iron Absorption in Chinese Patients with Anemia of Chronic Kidney Disease (CKD)	• Anemia of Chronic Kidney Disease	• AstraZeneca • Parexel
6	NCT02273726	Drug: Epoetin Alfa Drug: Roxadustat	Study to Evaluate the Efficacy and Safety of Roxadustat in the Treatment of Anemia in Participants with ESRD on Stable Dialysis	•CKD Anemia in Stable Dialysis Patients	• FibroGen • Astellas Pharma Europe B.V. • AstraZeneca
7	NCT02965040	Drug: Roxadustat	A Phase 1 Study of Roxadustat in Subjects with Different Degrees of Renal Function	• Normal Renal Function •Impaired Renal Function	• Astellas Pharma Europe B.V. • FibroGen •Astellas Pharma Inc
8	NCT04059913	Drug: Roxadustat	Evaluate the Efficacy and Safety of Multiple Roxadustat Dosing Regimens for the Treatment of Anemia in Dialysis Participants with Chronic Kidney Disease	CKD Anemia in Dialysis Participants	• FibroGen
9	NCT03960489	Drug: Roxadustat	A Study to Assess the Relative Bioavailability of Roxadustat Following a Single Dose of Pediatric Azo Dye-free Tablet Formulation and Pediatric Azo Dye-free Mini-tablet Formulation Compared to a Single Dose of Azo Dye-containing Tablet Formulation in Healthy Adult Subjects	• Healthy Adult Subjects	• Astellas Pharma Global Development, Inc. • FibroGen •Astellas Pharma Inc
10	NCT01887600	Drug: Roxadustat Drug: Placebo	Roxadustat in the Treatment of Anemia in Chronic Kidney Disease Patients Not Requiring Dialysis	Anemia in Chronic Kidney Disease in non-dialysisPatients	• Astellas Pharma Europe B.V. • FibroGen • Astellas Pharma Inc
11	NCT02052310	Drug: Roxadustat Drug: Epoetin Alfa	Safety and Efficacy Study of Roxadustat (FG-4592) for the Treatment of Anemia in End-Stage Renal Disease (ESRD) Newly Initiated Dialysis Participants	Anemia in Incident Dialysis Patients	• FibroGen • Astellas Pharma Europe B.V. •AstraZeneca
12	NCT01630889	Drug: Roxadustat	Open-Label Extension Study for the Long-Term Efficacy and Safety of Roxadustat in Participants with Dialysis and Non-Dialysis Chronic Kidney Disease	• Chronic Kidney Disease • End Stage Renal Disease •Anemia	• FibroGen •AstraZeneca • Astellas Pharma Inc
13	NCT02161224	Drug: FG-4592	A Study to Investigate the Exposure and Safety and Tolerability of a Single Dose of FG-4592 in Subjects With Moderately Diminished Liver Function Compared to Those with Normal Liver Function	• PK of FG-4592 • Hepatic Insufficiency • Healthy Subjects	• Astellas Pharma Europe B.V. • FibroGen •Astellas Pharma Inc
14	NCT02021318	Drug: Roxadustat Drug: Darbepoetin alfa	Roxadustat in the Treatment of Anemia in Chronic Kidney Disease (CKD) Patients, Not on Dialysis, in Comparison to Darbepoetin Alfa	• Anemia in Chronic Kidney Disease in non-dialysis Patients	• Astellas Pharma Europe B.V. •FibroGen • Astellas Pharma Inc
15	NCT02161796	Drug: FG-4592 Drug: Placebo	A Study to Evaluate the Dose proportionality and Effects of FG-4592 in Healthy Young and Elderly Male and Female Subjects	• PK for FG-4592 • Healthy Subjects	• Astellas Pharma Europe B.V. •FibroGen • Astellas Pharma Inc
16	NCT02278341	Drug: Roxadustat	Roxadustat in the Treatment of Anemia in End Stage Renal Disease (ESRD) Patients on Stable Dialysis	Anemia • End Stage Renal	• Astellas Pharma Europe B.V.

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Table 8 (*continued*)

Sr No.	NCT Number	Interventions	Study Title	Conditions	Sponsor:
		Drug: Epoetin alfa Drug: Darbepoetin alfa Drug: Iron		Disease (ESRD)	• FibroGen • Astellas Pharma Inc
17	NCT01750190	Drug: Roxadustat Drug: Placebo	A Study of Roxadustat for the Treatment of Anemia in Participants With Chronic Kidney Disease and Not Receiving Dialysis	CKD Anemia	• FibroGen • Astellas Pharma Europe B.V. • AstraZeneca
18	NCT01244763	Drug: Roxadustat	Study of Roxadustat in Non- Dialysis Chronic Kidney Disease Participants with Anemia	• Chronic Kidney Disease •Anemia	• FibroGen • Astellas Pharma Inc
19	NCT02252731	Drug: Warfarin Drug: FG-4592	A Study to Evaluate the Effects of Multiple Doses of FG-4592 on the Exposure, Safety and Tolerability and Effect of Warfarin in Healthy Subjects	Pharmacokinetics of FG-4592 • Healthy Subjects	Astellas Pharma Europe B.V. •FibroGen • Astellas Pharma Inc
20	NCT02174731	Drug: Roxadustat Drug: Epoetin alfa	Safety and Efficacy Study of Roxadustat to Treat Anemia in Patients with Chronic Kidney Disease, on Dialysis.	• Anemia	• AstraZeneca • FibroGen
21	NCT01147666	Drug: Roxadustat Drug: Epoetin Alfa Other: Placebo	Study of Roxadustat (FG-4592) in Participants with End-Stage Renal Disease Receiving Maintenance Hemodialysis	End Stage Renal Disease • Anemia	• FibroGen • AstraZeneca •Astellas Pharma Inc
22	NCT02174627	Drug: Roxadustat Drug: Placebo	Safety and Efficacy Study of Roxadustat to Treat Anemia in Patients with Chronic Kidney Disease (CKD), Not on Dialysis	• Anemia	AstraZeneca • FibroGen
23	NCT00761657	Drug: Roxadustat Drug: Placebo	Phase 2 Study of Roxadustat in Participants with Anemia and Chronic Kidney Disease Not Requiring Dialysis	• Chronic Kidney Disease •Anemia	• FibroGen • Astellas Pharma Inc
24	NCT01414075	Drug: Roxadustat Drug: Oral Iron Drug: IV Iron	Study of Roxadustat (FG-4592) to Correct Anemia in Newly Initiated Dialysis Participants Not on Erythropoiesis-Stimulating Agent Treatment	• Dialysis • Anemia	• FibroGen • Astellas Pharma Inc
25	NCT01376063	Drug: FG-4592	Study to Investigate the Interaction Between FG-4592 and Rosiglitazone in Healthy Adult Subjects	• Healthy Adult Subjects	• FibroGen
26	NCT01596855	Drug: FG-4592 Drug: Epoetin Alfa	Study of FG-4592 in Subjects with End-Stage Renal Disease Receiving Maintenance Hemodialysis in China	• Anemia in the End Stage Renal Disease	• FibroGen • Ruijin Hospital
27	NCT01599507	Drug: FG-4592 Drug: Placebo	Study of FG-4592 in Subjects with Chronic Kidney Disease in China	Anemia in Chronic Kidney Disease	• FibroGen
28	NCT02652806	Drug: FG-4592 Drug: Epoetin Alfa	FG-4592 for Treatment of Anemia in Subjects with Chronic Kidney Disease	• Anemia	• FibroGen
29	NCT02652819	Drug: FG-4592 Drug: Placebo	FG-4592 for Treatment of Anemia in Subjects with Chronic Kidney Disease Not on Dialysis	• Anemia	• FibroGen
30	NCT02988973	Drug: Roxadustat Drug: DA	A Study of Intermittent Oral Dosing of ASP1517 in Non-Dialysis Chronic Kidney Disease Patients with Anemia	Chronic Kidney Disease	• Astellas Pharma Inc •FibroGen
31	NCT02780726	Drug: Roxadustat	A Study of Intermittent Oral Dosing of ASP1517 in Peritoneal Dialysis Chronic Kidney Disease Patients With Anemia	Peritoneal Dialysis Chronic Kidney Disease Patients With Anemia	• Astellas Pharma Inc •FibroGen
32	NCT02780141	Drug: Roxadustat	A Study of Intermittent Oral Dosing of ASP1517 in Erythropoiesis Stimulating Agent (ESA)-Naive Hemodialysis Chronic Kidney Disease Patients with Anemia	ESA-naive Hemodialysis Chronic Kidney Disease Patients With Anemia	• Astellas Pharma Inc • FibroGen
33	NCT02964936	Drug: Roxadustat	A Study of Intermittent Oral Dosing of ASP1517 in ESA untreated Chronic Kidney Disease Patients with Anemia	• Chronic Kidney Disease	Astellas Pharma Inc •FibroGen
34	NCT02779764	Drug: Roxadustat	A Long-Term Study of Intermittent Oral Dosing of ASP1517 in Hemodialysis Chronic Kidney Disease Patients with Anemia Converted From Erythropoiesis Stimulating Agent (ESA) Treatment	Hemodialysis Patients With Renal Anemia	Astellas Pharma Inc • FibroGen

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Table 8 (*continued*)

Sr No.	NCT Number	Interventions	Study Title	Conditions	Sponsor:
35	NCT01083888	Drug: Roxadustat	ASP1517 Pharmacokinetics Study in Anemia Patients on Hemodialysis	• Anemia • Hemodialysis • Renal Impairment	Astellas Pharma Inc
36	NCT02952092	Drug: Roxadustat Drug: Darbepoetin alfa	A Study of Intermittent Oral Dosing of ASP1517 in Hemodialysis Chronic Kidney Disease Patients with Anemia	• Hemodialysis Chronic Kidney Disease Patients With Anemia Healthy Volunteers	• Astellas Pharma Inc • FibroGen
37	NCT00978198	Drug: ASP1517 (Roxadustat) Drug: Placebo	Safety, Tolerability, and Pharmacokinetic Study of ASP1517 in Healthy Non-elderly Male Volunteers		Astellas Pharma Inc
38	NCT01888445	Drug: Roxadustat Drug: darbepoetin alfa	A Study to Investigate the Effect of ASP1517 After Intermittent Oral Dosing in Dialysis Chronic Kidney Disease Patients with Anemia Compared With Darbepoetin as a Reference Drug	• Renal Anemia Associated With Chronic Renal Failure (CRF)	Astellas Pharma Inc

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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