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SHORT REVIEW ON GENOTOXIC IMPURITIES IN SARTANS

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ABSTRACT

The nitrosodimethylamine (NDMA), a confirmed genotoxic impurity, was first reported in valsartan active pharmaceutical ingredient (API) by the European Medicines Agency (EMA) in 2018. Manufacturers performed voluntary recalls followed by investigations of contamination by health regulatory authorities. In this review, we discussed potential sources, mechanism of the formation of NDMA, and other nitrosamines impurities in valsartan, losartan, irbesartan, candesartan, and olmesartan. Additionally, we summarized currently available validated analytical techniques for controlling these impurities as suggested by regulatory authorities. An overview of measurements by regulatory authorities was mentioned, and we also outlined suggestions for viable solutions to avoid future crises.

KEYWORDS: Nitrosamine, ARBs, genotoxic impurities, valsartan recalls.

1.0 INTRODUCTION

Hypertension, also known as high blood pressure, is characterized by systolic blood pressure (SBP) of 140 mmHg or above and diastolic blood pressure (DBP) of 90mmHg or more. According to World Health Organization (WHO) statistics, approximately 1.3 billion people globally suffer from hypertension, which substantially elevates the risk of renal, cardiovascular, brain, and other diseases.[1] For this reason, it is recognized as the primary cause of premature death globally. Commonly prescribed medications for tackling hypertension include angiotensin-converting enzyme (ACE) inhibitors, angiotensin-2-receptor blockers (ARBs), diuretics, and calcium channel blockers. Angiotensin-2-receptor blockers (Fig.1) also known as sartans, are antagonists of angiotensin-2, which causes vasoconstriction and consequently raises blood pressure (BP).[2] Because of their

effectiveness, they are listed among the top prescribed drugs in the USA. For example, in 2018, the total number of prescriptions for losartan exceeded 50 Million.[3]

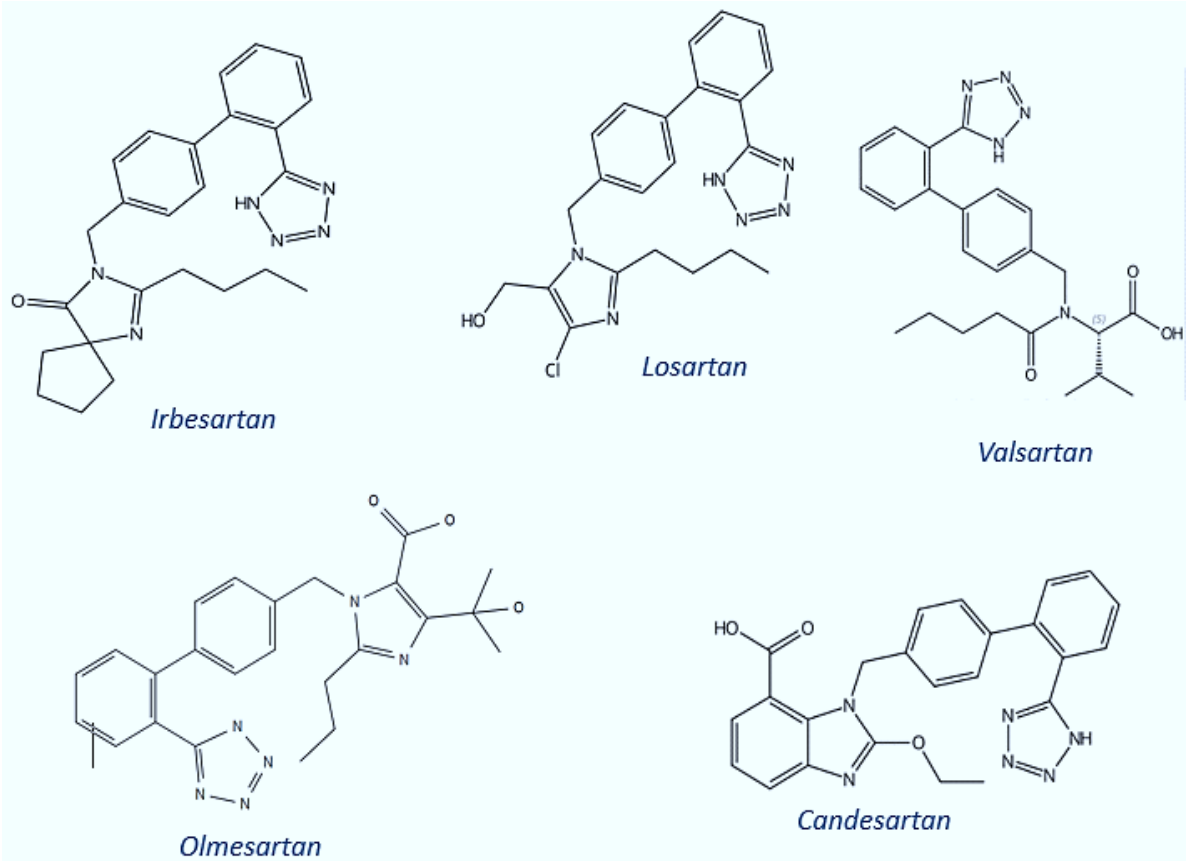


Figure 1: Five representatives of ARBs.

1.1 Valsartan recalls

In 2018 multiple pharmaceutical distributors and companies performed voluntary recalls of valsartan-containing products following command by the EMA and the US Foods and Drug Administration (FDA) after the EMA discovered N- nitrosodimethylamine (NDMA) in valsartan API.[4] Almost 2300 batches distributed worldwide to countries in the EU, Canada, and Bahrain were recalled.[5] In August 2018, the FDA outlined sixteen companies distributing valsartan API implicated in containing nitrosamine impurities.

Following the actions taken by the pharmaceutical authorities, the number of patients using valsartan decreased sharply due to two main reasons. First, recalls affected the number of uncontaminated valsartan products due to the limited number of companies producing the API. This crisis implied the risks of dependence on a few API manufacturers of life-saving drugs. Second, the number of patients willing to take the drug decreased due to heightened misinterpretation of the news regarding the risk associated with carcinogenicity of

nitrosamines. Because of the importance of sartans to patients with hypertension and the inadequate quantity remaining due to the recalls, the FDA had to perform a risk-to-benefit evaluation of using contaminated products. The benefits outweighed the risks, and interim acceptable intake (AI) levels ranging from 26.5-96ng/day were established for manufacturers to guarantee the safety of the finished drug products.[5] At the same time, manufacturers were required to establish strategies to eliminate the impurities from their production process.

2.0 Discovery of Other Nitrosamines

In August 2018, N-nitrosodiethylamine (NDEA), a new type of nitrosamine impurity in valsartan API, was reported by Zhejiang Hua Hai pharmaceutical company to the European regulatory network. Subsequently, NDEA was also found in losartan and irbesartan.[6][7] By January 2019 the EMA reported pioglitazone, the first known non-ARB to contain NDMA, since the detected quantities were below the interim acceptable levels, they issued no recalls.[8] This emphasized the necessity of testing other medicinal products and APIs for possible contamination with nitrosamines. Shortly after, in March 2019, N-Nitroso-N-methylamino butyric acid (NMBA) was reported in losartan followed by the detection of N-nitroso-N-methylaniline (NMPA) in valsartan in July 2019 by the FDA. From September 2019 to January 2020 three other non-ARB drugs (ranitidine, metformin, and nizatidine) were reported to contain NDMA. Rifampin and Rifapentine antibiotics for tuberculosis treatment also showed potential to contain 1-methyl-4-nitrosopiperazine (MNP) and 1-cyclopentyl-4-nitroso- piperazine (CPNP). In June 2020 EMA provided guidelines on precautions for avoiding nitrosamine impurities in pharmaceuticals.[9] In December 2020 the European Pharmacopoeia adopted a new general chapter for N-nitrosamine impurities and it was immediately available on the website of European Directorate for the Quality of Medicines and HealthCare (EDQM) because of its necessity and it is expected to be published by July 2021 in European Pharmacopoeia (Ph.Eur)[10] (Figure 2).

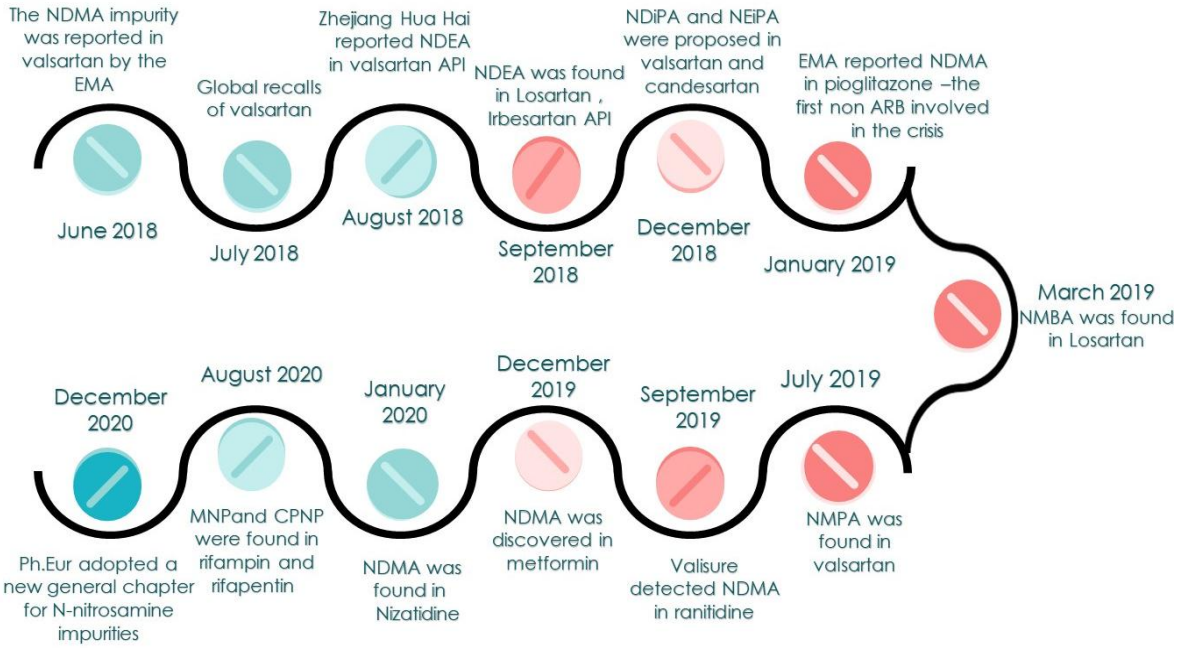


Figure 2: Timeline of nitrosamines discovery.

3.0 Genotoxicity of Nitrosamine Impurities

Although the contamination of valsartan by N-Nitroso-compounds came as shocking news to the scientific community, these contaminants have long been reported in drinking water and food products since the 1980s by the International Agency for Research on Cancer (ICRA) which classified them as "probable human carcinogens". N-Nitroso-compounds are considered as a member of the "cohort of concern" by the International Council for Harmonization (ICH) of technical requirements for pharmaceuticals for human use.[7][11] This means that they are regarded as highly carcinogenic, toxicogenic, and mutagenic (genotoxic) substances.[12]

We can illustrate the genotoxicity of these compounds in their ability to impair replication of cellular genetic materials leading to the formation of tumors. Their genotoxic pathway needs to be activated by the hepatic microsomal enzymes through the formation of alpha-hydroxynitrosamines, which after several transformations lead to the formation of alkyl diazonium ions: strong alkylating electrophiles, which can form adducts with nucleophilic constituents of genetic materials. In vivo, NDMA gives rise to methyl diazonium ions, which form an adduct with guanine resulting in O6-methyl guanine, which can be mistaken with adenine during base paring resulting in guanine: cytosine to adenine: thymine translation mutation. Formaldehyde formed because of NDMA biotransformation is a known genotoxic compound in humans.[13] Detailed mechanisms are illustrated in the Figure 3.

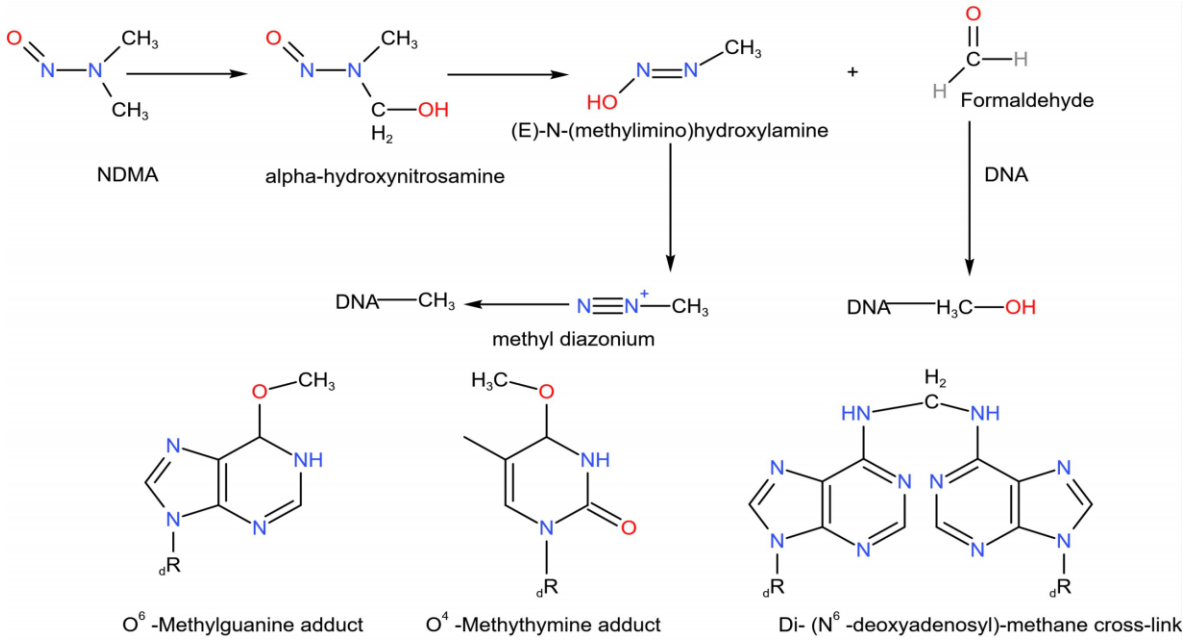


Figure 3: Bio activation of nitrosamines to reactive species and the major nucleobase adducts subsequently formed.

3.1 Mechanism of formation

Nitrosamine formation requires two key ingredients, which are a nitrosating agent with a secondary or tertiary amine under acidic conditions[12] (Figure 4 and figure 5).Tetrazole ring is an essential component of the structures in some ARB's such as valsartan, losartan, irbesartan, olmesartan, and candesartan, however, the formation of tetrazole ring requires the utilization of azide reagents such as tributyltin azide, sodium azide, and trimethyltin azide.[14] These azides are hazardous to the environment (highly explosive) and toxic in human, hence any residual amount needs to be eliminated by adding sodium nitrite in acidic conditions, upon which nitrous oxide and nitrogen gas are released. Under acidic conditions, nitrite can form nitric acid, which is a nitrating agent. Synthesis of sartans requires using solvents such as DMF (dimethylfomamide), NMP (N-Methyl-2-Pyrrolidone), TEA (triethylamine), which can contain trace amounts of amines as contaminants. Amines arising from the solvent and nitrating agents originating from the added nitrites give rise to nitrosamines.[15]

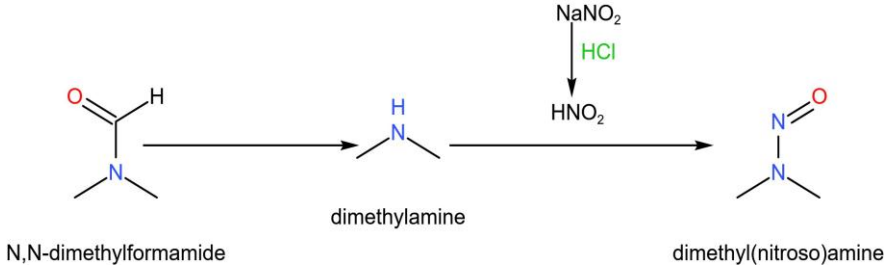


Figure 1: Mechanism of formation of NDMA.

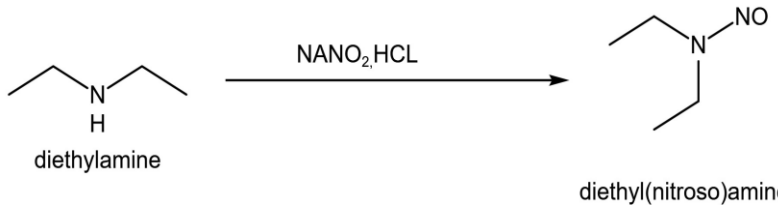
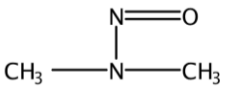
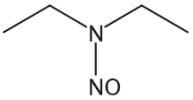
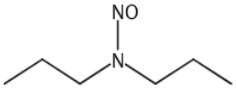


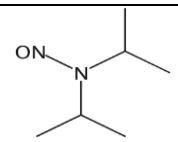
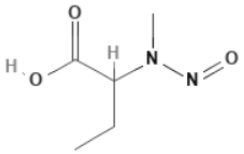
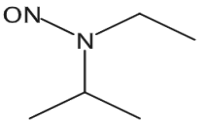
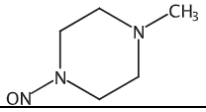
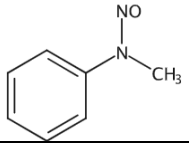
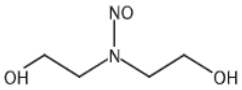
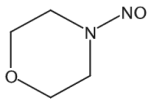
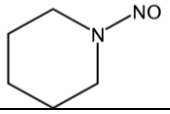
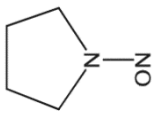
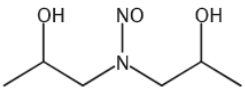
Figure 2: General reaction scheme for formation of NDEA from diethyl amine.

3.2 Limits of nitrosamines

As has been mentioned earlier, nitrosamine contamination in sartan APIs and drug products can cause a potential risk of cancer development in patients. Health regulatory authorities developed special guidelines for both manufacturers and health care professionals to perform a risk assessment based on the genotoxic potency, course of treatment, maximum daily dose, and the number of patients. ICH M7(R1) guidelines applied to determine the acceptable intake limits (AI), which show that one in a hundred thousand patients is at risk of getting cancer after continuous daily exposure to nitrosamine impurities for 70 years.[16] The range of the AI is between 26.5 to 96 ng/d depending on the impurity. This is only relevant when a single nitrosamine is present in active pharmaceutical product (API) or drug product (DP). The recommended limit has to be adjusted if multiple impurities are present in the API/DP and the overall level should not exceed 26.5 ng/d based on the maximum daily dose (MDD). The following formula expresses AI in ppm (parts per million) based on the drugs maximum daily dose as labeled by the manufacturer (ppm=(AI(ng)/MDD(mg)). When the MDD is less than 800 mg/day in a drug product, the total amount of nitrosamines should be less than 0.03 ppm, otherwise if the MDD exceeds 880 mg/day the limit for nitrosamines should not exceed 26.5 ng/day.[17]

Table 1: Structures of nitrosamine impurities with AIs.

Chemical name	Structure	Abbreviation	Allowable intake (ng/day)
N-Nitrosodimethylamine		NDMA	96.0
N-Nitrosodiethylamine		NDEA	26.5
N-Nitrosodipropylamine		DPNA	26.5

N-Nitrosodiisopropylamine		DIPNA	26.5
N-Nitroso-N-methylamino butyric acid		NMBA	96.0
N-Nitrosoethylisopropylamine		EIPNA	26.5
1-methyl-4-nitrosopiperazine		MNP	96.0
N-nitroso-N-methylaniline		NMPA	34.3
N-Nitrosodiethanolamine		NDELA	NA
N-Nitrosomorpholine		NMOR	NA
N-Nitrosopiperidine		NPIP	NA
N-Nitrosopyrrolidine		NPYR	NA
N-Nitrosodiisopropanolamine		NDiPLA	NA

Key: NA- not available.

4.0 Methods Used for Analysis of Nitrosamines

Lack of methods and equipment that could selectively and sensitively detect trace amounts of contaminants at ppm levels might have been one of the reasons for NDMA not being detected in valsartan API produced by Zhejiang Hua Hai since 2011, when they changed their synthesis route.[2] After the EMA recalls of valsartan different regulatory authorities and laboratories designed and validated various methods for screening and quantifying NDMA. Methods developed were mostly based on liquid chromatography (LC) and gas chromatography (GC).[18] Selection of method should contemplate polarity, thermal stability,

and complexity of the sample. Parameters such as sensitivity, selectivity, recovery, accuracy, precision, linearity, and robustness should be assessed according to ICH guidelines. For example, GC methods cannot be applied for detection of NMBA, because it is thermally unstable. Degradation of NMBA arises from the high temperature during sample injection hindering the correct quantification of the compound.[19]

On May 1st, 2021 the FDA has published a general chapter on its website regarding the control of six nitrosamines (NDMA, NDEA, NEIPA, NDIPA, NMBA, and NDBA).[20] This chapter suggests four analytical methods: LC-HRMS (liquid chromatography-high resolution mass spectrometry), GC-HS-MS/MS (headspace-gas chromatography/mass spectrometry), LC-MS/MS (liquid chromatography with tandem mass spectrometry), and GC-MS/MS (Gas Chromatography - Tandem Mass Spectrometry) depending on the type of impurity analyzed. For LC-HRMS and LC-MS/MS (triple-quadrupole detector), all six impurities can be quantified with LOQ (limit of quantitation) of 0.05 ppm and 0.02ppm respectively. Regarding GC-HS-MS/MS (triple-quadrupole detector) and GC-MS/MS (triple-quadrupole detector), both were not applicable for detection of NMBA while headspace injection mode only could not assess NDBA.GC methods were more sensitive than the LC methods and could detect at LOQ of 0.005 ppm for GC-MS/MS and 0.02 ppm for the GC-HS-MS/MS. The FDA suggested using an isotopically labeled internal standard for GC-HS-MS/MS, GC-MS/MS, and LC-MS/MS.[20]

The European Directorate for the Quality of Medicine and healthcare (EDQM) has taken measures for creating risk-based sampling and testing programs that ensure adequate control of nitrosamines. EDQM coordinated the Official Medicines Control Laboratories (OMCLs) throughout Europe and begun developing new methods for testing nitrosamines in sartans.[21] The French OMCL at the ANSM developed two HPLC-UV methods, one being suitable for NDMA valsartan only and the other for simultaneous detection of NDMA and NDEA in five sartans drug substances namely losartan, valsartan, irbesartan, candesartan, and olmesartan.[22][23] Both methods used Inertsil ODS-3 column, mobile phase comprising methanol and water at different ratios with gradient elution to ensure unexpected peaks will not appear on the chromatogram. The LOD (limit of detection) for NMDA in valsartan only was 0.1 ppm.

Germany OMCL (CVUA Karlsruhe) developed the first efficient method for simultaneous analysis of NDMA and NDEA in sartans for both film-coated tablets and drug substances

having a lower LOD and LOQ for NDEA compared to the France OMCL method mentioned above.[24] Before injection the sample was passed through a membrane filter and then separated and analyzed using UHPLC -APCI-MS/MS in a multiple reaction monitoring mode (MRM). Isotope labeled internal standard NDMA-d6 and NDEA-d10 were used for the quantification of both impurities.

The OMCL at SWISS MEDIC established a new method for the determination of six N-nitrosamine impurities (NDMA, NDEA EIPNA, DIPNA, DPNA, and DBNA) in sartan API and tablets (losartan, valsartan, irbesartan, candesartan, olmesartan).[25] They used GC tandem MS/MS detector in multiple reaction monitoring mode (MRM) which enabled them to quantify the above nitrosamines at a concentration level of 0.02ppm (LOQ).

Taiwan Food and Drug Administration developed a method for NMBA analysis in sartan drug substances and drug products (candesartan, irbesartan, losartan, olmesartan, telmisartan, and valsartan). Taking into consideration NMBA's thermal instability Taiwan FDA recommended liquid chromatography-tandem mass spectrometry (LC-MS/MS)method, which can accurately quantify NMBA level at 0.05 ppm(LOQ)[26]. In 2020 Taiwan Food and Drug Administration published "method of test of Nitrosamine in medicines–multiple analysis" that outlined successful determination of twelve nitrosamine (NDELA, NDEA, NDIPLA, NDMA, NDPA, NEIPA, NMBA, NMEIA, NMOR, NPIP, NPYR, NDIPA) by using LC-MS/MS. Isotope -labelled standard method was applied and all nitrosamines could be quantified with LOQ of 0.05 ppm.[27]

5.0 CONCLUSION

The valsartan contamination has prompted the need for regular and detailed risk assessments of genotoxic impurities in pharmaceutical preparations. Manufacturers should implement quality by design (QbD) when planning or changing the synthesis route to avoid all possible risks of nitrosamine contamination. Regulatory authorities such as EMA and FDA have responded accordingly by providing reliable testing methods and guidelines for the industries to control nitrosamine impurities.[17][28] Regulatory authorities should co-operate and form a single database for reported cases and practices that can cause a risk of contamination by carcinogenic impurities. This approach will ensure that other manufacturers and authorities will not repeat avoidable and controllable shortcomings. Consequently, patients will have safe, timely, and ensured availability of life-saving drugs.

Conflict of interest

The authors declare no conflict of interest.

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