

Impurities in Anti Diabetic Drugs: Modern Analytical Approaches and Regulatory Perspectives

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Abstract: Diabetes mellitus is one of the most prevalent chronic metabolic disorders worldwide, requiring long-term pharmacotherapy with a wide range of antidiabetic agents. The safety, efficacy, and quality of these drugs are highly dependent on the control of pharmaceutical impurities that may arise during synthesis, formulation, storage, or degradation processes. Impurities, including process-related impurities, degradation products, residual solvents, elemental contaminants, genotoxic impurities, nitrosamines, melamine, and cyanoguanidine, can adversely affect drug stability and patient safety even at trace levels. Therefore, impurity profiling has become a critical component of pharmaceutical quality assurance and regulatory compliance. This review summarizes the sources, classification, toxicological significance, and regulatory aspects of impurities in antidiabetic drugs, with emphasis on guidelines issued by the International Council for Harmonisation (ICH), including Q3A, Q3B, Q3C, Q3D, and M7. The article also highlights modern analytical techniques employed for impurity detection, identification, characterization, and quantification, such as HPLC, HPTLC, LC–MS/MS, GC–MS, CE–MS, and NMR spectroscopy. Furthermore, recent advances in green analytical chemistry and in silico toxicity assessment approaches are discussed. A comprehensive overview of impurity profiling studies reported for major antidiabetic drugs, including metformin, sitagliptin, pioglitazone, empagliflozin, vildagliptin, and related fixed-dose combinations, is presented. The review emphasizes the importance of robust analytical methodologies and regulatory-driven impurity control strategies to ensure the quality, safety, and therapeutic effectiveness of antidiabetic pharmaceutical products.

Keywords: Diabetes Mellitus, Antidiabetic Drugs, Impurity Profiling, Genotoxic Impurities, Nitrosamine Impurities, Melamine, Cyanoguanidine, Green Analytical Chemistry.

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I. INTRODUCTION

Diabetes mellitus is a progressive metabolic condition that involves persistently elevated glucose levels in the blood and associated with severe consequences in several organ systems. Chronic diabetes mellitus necessitates ongoing medical and lifestyle care due to its serious long-term effects. Since a number of new medications have been released recently, it is critical to assess their efficacy and safety^[1].

By controlling blood sugar levels and fostering overall disease control, a different class of antidiabetic medications, such as biguanides, sulfonylureas, meglitinides, thiazolidinediones, DPP-4 inhibitors, SGLT-2 inhibitors, and insulin analogues, are used to treat this illness. Because of

their extensive use and lengthy administration, it is imperative to maintain the therapeutic efficacy, safety, and quality of these drugs^[2].

Antidiabetic medicines may acquire contaminants from raw materials, intermediates, reaction byproducts, degradation mechanisms, or interactions with excipients during the synthesis, formulation, storage, and distribution processes. Such contaminants may disrupt drug stability, lower therapeutic efficacy, and endanger patient health in just small doses. As a result, one important aspect of pharmaceutical quality assurance is impurity profiling, which includes the methodical identification, structural clarification, and quantitative measurement of impurities. Regulatory organizations like the International Council for

Harmonization (ICH) have established stringent standards (ICH Q3A, Q3B, and Q3C) controlling impurity levels in both active pharmaceutical ingredients as well as finished dosage forms in order to protect patient safety. Modern methodologies for analysis are frequently used in the impurity profiling of antidiabetic medications, such as nuclear magnetic resonance (NMR), gas chromatography (GC), high-performance liquid chromatography (HPLC), and liquid chromatography–mass spectrometry (LC–MS). Furthermore, latest research suggests an increasing prevalence of green analytical chemistry techniques, which seek to lessen environmental effect while maintaining analytical accuracy and dependability^[3].

On a global scale 11.11% of the population (~589 million) had diabetes in 2024; by 2050, the prevalence is expected to increase to 12.96% (~853 million). The highest rates were 23.72% for people over 65 and 24.79% for those between the ages of 75 and 79. Men were more likely than women to have it (11.55% vs. 10.68), and metropolitan regions had a higher prevalence than rural ones (12.26% vs. 9.23). Middle-income nations had the most prevalence (11.46%), followed by high-income nations (10.21%) and low-income nations (7.47%)^[4].

II. IMPURITY PROFILING

In pharmaceutical industry unintended organic or inorganic compounds that can arise during formulation and storage or stay in active pharmaceutical ingredients (APIs) are referred to as impurities. Purity evaluation has opened the way to impurity profiling, which takes consideration of genotoxic and degradation products that may have an impact on a drug's ADMET parameters. Strict impurity detection and control are stressed by regulatory bodies like the USFDA, Health Canada, and ICH in both APIs and final products. TLC, HPLC, and HPTLC are examples of modern analytical procedures that are essential for identifying and characterizing impurities, and their value in pharmaceutical research is only advancing^[5].

➤ *Impurities Limits*

The ICH Suggestions for impurities in new medicinal drugs specify that, unless suspected impurities are anticipated to be exceptionally harmful, identification of impurities below the 0.1% limit is not deemed necessary. The ICH states that the maximum daily dose qualifying criteria to be taken into consideration is as follows: < 2g/day 0.1% or 1 mg per day consumption (whichever percentage is lower) >2g/day 0.05%^[6].

➤ *Impurity Control Framework for Regulation*

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) developed multidisciplinary rules that regulate impurity management in pharmaceuticals. ICH Q3A, Q3B, Q3C, Q3D, and Q6A/Q6B, along with M3 and M7, are the most significant articles.

➤ *ICH Q3A – Impurities in New Active Substances*

The intention of this guideline is to observe, categorize, and evaluate contaminants in recently created active pharmaceutical ingredients (APIs). From raw materials, reagents, intermediates, by-products, filtering aids, or degradation, organic contaminants can develop during synthesis or storage. It describes how contaminants should be classified (known vs. unknown), recorded in specifications (connected to Q6A/Q6B), and found using the proper analytical methods. To evaluate impurities in clinical or toxicological batches, safety studies are also necessary. Stereo Chemistry purity, including enantiomeric impurities and other non-chiral elements, must be given special consideration^[7].

➤ *ICH Q3B – Impurities in New Drug Products*

Similar to Q3A, the following regulation is applicable for finished dosage forms. Drug product impurities typically develop during production or storage, frequently as API degradation products or as a result of interactions with excipients, processing aids, or packaging materials. These degradation products must be specified in specifications, in addition to permissible limits^[8].

➤ *ICH Q3C – Residual Solvents*

Regarding solvents that might still be present in APIs or pharmaceutical products, Q3C establishes limitations. Managing the solvent used during synthesis is crucial since it can affect essential features like yield, purity, solubility, and crystal structure. There are three distinct groups for solvents:

- Class 1: Avoid highly poisonous substances, such as benzene.
- Class 2: Permitted daily exposure (PDE) with restricted use.
- Class 3: Low toxicity; ALARP (As Low as Reasonably Practicable) guidelines are used for administration. Regulators typically expect process capability evaluations for all solvent categories, although ALARP is officially only required for Class 3^[9].

➤ *ICH Q3D – Elemental Impurities*

The permitted limits of elemental contaminants in APIs and pharmaceutical products are outlined in this guideline. Leaching solvents from machinery or packing, residual catalysts from chemical reactions, and contaminants in excipients or active substances are examples of possible sources. Toxicity data evaluation, PDE determination for each component, and a risk-based control strategy are all necessary. Catalyst use is emphasized as having benefits for the economy and the environment, increasing yields and decreasing waste^[10].

➤ *ICH Q6A & Q6B – Specifications*

- Q6A: Specifies a framework for specifying requirements for recently developed chemically synthesized medication ingredients and the final products.
- Q6B: Highlights biological and biotechnological items. When taken as a whole, these recommendations help

manage impurities by defining the proper way to create and justify specifications ^[11,12].

III. IMPORTANCE OF IMPURITY PROFILING

In order to ensure the safety, effectiveness, stability, and overall quality of APIs and their formulations, impurity profiling is essential in the pharmaceutical industry. It is now a requirement for new medication submissions. Impurities that may occur during synthesis, manufacture, or storage are systematically identified, described, and quantified as part of the process. Advanced analytical techniques including mass spectrometry, spectroscopy, and chromatography are utilized for quality control to identify and trace contaminants during the production process. This guarantees that the finished pharmaceutical product maintains its high quality and complies with regulatory requirements.

Quality control and assurance detect, measures, and controls contaminants in order to assure the quality of medications ^[13].

Regulatory Compliance Strict adherence to GMP guidelines is necessary, as mandated by organizations like the FDA, to preserve API integrity and safeguard public health ^[14].

Stability Studies evaluates shelf life and guides storage conditions by revealing degradation routes and products ^[15].

Process Optimization assists in reducing process-related contaminants by emphasizing the various manners in which manufacturing variables affect impurity advancement ^[16].

Formulation Development guarantees robust formulations by aiding excipient selection and process design via an understanding of impurity sources ^[17].

Patient Safety optimizes reliability and efficacy by strictly identifying and handling contaminants to comply with legal standards. Drug contaminants may have unfavourable pharmacological or toxicological effects that might reduce the medication's beneficial effects ^[18-19].

Analytical Method Validation Verifies the precision, accuracy, and dependability of the techniques used to identify and measure contaminants ^[20].

Comparability Studies provides consistency through assessment of changes in impurity profiles brought about by process or formulation alterations ^[21].

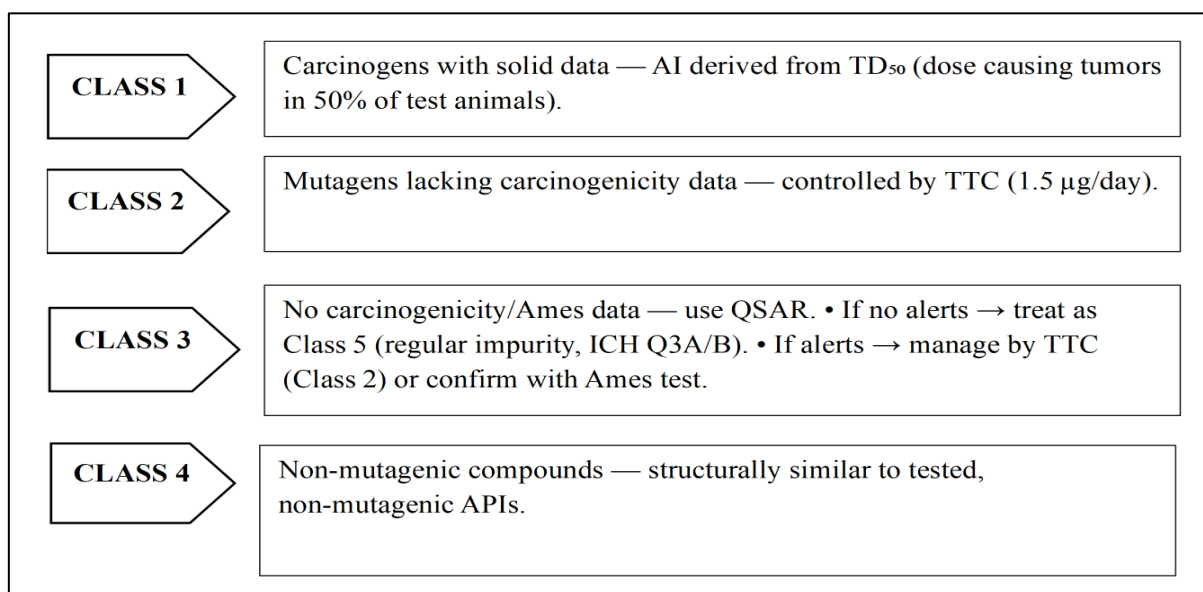
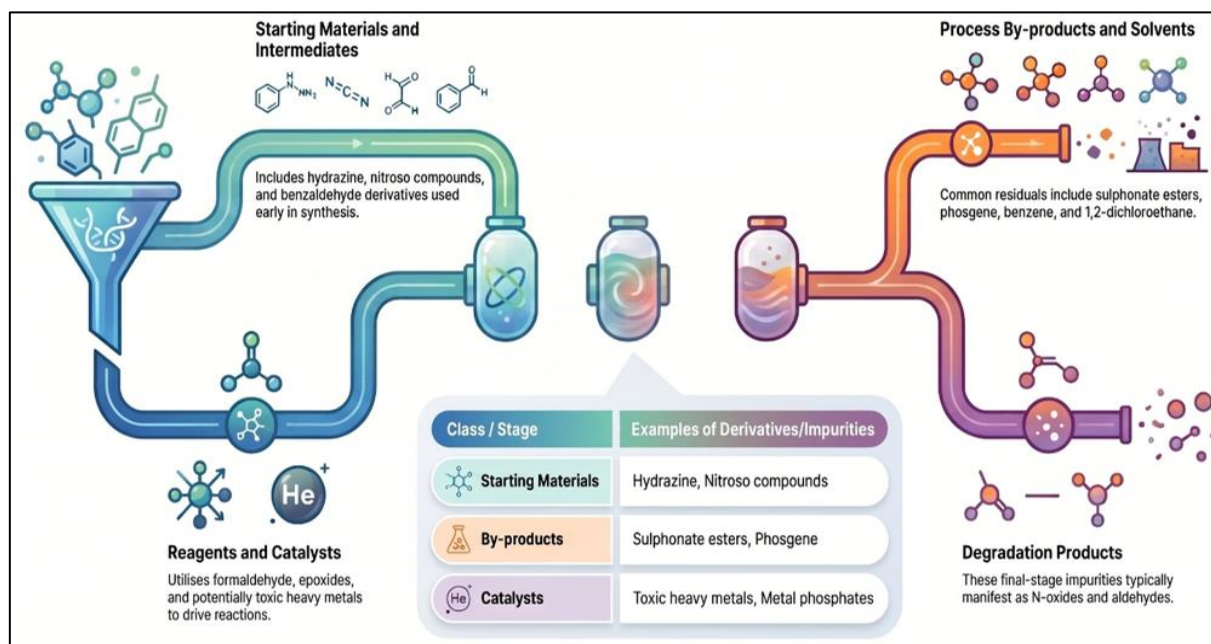


Fig 1 Impurity Categorization and Management ^[22]

➤ Sources of Genotoxic Impurities

The raw material utilized to make pharmaceutical compounds and their impurities is the main source of genotoxic impurities that can be integrated into drug substances. Similar to this, genotoxic intermediates and byproducts that are created during the manufacturing process can end up as genotoxic contaminants in the therapeutic ingredients. In addition to these, genotoxic contaminants in therapeutic compounds may also originate from solvents, catalysts, and reagents utilized during the synthesis process.

Impurities are created in pharmacological compounds as a result of degradation products produced during storage and transportation, as well as upon exposure to light, air oxidation, or hydrolysis. Stereoisomers of the raw material and intermediate also participate in the formation of chiral impurities in drug substances if a particular isomer of the therapeutic substance is needed ^[23].

Fig 2 Genotoxic Compounds that Occur in Drug Substances ^[24]➤ *Chemical Classes of Common Genotoxic Impurities:*

- Alkyl halides: Methyl halide, ethyl halide, and propyl halide.
- Epoxides: 2,3-Epoxypropanol (glycidol), 1-chloro-2,3-epoxypropane (epichlorohydrin), and 1,2-epoxy-3-butene.
- Hydrazines: 1,2-Dimethylhydrazine and 1,1-dimethylhydrazine.
- TEMPO derivatives: 2,2,6,6-Tetramethylpiperidin-1-oxyl (TEMPO), which may occur during the oxidation of alcohol intermediates.
- Aromatic amines: 4-Nitroaniline and 4-amino-2-nitrophenol.
- Boronic acids: Carboxyphenylboronic acid and methylphenylboronic acid.
- Sulfonate esters and their precursors: Methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS), methyl benzenesulfonate (MBS), and benzenesulfonic acid.
- Nitrosamines: *N*-Nitrosodimethylamine (NDMA), *N*-Nitrosodiethylamine (NDEA), and *N*-nitrosomethylphenylamine (NMPA) ^[25-26].

IV. ANALYTICAL TOOLS FOR DETERMINATION AND IDENTIFICATION

As a result of very low allowable levels, genotoxic contaminants require highly sensitive analytical procedures for accurate detection.

➤ *High-Performance Liquid Chromatography (HPLC)*

- Used for quantitative impurity profiling.
- Ideal for polar, non-volatile impurities ^[27].

➤ *Liquid Chromatography- Mass Spectrometry (LC-MS/MS)*

- High sensitivity for low-level impurity detection.
- Detects specific fragmentation patterns of genotoxic compounds ^[28].

➤ *Gas-Chromatography – Mass Spectrometry (GC-MS)*

- Traceable impurities in pharmaceutical materials to be deteriorated beyond analytical categorization are now feasible to identify according to this technique ^[29].

➤ *Capillary Electrophoresis – Mass Spectrometry (CE-MS)*

- Employed to distinguish and analyze enantiomeric contaminants in medications with stereochemical centers ^[30].

➤ *High Performance Thin Layer Chromatography (HPTLC)*

- Allows for the identification and quantification of even trace level of impurities.
- Enables parallel analysis of multiple impurities in a single run.

➤ *Nuclear Magnetic Resonance Spectroscopy (NMR)*

- By unveiling to the toxic context and relationship of organic contaminants, methods allow for their reliable detection.
- Facilitate comprehensive structural characterization of impurities ^[31].

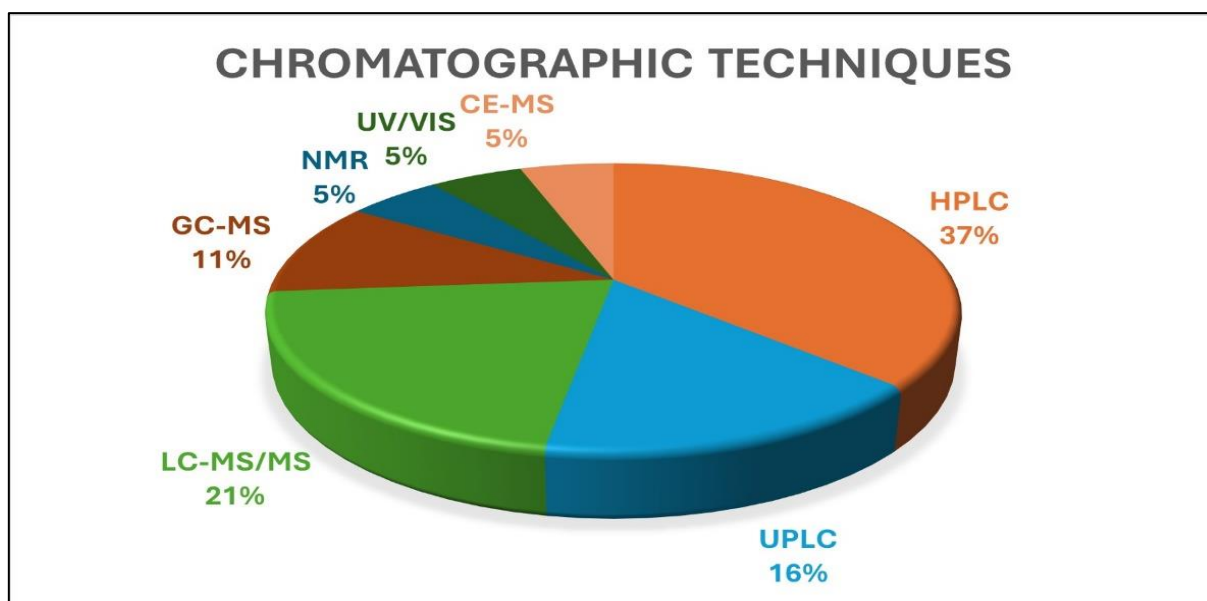
Table 1 The Minimum threshold for ICH-Compliant Impurity Detection, Standard Reporting, and Quantification ^[32].

Maximum dosage per day in (g)	Reporting threshold limit%	Limit for Identification threshold	Limit for Qualification threshold
≤2g/day	0.05%	0.1 or 1.0 mg/day (whichever is lower)	0.15% or 1.0 mg/day (whichever is lower)
≥2g/day	0.03%	0.05%	0.05%

The (ICH) M7 (R1) guideline, "Evaluation and Management of Mutagenic DNA Reactivity," An approach to impurity categorization, assessment, and control that is based on risk is offered by "Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk." The following are main tenets of ICH M7 (R1):

- Use in vitro, in silico and in vivo tests to detect genotoxic contaminants.

- To determine acceptable exposure limits, apply the threshold of toxicological concern (TTC).
- A system for categorizing impurities that utilizes data concerning mutagenicity.
- Strategies for control that take into account the probability of patient exposure ^[33].

Fig 3 Methods Reported in Various Analytical Techniques ^[34]Table 2 Comparative Studies of Analytical Techniques for Impurities ^[35]

S.NO	METHODS	DETECTION LIMIT	ADVANTAGES	LIMITATIONS	APPLICATIONS
1	Thin layer chromatography (TLC)	~0.5%	Economical, Resource-efficient.	Lower sensitivity because detection relies on visual spots	Rapid screening of mixtures and early-stage analysis.
2	High-performance thin layer chromatography (HPTLC)	~0.1%	Higher resolution. with sharper image and detailed view.	Requires specialized equipment for automated sample applicators, sophisticated development chambers, and densitometric scanners.	Simultaneous multi-sample comparison and Quantitative analysis with densitometry.
3	High-performance liquid chromatography (HPLC)	~0.01%	Excellent specificity and sensitivity.	Expensive cost and maintenance.	Impurity and degradation analysis in drugs.
4	Gas chromatography (GC)	~0.001%	Outstanding for volatile chemicals.	High resolution separation of volatile and thermally stable compounds.	Separation of volatile and semi-volatile compounds.

5	Liquid chromatography coupled with mass spectrometry (LC-MS)	~0.001%	Incomparable sensitivity as well as specificity.	Ultra-high sensitivity for trace and genotoxic impurity detection.	Ultra-sensitive detection of trace impurities (e.g., nitrosamines)
6	Nuclear magnetic resonance spectroscopy (NMR)	~0.1%	Structural elucidation chemical environment of nuclei and molecular framework.	Chemical environment analysis, detailed structural information and Quantitative accuracy.	Detailed structural elucidation and Stereochemistry and conformational analysis.

Table 3 Classification of Antidiabetic Drugs with US-FDA Approval [36]

S.NO	CLASSIFICATION OF DRUG	DRUGS NAME	FDA APPROVAL
1.	Sulfonylureas	Glyburide	1984
		Glipizide	1984
		Glimepiride	1995
2.	Alpha Glucosidase Inhibitors	Acarbose	1996
		Miglitol	1999
3.	Biguanide	Metformin	1999
4.	Biguanide and Sulfonyl urea combination	Metformin: Glyburide	2004
		Metformin: Glipizide	2005
5.	Biguanide and TZD combination	Metformin: Pioglitazone	2005
6.	Biguanide and DPP-4i combination	Metformin: Sitagliptin	2007
		Metformin: Sitagliptin	2010
		Metformin: Linagliptin	2012
		Metformin: Alogliptin	2013
7.	Biguanide and SGLT2i combination	Metformin: Canagliflozin	2014
		Metformin: Dapagliflozin	2014
		Metformin: Empagliflozin	2015
		Metformin: Ertugliflozin	2017
8.	Biguanide, SGLT2i and DPP-4i combination	Metformin: Saxagliptin: Dapagliflozin	2019
		Metformin: Linagliptin: Empagliflozin	2020
9.	Amylin analogue GLP1R agonist	Pramlintide	2005
		Exenatide	2005
		Liraglutide	2010
		Dulaglutide	2014
		Albiglutide	2014
		Lixisenatide	2016
		Semaglutide	2017
10.	Sulfonyl urea and TZD combination DPP-4i	Glimepiride: Pioglitazone	2006
		Sitagliptin	2006
		Saxagliptin	2009
		Linagliptin	2011
		Alogliptin	2013
11.	Meglitinides	Nateglinide	2009
		Repaglinide	2013
12.	Bile acid sequestrant Dopamine Receptor agonist	Colesevelam	2008
		Bromocriptine	2009
13.	SGLT2 inhibitor	Canagliflozin	2013
		Dapagliflozin	2014
		Empagliflozin	2014
		Ertugliflozin	2017
14.	DPP-4i and TZD	Alogliptin: Pioglitazone	2013
15.	DPP-4i and SGLT2 inhibitor	Linagliptin: Empagliflozin	2015
		Sitagliptin: Ertugliflozin	2017
		Saxagliptin: Dapagliflozin	2017
16.	SGLT2 inhibitor	Bexagliflozin	2023

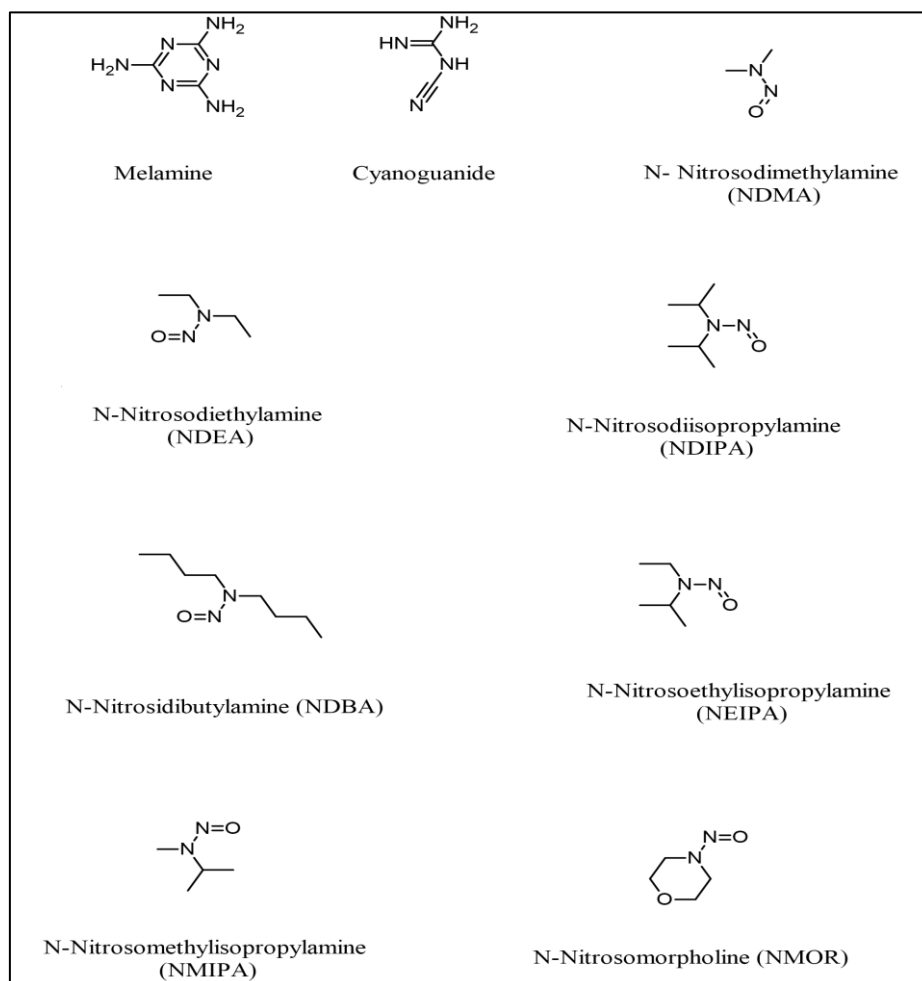


Fig 4 Structures of Impurities

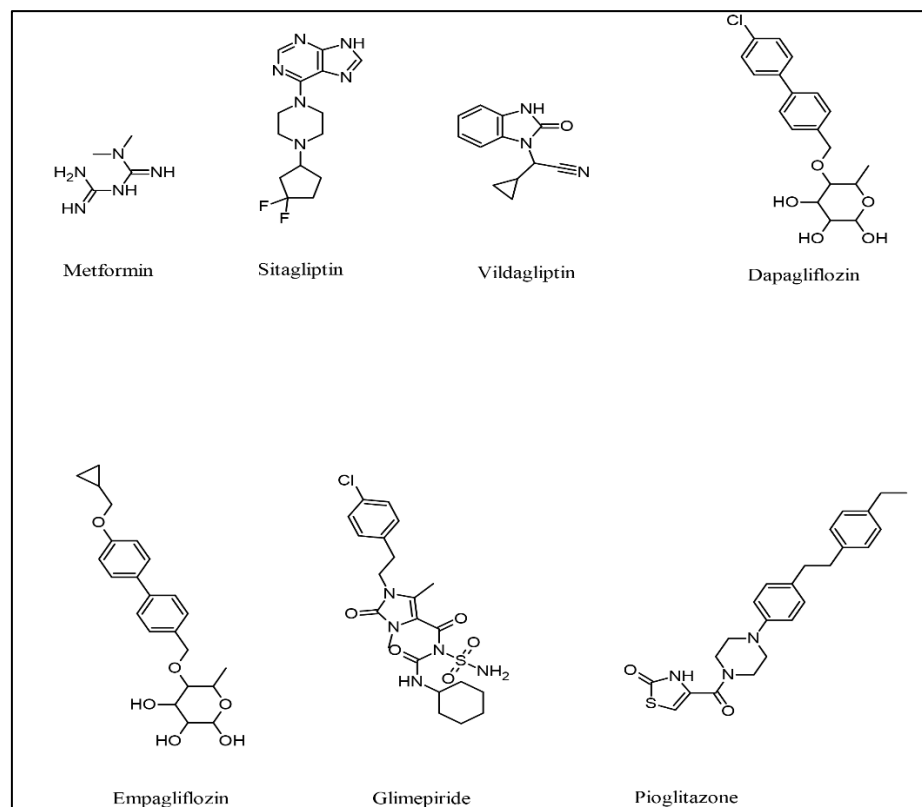


Fig 5 Structure of Some Antidiabetic Drugs

➤ *UV Methods for Impurities Profiling:*

Abdallah FF et al. presents a novel quadruple divisor spectrophotometric method (QD) for the simultaneous determination of metformin (MET), its toxic impurities (melamine, cyanoguanidine), and co-formulated drugs (glipizide, sitagliptin) in pharmaceutical mixtures. The authors emphasize that conventional chromatographic methods, though reliable, are costly and require separation steps. In contrast, the QD method is eco-friendly (using water as solvent), simple, and does not require prior extraction or separation, making it highly suitable for routine analysis. The method effectively resolves severe spectral overlap among the five analytes. Validation studies demonstrated excellent linearity (correlation coefficients ≥ 0.9996), high accuracy (~ 99 – 101% recovery), and precision (RSD $< 2\%$), with very low detection limits (e.g., melamine LOD = $0.133 \mu\text{g/mL}$). The approach worked well with conventional formulations like Janumet and Engilor, with results statistically comparable to RP-HPLC, confirming its robustness. In conclusion, El-Ghobashy et al. show that the QD spectrophotometric method is a green, sensitive, precise, and cost-effective alternative to chromatographic techniques, aligning with ICH validation guidelines and offering a practical solution for quality control and impurity monitoring in metformin-based combinations [37].

Kafrawy A et al. presents validated UV spectrophotometric methods for the simultaneous determination of metformin (MET), pioglitazone (PIO), and the toxic impurity melamine (MEL). The authors developed three approaches to overcome spectral overlap among these compounds. The zero-order method allowed direct quantification of PIO at 268 nm, where MET and MEL show negligible absorbance. For MET, a double divisor ratio spectra method was applied, using a binary mixture of PIO and MEL as the divisor and measuring the second derivative at 254 nm. Additionally, an isoabsorptive point method was employed at 255 nm, where MET and PIO have equal absorptivity; PIO was first determined at 268 nm, and its contribution subtracted to yield MET's absorbance. All methods demonstrated excellent linearity, accuracy, and precision, with recovery values close to 100% and relative standard deviations within acceptable limits. They were successfully applied to Bioglita Plus tablets, showing no interference from excipients and results comparable to TLC–densitometric and reported HPLC methods. In conclusion, these UV spectrophotometric methods are simple, rapid, eco-friendly, and reliable alternatives for routine quality control of metformin–pioglitazone combinations in the presence of melamine impurity [38].

Table 4 RP-HPLC/HPLC Methods for Organic and Process-Related Impurities in Antidiabetic Drugs

Sl. No.	Author	Drug	Impurity	Analytical Method	Major Chromatographic Conditions	Major Findings	Ref.
1	Yu Yuan et al.	Pramlintide acetate	Process & degradation impurities	RP-HPLC/SCX-HPLC/LC-MS	C8 column, phosphate buffer–ACN gradient	13 impurities separated; LC-MS confirmation	[45]
2	Tudi Avinash Reddy et al.	Liraglutide	Impurity A & B	RP-HPLC	C18, phosphate buffer–MeOH gradient	Validated stability-indicating method	[46]
3	Kun Zhang et al.	Alogliptin benzoate	Seven impurities	HPLC	CN column, water–ACN–TFA gradient	Seven impurities characterized	[47]
4	A. Madhavi et al.	Nateglinide	Imp-1 & Imp-2	HPLC	C8, phosphate buffer: ACN (50:50)	Stability-indicating method	[48]
5	Sakshi Gupta et al.	Glipizide	Four degradation products	RP-HPLC	C18, KH_2PO_4 –MeOH gradient	Four DPs identified	[49]
6	Lixiang Zhang et al.	Liraglutide	Five peptide impurities	HPLC	C8, phosphate buffer–ACN	Validated QC method	[50]
7	Krishna Reddy et al.	Repaglinide	Four impurities	HPLC	C18, KH_2PO_4 : ACN	Impurities isolated & characterized	[52]
8	G. Navaneethan et al.	Pioglitazone + Glimepiride + Metformin	Related compounds	RP-HPLC	Cyano column, phosphate buffer–ACN	Validated stability-indicating method	[53]
9	Patil Pallavi et al.	Nateglinide	Unknown impurities	HPTLC	Chloroform: Methanol (8:2)	Impurity profiling	[55]
10	Prasad Kancherla et al.	Repaglinide	Seven novel impurities	UPLC	BEH C18, formic acid gradient	Novel impurities characterized	[56]

➤ *Green UV Method:*

Abdallah et al. (2026) propose a novel green spectrophotometric approach for simultaneous quantification of metformin (MET), empagliflozin (EMP), and melamine (MEL). The authors highlight the analytical challenge posed by severe spectral overlap between MET and MEL due to structural similarity, which traditionally necessitated separation techniques. Three complementary spectrophotometric approaches were established in phosphate buffer (pH 3.6): the ratio difference method for metformin at 238–244 nm, first-derivative spectrophotometry for meloxicam at 217 nm, and second-derivative ratio spectra for empagliflozin at 284 nm. Validation in accordance with ICH Q2(R1) confirmed excellent linearity, accuracy (98–102% recovery), precision ($RSD \leq 1.3\%$), and low detection limits. Robustness was demonstrated by successful application to Empagliflozin tablets without the need for chromatographic separation. Environmental sustainability was assessed using NEMI, Eco-Scale, GAPI, AGREE, and BAGI, all of which indicated minimal ecological impact. This represents the first separation-free, eco-friendly spectrophotometric protocol for these drugs, offering an affordable and globally applicable quality-control solution, particularly valuable in resource-limited settings [39].

➤ *TLC Methods:*

Kafrawy A et al. focuses on the simultaneous determination of metformin (MET) and pioglitazone (PIO) in the presence of metformin impurity (melamine, MEL) using different spectrophotometric and TLC–densitometric methods. The study highlights those conventional chromatographic methods, while accurate, are resource-intensive. To address this, the authors developed simple, cost-effective, and eco-friendly spectrophotometric approaches alongside a TLC–densitometric method. These methods successfully resolved spectral overlap between MET, PIO, and MEL without requiring prior separation. Validation results confirmed excellent linearity, accuracy, and precision, with recovery values close to 100% and relative standard deviations within acceptable limits. The TLC–densitometric method provided clear separation and quantification of the three analytes, while spectrophotometric techniques offered rapid and reliable alternatives. The methods were applied to commercial tablet formulations, showing no interference from excipients and yielding results comparable to reference methods. In conclusion, Khorshid *et al.* demonstrated that spectrophotometric and TLC–densitometric methods are robust, sensitive, and practical tools for routine quality control, ensuring reliable monitoring of metformin and pioglitazone in the presence of toxic impurities [38].

Abdelwahab et al. (2019) explains two proven chromatographic techniques for measuring metformin (MET) and vildagliptin (VIL) in the presence of the hazardous contaminant melamine (MEL). Using a methanol–chloroform–formic acid system (7:3:0.3 v/v) on silica gel plates scanned at 215 nm, the first technique is a thin-layer chromatography (TLC) densitometric approach. For VIL (0.2–2.6 $\mu\text{g}/\text{band}$), MET (0.4–4.5 $\mu\text{g}/\text{band}$), and MEL (0.05–1.4 $\mu\text{g}/\text{band}$), linearity was demonstrated. The second technique is reversed-phase ultra-performance liquid

chromatography (RP-UPLC), which is carried out on a C18 column using a mobile phase of methanol–acetonitrile–phosphate buffer that contains sodium lauryl sulfate (pH 5) and is measured at 205 nm. Excellent linearity was demonstrated by calibration curves over 1–50 $\mu\text{g}/\text{mL}$ (VIL), 2–70 $\mu\text{g}/\text{mL}$ (MET), and 0.5–30 $\mu\text{g}/\text{mL}$ (MEL). Both methods were successfully applied to Galvus Met tablets without interference from excipients. Validation in line with ICH guidelines confirmed robustness, accuracy, and precision, with results showing strong agreement with previously reported approaches. Overall, the study demonstrates that RP-UPLC and TLC densitometry are simple, reliable, and accurate techniques for the simultaneous determination of antidiabetic drugs and hazardous contaminants, establishing them as valuable tools for routine pharmaceutical quality control [40].

➤ *In-silico Methods for Toxicity Evaluation:*

Baira SM et al. reported that Anagliptin was systematically assessed under hydrolytic, oxidative, photolytic, and thermal stress conditions. Significant degradation was observed in acidic, basic, neutral, and oxidative environments, whereas the drug remained stable under thermal and photolytic stress. LC/ESI/QTOF/MS/MS analysis enabled the identification and structural characterization of seven degradation products (DP1–DP7) through precise mass measurements. Furthermore, molecular docking and in silico toxicity prediction were employed to compare the biological activity and toxicity of these products with the parent compound. Toxicity evaluation was performed using TOPKAT software, which applies quantitative structure–toxicity relationship (QSTR) models to generate probability values across multiple animal systems. Toxicological profiling of the degradation products (DPs) was performed using TOPKAT, with endpoints classified as high probability (positive; ≥ 0.7) or low probability (negative; ≤ 0.3). Hazardous responses were observed for DP2, DP3, and DP5 across several animal models. Molecular docking analysis revealed that DP4 exhibited a superior glide score compared to the parent drug and established broader interactions with key residues of the target protein, suggesting its potential as a lead scaffold for future DPP-4 inhibitor development. The UPLC/PDA method employed was validated according to ICH guidelines and proven suitable for quantitative determination of anagliptin in bulk drug, pharmaceutical formulations, and stability samples [41].

Gumieniczek A et al. A novel stability-indicating LC method was developed and validated for the determination of empagliflozin (EMPA) and its major impurities in coated tablets, in compliance with ICH guidelines. The technique exhibited high sensitivity, with LOD values of 35 $\text{ng}\cdot\text{mL}^{-1}$ and 115 $\text{ng}\cdot\text{mL}^{-1}$ for impurities A and B, respectively. Strong linearity ($r > 0.999$), selectivity, precision ($< 2.0\%$ for EMPA and $< 5.0\%$ for synthetic impurities), accuracy (99.09–119.58%), and robustness were achieved. These attributes confirm its suitability for routine quality control applications. In addition, in silico toxicity assessments were performed to evaluate the potential toxicological risks associated with the detected impurities. AMES mutagenicity, hepatotoxicity, and skin sensitization were predicted using pkCSM, whereas

mutagenic, tumorigenic, irritant, and reproductive toxicity potential was evaluated using OSIRIS Property Explorer. Impurities A and B had no discernible theoretical harmful potential when compared to the toxicity profile of EMPA;

OSIRIS results ranged from none to moderate to medium, while pkCSM predictions indicated either positive or negative effects depending on the parameter evaluated ^[42].

Table 5 Methods for Melamine and Cyanoguanidine Impurity Determination

Sl. No.	Author	Drug	Target Impurity	Method	Major Conditions	Key Findings	Ref.
1	Michael G. Fawzy et al.	Metformin, Empagliflozin, Canagliflozin	Melamine	HPLC	Symmetry column, KH ₂ PO ₄ /heptane sulfonic acid–ACN	Simultaneous determination	[57]
2	El-Zaher AA et al.	Metformin combinations	1-Cyanoguanidine	RP-HPLC	CN column, phosphate buffer–ACN	Routine QC method	[58]
3	Gabriela Kłaczko et al.	Metformin HCl	Cyanoguanidine, Melamine, Triazine impurities	RP-HPLC	SCX column	Trace impurities quantified	[61]
4	NS Abdelhamid et al.	Repaglinide + Metformin	Melamine	HPLC	ACN: MeOH: Buffer	8 min separation	[62]
5	Y. Zhou et al.	Metformin HCl	Melamine, Dicyandiamide	SPE-HPLC-MS	C18 with SPE cleanup	Sensitive trace analysis	[63]
7	B.D. Musmade et al.	Metformin + Teneligliptin	Melamine, Cyanoguanidine	RP-HPLC	BDS C18, ion-pair gradient	Validated impurity profiling	[64]

Table 6 Nitrosamine and Genotoxic Impurity Methods

Sl. No.	Author	Drug	Genotoxic/Nitrosamine Impurity	Method	Findings	Ref.
1	M. Manivannan et al.	Teneligliptin	p-Anisaldehyde	GC-MS	LOD 0.12 ppm	[51]
2	Bashar Al-Sabti et al.	Alogliptin	Pyridine, DMAP, 3-AP, DMA	LC-MS	PGI determination	[54]
3	Zhen Wang et al.	Sitagliptin	NTTP	UPLC-MS/MS	LOD 0.037 ppm	[65]
4	Chittireddy et al.	Sitagliptin	NTTP	UPLC-TQ-MS	LOD 0.002 ppm	[66]
5	Xiao T et al.	Metformin + Empagliflozin	Benzyl peroxide	UPLC-MS	Trace PGI determination	[67]
6	Ferrazza Alves Giordani et al.	Vildagliptin	N-Nitroso-vildagliptin	UPLC-QTOF-MS/MS	Nitrosamine profiling	[72]
7	Gosar A et al.	Canagliflozin	Hydroperoxide impurity	RP-HPLC	Validated method	[73]

Table 7 Stability-Indicating and Degradation Studies

Sl. No.	Author	Drug	Method	Major Outcome	Ref.
1	Mahajan N et al.	Vildagliptin	RP-HPLC	Stress degradation profiling	[68]
2	Patel BD et al.	Vildagliptin	RP-HPLC	Impurity B & G quantified	[69]
3	Gogikar SK et al.	Imeglimin	LC-MS/MS	Four degradation products identified	[70]
4	Shah D et al.	Rosuvastatin + Teneligliptin	RP-HPLC	Teneligliptin impurity A	[71]
5	Machairas G et al.	Rosuvastatin + Metformin	HILIC	Impurity profiling	[59]
6	Uber et al.	Vildagliptin + Metformin	HPLC-MS/MS	Metformin impurities A–C	[60]

Table 8 Reported Green Chromatographic Methods for Determination of Melamine, Cyanoguanidine, Nitrosamine and Related Impurities in Antidiabetic Drugs.

Authors	Drug(s)	Impurity/Related Compound	Analytical Method & Chromatographic Conditions	Key Findings	Ref.
Aml A. Emam <i>et al.</i>	Metformin, Glipizide, Sitagliptin	Melamine, Cyanoguanidine	HPTLC ; Silica gel 60 F254; Mobile phase: Ethyl acetate: methanol: ammonia: formic acid (7:2:0.2:0.2, v/v); Detection: 235 nm	Eco-friendly and ICH Q2(R1)-validated method with excellent linearity ($r > 0.999$); successfully applied to marketed tablets.	[74]
Naglaa A.S. Kabil <i>et al.</i>	Metformin, Bisoprolol	Melamine, Cyanoguanidine	RP-HPLC ; Symmetry C18 (2.1 × 50 mm, 1.7 μ m); Mobile phase: SDS-OPA buffer (pH 5) + 10% IPA; UV 230 nm	Accurate and selective method with excellent greenness scores (Eco-scale 81, MoGAPI 89).	[75]
Nashwa A. Ibrahim <i>et al.</i>	Dapagliflozin, Metformin	Melamine, Cyanoguanidine	RP-HPLC : C18; Methanol: Water (90:10, pH 3.5); 225 nm. HPTLC : Ethanol: Ethyl acetate (1:9)	Both HPLC and HPTLC methods were validated, accurate, and environmentally friendly for tablet analysis.	[76]
Raghda A. Emam, Aml A. Emam	Canagliflozin, Metformin	Melamine, Cyanoguanidine (degradation product)	TLC ; Acetone: Ethyl acetate: Acetic acid (8:2:0.2); Detection: 205 nm	Stability-indicating TLC assay validated per ICH; greenness evaluated using Eco-Scale and GAPI.	[77]
Dina S. El-Kafrawy <i>et al.</i>	Empagliflozin, Linagliptin, Metformin	Cyanoguanidine, Melamine	HPLC-DAD : Agilent C18; phosphate buffer–methanol gradient. HPTLC : Ethanol: Ethyl acetate: Water (3.5:3:1)	First eco-friendly stability-indicating methods for simultaneous estimation of APIs and impurities.	[78]
Sriram Pepakayala <i>et al.</i>	Sitagliptin API	NDMA, NDIPA, NIPEA, NMAP	LC-APCI-MS/MS ; Poroshell EC-C18; FA-water/MeOH–ACN gradient	Highly sensitive ICH Q2(R2)-validated method; no nitrosamines detected in tested batches.	[79]
Haydi S. Elbordiny <i>et al.</i>	Metformin, Rosuvastatin	Cyanoguanidine, RSV impurity B	Capillary electrophoresis and RP-HPLC-DAD using gradient phosphate buffer–ACN–MeOH	Stability-indicating methods with excellent greenness and rapid analysis.	[80]
Vaishnavi Chintala <i>et al.</i>	Vildagliptin	VLN-1, VLN-2	UHPLC-MS/MS ; XBridge C18; FA–ammonium acetate/ACN–MeOH gradient	Trace-level impurity determination with LOQ 60 ng/mL and satisfactory greenness.	[81]
Mohammed Shahid Ali <i>et al.</i>	Metformin	Cyanoguanidine, Melamine	Mixed-mode HILIC ; Silica column; ACN–buffer mobile phase	Stability-indicating method with effective impurity separation under stress conditions.	[82]

V. DISCUSSION

The studies reviewed demonstrate a clear transition in impurity profiling of antidiabetic drugs from conventional quality control toward comprehensive risk-based assessment. Although process-related impurities, degradation products, residual solvents, and elemental impurities continue to require routine monitoring, increasing attention has been directed toward genotoxic impurities, particularly nitrosamines, melamine, and cyanoguanidine, because of their potential toxicological impact at trace concentrations. This shift has expanded the scope of impurity profiling beyond regulatory compliance to include proactive evaluation of patient safety throughout the drug lifecycle. Comparison

of the reported analytical methods indicates that the choice of technique depends primarily on the nature and concentration of the impurity. RP-HPLC remains the preferred method for routine impurity determination because of its robustness, reproducibility, and wide regulatory acceptance, whereas LC-MS/MS and UPLC-MS/MS have become indispensable for ultra-trace analysis and structural confirmation of genotoxic impurities. HPTLC and advanced spectrophotometric methods offer economical and environmentally friendly alternatives for routine quality control but are generally less suitable for trace-level impurity determination. Recent studies also highlight the growing integration of green analytical chemistry principles and in silico toxicity

prediction, enabling sustainable analytical workflows while supporting regulatory risk assessment.

Despite substantial advances, significant analytical challenges remain due to the diverse physicochemical properties of antidiabetic drugs and their associated impurities, making simultaneous determination within a single analytical platform difficult. The literature indicates an increasing emphasis on developing stability-indicating,

highly sensitive, and environmentally sustainable analytical methods capable of meeting evolving regulatory expectations. Therefore, future impurity profiling strategies should focus on combining advanced chromatographic techniques with green analytical approaches and predictive computational tools to achieve reliable, efficient, and comprehensive impurity monitoring in antidiabetic pharmaceuticals.

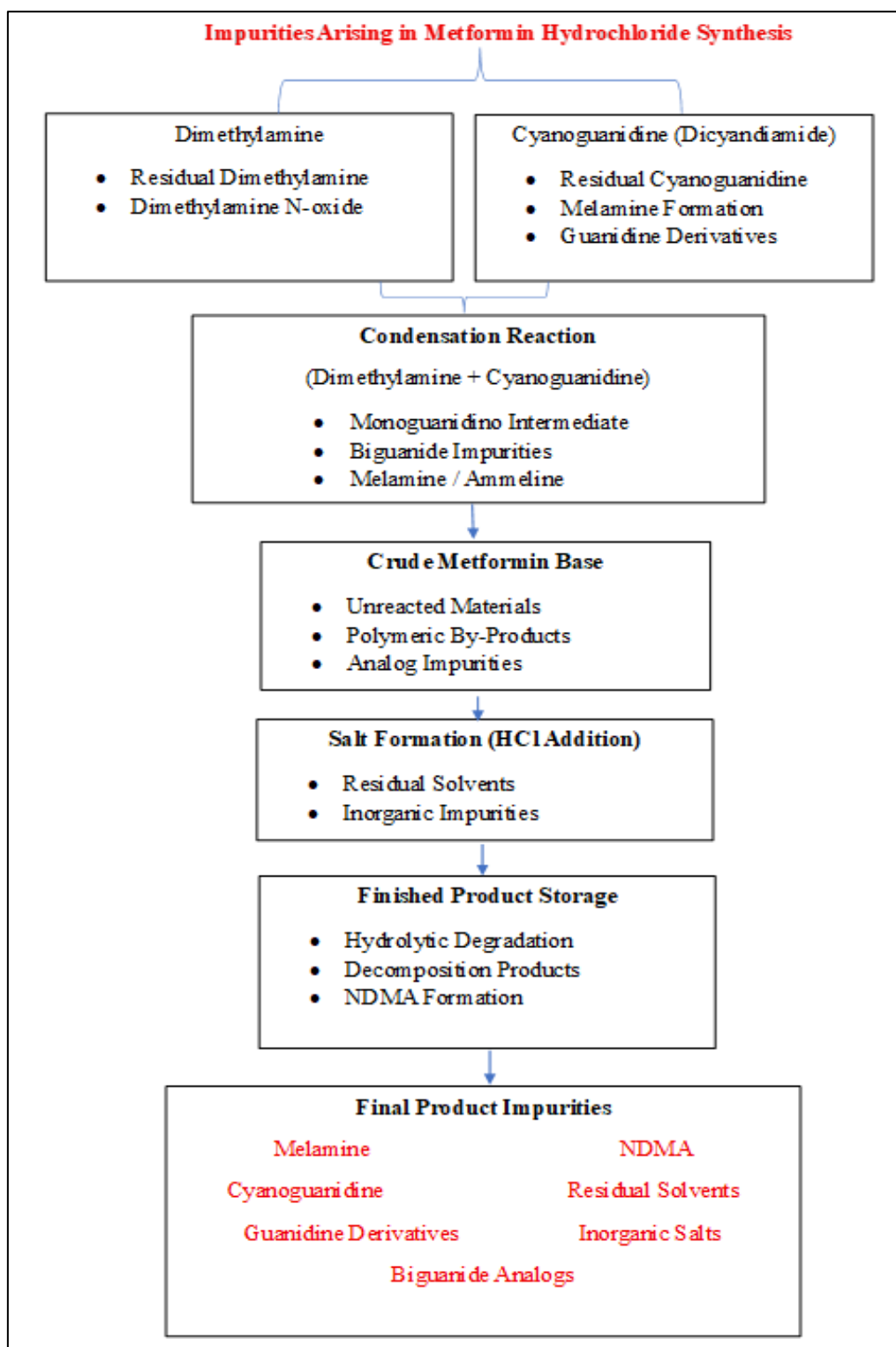


Fig 6 Impurity Arising in Metformin Synthesis ^[43-44]

➤ Future Perspective

Three key pillars of the future direction of impurity profiling of antidiabetic drugs are likely to be increased sensitivity, harmonization of regulations, and sustainability. As the number of nitrosamine and other genotoxic impurities in medications like metformin and sitagliptin are increasingly detected, regulatory requirements based on the Food and Drug Administration and ICH M7 requirements will persist to necessitate ultra-trace quantification on highly selective LC–MS/MS and high-resolution mass spectrometry systems. At the same time, the principles of green analytical chemistry are likely to transform the chromatographic procedures by introducing the more extensive use of micellar systems, mobile phases based on ethanol, miniaturization of the extraction, and the strategy of solvent-reduction. New instruments like supercritical fluid chromatography, coupled with chemometrics near-infrared spectroscopy, and in silico toxicity prediction models can be used to enhance proactive impurity risk assessment. The next revolution of the sustainable and regulatory-conforming pharmaceutical testing could be the integration of artificial intelligence that will enable constructive modelling of predictive impurity formation and real-time quality control.

VI. CONCLUSION

The quality, safety, and efficacy of antidiabetic medications depend on impurity profiling. In accordance with regulations and safeguard patient health, impurities resulting from synthesis, degradation, residual solvents, elemental pollutants, and genotoxic chemicals must be closely watched and managed. The detection and characterization of contaminants at trace levels have greatly improved thanks to sophisticated analytical methods as HPLC, LC–MS/MS, GC–MS, HPTLC, and NMR. In addition, sustainable and effective impurity monitoring has been improved by the use of green analytical techniques and in silico toxicity evaluation. The development of safer antidiabetic treatments will be aided by ongoing improvements in analytical technologies and regulatory approaches, which will also reinforce pharmaceutical quality assurance.

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