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A Brief Review on Nitrosamine Impurity in Pharmaceuticals

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ABSTRACT

Nitrosamine impurities are referred as carcinogenic and mutagenic impurities, very less amount of exposure of those impurities can cause cancer. Nitrosamine impurities considered as a human cancer-causing agent by the International Agency for Research on Cancer (IARC). Nitrosamine impurities are detected in different pharmaceutical products in recent days. This led to the recall of pharmaceutical products. Various sartans, nizatidine, ranitidine, and metformin are recalled from the markets because of the high limit of Nitrosamine impurities. These impurities are formed and get incorporated into drug substance or drug product through catalyst, reagent, solvent or raw materials used in the manufacturing process. Nitrosamine impurities is avoided by taking precaution within the manufacturing of drug substance and drug products. Validated analytical methods are to be used to identification and quantification of Nitrosamine impurities hence it needs sensitive instrument which may detect Nitrosamine impurities to the trace level at given interim limit. Gas chromatography or Liquid chromatography together with mass detector is widely used for their determination and identification. Various regulatory authorities like USFDA, ICH Canadian Drug and Health Agencies are focusing on the purity requirements and identification of impurities in active pharmaceutical ingredients as presence of impurities even in less amounts may affect on the safety and efficacy of the pharmaceutical products. Regulatory Authorities has improved for educating the health care professional, manufacturers and also public about the adverse effect(carcinogenic) of the Nitrosamine impurity consumption.

KEY WORDS: Nitrosamine, Impurities, Carcinogens, Guidelines, Analytical methods

INTRODUCTION [1]

Pharmaceutical impurities are the undesirable chemicals that remain within the active pharmaceutical ingredients (APIs) which are developed during formulation or due to the aging of both API as well as formulated APIs to medicines.

INTRODUCTION OF GENOTOXIC IMPURITY [2]

International Council for Harmonisation in its guideline ICH S2 (R1) defines genotoxicity as “a broad term that refers to any undesirable change within the genetic material, consideration for the mechanism by which the change is induced.” While genotoxic impurities are defined as “Impurity that has been indicated to be genotoxic in a relevant genotoxicity test model, e, g., bacterial gene mutation (Ames)test.’’

CLASSIFICATION OF GENOTOXIC IMPURITY [2]

Genotoxic impurities are classified as on the basis of their risk analysis comprises a preliminary analysis of actual and potential impurities by performing database and literature searches for carcinogenicity and bacterial mutagenicity data which classify genotoxic impurities into Class 1, 2, or 5. If data of classification is not

available, we can perform Structure-Activity Relationships (SAR) to predict the bacterial mutagenicity. This shall be classifieds it into Class 3, 4, or 5. Each class is defined as below.

Class 1: These impurities have established mutagenic and carcinogenic data and are known to be more serious risk and essential to eliminate them by modifying the process. If the aforementioned is not possible the impurities are to be limited at "threshold of toxicological Concern (TTC)" as a last option.

Class 2: These impurities have the well-established mutagenic data, but their potential to cause carcinogen isn't known. Hence, these impurities must be controlled using the TTC approach.

Class 3: These adulterated substances are having viligant structures unrelated to the structure of the drug substances and of unspecified genotoxic potential. On the basis of functional groups within their molecule, they will be classified as genotoxic. The toxicity of those impurities is identified on the basis of the structure-activity relationship (SAR).

Class 4: The structure of impurities is having similarity with the structure of drug substances and also includes functional or moiety which has potentially alert shared with the parent structure and consider to be non-genotoxic.

Class 5: These impurities haven't any alert structures, and evidence indicates the absence of genotoxicity. These impurities should be considered as a normal impurities and should be checked according to ICH guideline.

INTRODUCTION OF NITROSAMINES [3,4]

Nitrosamines, or N-nitrosoamines, is an any molecule that containing the (N–NO) nitroso functional group. N-Nitrosamines are polar hydrophilic, uncharged molecule which have high vapor pressure and very high solubility in water. N -nitroso compounds are a large group of potent carcinogens which includes N-nitrosamine. These N-Nitrosamines, which are derived from alkyl, alkaryl, aryl or cyclic amines. It is a group they share with N-nitrosamides which are derived from N-alkylureas, N-alkylcarbamates, and simple N-alkylamides. These compounds are related to nitrosamine impurities and that nitrosamine impurities are probable human mutagenicity, teratogenicity, and carcinogenicity.

INTRODUCTION OF NITROSAMINE IMPURITY [5,6]

ICH M7 (R1) classifies Nitrosamine impurities as Class 1 Genotoxic impurities, which is known to be mutagenic and carcinogenic, on the basis of both rodent carcinogenicity and mutagenicity data. These Nitrosamine impurities produce effect on the genetic material by means of mutations through chromosomal breaks, rearrangements, covalent binding or insertion into the DNA during replication. These changes within the genetic materials produce by the exposure to very low levels of Nitrosamine impurities can result in cancer. Thus, it's important to identify Nitrosamine impurities in drugs at very low levels to make sure safety to the general public.

A total of 10 nitrosamine impurities is reported for mutagenic potential.

1. N-nitrosodimethylamine
2. N-nitrosodiethylamine
3. N-nitrosomethylethylamine
4. N-nitrosodi-n-propylamine
5. N-nitrosodiisopropylamine
6. N-nitrosodi-n-butylamine
7. N-nitrosodiphenylamine
8. N-nitrosopyrrolidine
9. N-nitrosopiperidine
10. N-nitrosomorpholine

ICH M7 GUIDELINE ON MUTAGENIC IMPURITIES [7]

ICH M7 guideline incorporated a concept first introduced by the Safety Working Party (SWP) within the EU guideline on genotoxic impurities. This is the threshold of toxicological concern (TTC) first developed within the related to food safety. The TTC thereby defined an appropriate intake (or risk) for any chemical with limited or no supporting safety data that may pose "a negligible risk of carcinogenicity or other toxic effects". A TTC value of 1.5µg/day, which corresponds to carcinogens noted because the "cohort of concern" (COC), comprises aflatoxin-like-, N-nitroso-, and alkyl-azoxy compounds; benzidine derivatives may additionally usefully be included. Industry had always argued that the "cohort of concern" structural classes were not possible to be encountered during the routine synthesis of pharmaceutical drug substances. TTC is, "(a) inappropriately impact by various type of potent carcinogens of historic interest that is difficult to create

unknowingly as pharmaceutical impurities, and (b) that the many of reactive chemicals that might be useful to synthetic chemists are among the smallest amount potent carcinogens within the underpinning supportive analyses".

WHAT ARE THE PHARMACOLOGICAL MECHANISMS FOR NDMA HARM[8]

In animal models NDMA has been shown to cause liver damage and fibrosis. When exposure to NDMA in one animal model that caused centrilobular congestion, Kupffer cell hyperplasia, and liver fat accumulation after 7 days and severe neutrophilic infiltration, multifocal collapse of liver parenchyma, and deposition of collagenous fibrosis after14 days. There are some cases where people around the world purposely poisoned by unknowingly consuming several 100 milligrams of NDMA, which further leads to diarrhoea, hepatotoxicity, vomiting, and/or death. The International Agency for Research on Cancer has been categorized NDMA as a probable cause of carcinogen, within animal researches disclosing tumour formation mostly within the gastrointestinal tract and liver but also within the lungs and kidneys. NDMA, like other nitrosamine impurities, activates ras oncogenes, and other NDMA metabolites by enzymeCYP2E1 which generate methyldiazonium, a known mutation inducer through methylation. As such, NDMA is believed to induce both localized as well as systemic carcinogenic effects.

SOURCES OF NITROSAMINE IMPURITY [9]

Formation of Nitrosamine impurity can occur when the use of sodium nitrite (NaNO2), or other nitrosating agents, in the presence of secondary or tertiary amines within the identical or different steps of the manufacturing process.

By Using sodium nitrite (NaNO2), or other nitrosating agents, in combination with catalysts, reagents, and solvents (e.g. DMAc, DMF, and NMP), which are vulnerable to degradation to secondary or tertiary amines, within the identical or different process steps.

Formation of Nitrosamine impurity can occur in presence of degraded raw materials within the API manufacturing process (e.g. solvents, reagents and catalysts).

Use of contaminated recovered material and contaminated recycled materials (e.g. solvents, reagents and catalysts), including recovery outsourced to 3rd parties who are do not seem to be tuned in to content of the materials they are processing and recovery processes carried out in non-dedicated equipment.

By using contaminated starting materials and intermediates supplied by vendors who use processes or raw materials which can contain residual nitrosamines or nitrosating agents.

Carry-over of nitrosamines intentionally generated (e.g. as intermediates) during the manufacturing process.

Cross-contamination because of different processes being run successively on the identical manufacturing line.

Carry-over of impurities between manufacturing process steps due to operator- related errors or inadequate detailed batch records such as inadequate phase separations during work-up procedures.

Degradation processes of active substances, starting materials and intermediates including those effected by intrinsic reactivity (e.g. presence of oxime, nitro, or other functionality) or by the presence of an exogenous nitrosating agent. This might potentially occur also during finished product formulation or storage and will be influenced by crystal structure, crystal habit and storage conditions (temperature, humidity etc.).

Use of specific packaging materials. Nitrosamine impurity has been observed in finished drug products stored in blister packs with lidding foil containing nitrocellulose.

Nitrosamines are shown to make from nitrocellulose degradation products and low molecular weight amines present either in printing ink or within the Finished drug Product during the blister heat-sealing process and to transfer to the product within the blister.

Reaction of nitrosatable nitrogen functionality in APIs or their impurities with nitrosating agents present in components of the drug Product during formulation or storage.

KNOWN SOURCES OF NITROSAMINES [10-14]

Food

Sodium nitrite and sodium chloride are commonly used as the curing agent in meats, however, because the meats contain amines the combination of these

chemicals can form nitrosamines. Intake levels range from 0.0004 to 0.23µg in cured meat, 0.0004– 1.02µg in smoked meat, 0.0006–0.13µg in grilled meat and 0.07–0.07µg in bacon. NDMA level in a variety of fish and fish products were found between <0.1 to 4.2 µg/kg. Average NDMA levels from vegetables was reported to be 0.075 µg/kg. In milk, NDMA was found to be below 0.1 µg/kg (detection limit) in most cases, while skim milk, powders, and other milk products had NDMA up to 0.7 µg/kg.

Water

N-Nitrosamines such as NDMA are reported as by-products formed after the disinfection of wastewater effluents by chlorine and the drinking water chlorination and chloramination processes in the presence of nitrogen-containing organic matter. N- Nitrosamines might equally pose a risk to water resources and given their potential adverse effects on human health the presence of these compounds is of more concern in drinking water than in wastewater. NDMA is introduced into drinking water as a by- product of chlorination, exposing people to roughly 0.0003–0.001µg/kg of body weight per day per a World Health Organization (WHO) estimate. In the Integrated Risk Information Service (IRIS) database of the United States Environmental Protection Agency (U. S. EPA), NDMA has been identified to have an estimated 10−6 lifetime cancer risk level at a concentration of 0.7 ng/L in drinking water.

Cosmetics

Raw materials for the production of cosmetics have been found to contain nitrosamine impurities., therefore, these raw materials need to be screened before use. Both Europe and Canada have banned nitrosamines in any cosmetic product. Formation of secondary nitrosamines can occur from reactions between some proteins and the preservatives, such as diethanolamine or triethanolamine. These compounds are common additives in cosmetics used to adjust pH or act as a wetting agent. NDMA was detected in various cosmetics and personal care products, including shampoos, hair conditioners and toners, bath and shower gels, creams and oils, facial tonic sand cleansers. A past study showed that 50 of145 cosmetic sand personal care products in Germany contained NDMA, at a maximum of 24 µg/kg in a shampoo.

Atmospheric nitrosamines

The inhalation of airborne nitrosamines formed in workplace atmospheres can present a significant risk to workers. The presence of amines and nitrogen oxides with certain levels of humidity and temperature can cause the formation of airborne nitrosamines.

FORMATION AND DISTRUCTION OF NITROSAMINE IMPURITY AND TOXICITY OF NITROSAMINE [15-17]

FORMATION OF NITROSAMINES

The chemistry of nitrosamine formation is very complex. Formation of N-nitrosamines are done by the reactions of organic amines and their derivatives with nitrosating compounds; however, the most stable nitrosamines are formed from secondary amines. Generally, Amines are categories as primary, secondary, and tertiaryamine.

Nitrosating agents can react with Primary amines and to generate highly reactive, unstable diazonium ions, which continually decompose to release molecular nitrogen. It's also feasible for the occurring diazonium ion to reacting with the starting primary amine to formation of a secondary amine, which might then undergo nitrosamine formation. In the case of a molecule with two primary amines that are segregated by 4 to 5 carbons, a cyclic nitrosamine can form. However, indirect nitrosation of primary amines is low producing because of the instability of the diazonium ion and the requirement for two continuous reactions to take place.

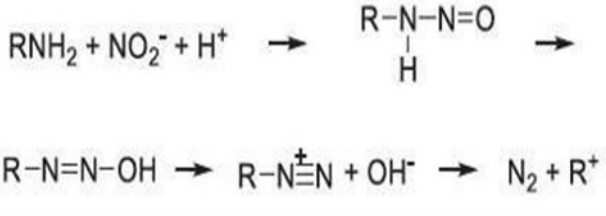

Figure 1 Nitrosamine formation from primary amine

Secondary amines are the foremost likely amines to react and formation of nitrosamines occur, however the rate of reaction is dependent on the both reactivity and concentration of starting materials. A characteristic chemical reaction procedure for secondary amines to formation of nitrosamines is provided in figure.

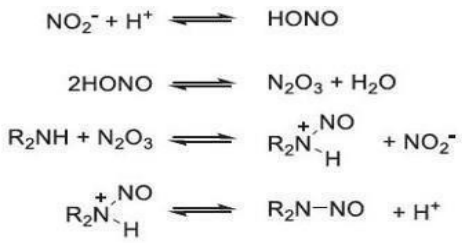

Figure 2 Nitrosamine formation from secondary amines

Tertiary amines are not directly reacting with nitrosating agents, but they can primary undergo nitrosative detachment to secondary amines, which may then after form nitrosamines. While this can be chemically feasible, the reaction is slow and generally requires more amount of the nitrosating agent and high temperatures. It is critical to note, that the tertiary amines (such as generally used diisopropylethylamine and triethylamine) can contain secondary amines as impurities and/or can decompose into secondary amines that may then proceed to more readily form nitrosamines. Further Other compounds that can contain a secondary amine as impurities or degradants and contain amide solvents like N-methyl-2-pyrrolidone (NMP), dimethylformamide (DMF), dimethylacetamide (DMAC), quaternary ammonium salts like as a tetra-n-butylammoniumfluoride (TBAF) and tetra-n-butylammoniumbromide (TBAB), and also primary amines. Generally nitrosating agents are nitrites like as sodium tert-butyl nitrite (t-BuONO), sodium nitrite (NaNO2), nitric oxide (NO), nitrous acid (HNO2), dinitrogen trioxide (N2O3), and dinitrogen tetroxide (N2O4) and nitrosyl halides (XNO, X=halogen). However, nitrosation can occur whether or not these reagents are not used into a reaction. Nitric acid (HNO3) can contain nitric oxide as an impurity and/or can convert into nitrous acid if exposed to reducing agents. chloramines (e.g. NH2Cl), Hydroxylamine (NH2OH), nitrates such as sodium nitrate (NaNO3), and ozone (O3), can act as indirect nitrosating agents under several conditions. Also, azides are generally quenched with nitrous acid or nitrites and these quenching reagents can produce nitrosate amines. Under oxidative conditions hydrazine's can form nitrosamines andin absence of oxygen they act as nitrosating agents.

Acidic conditions are generally assigned for nitrosation to take place, although basic or neutral conditions can cause nitrosation if only in a presence of catalyst like an aldehyde (especially formaldehyde). An aldehyde can act as a catalyst by formation of an iminium ion intermediate with the amine first, and also the iminium ion can undergo nitrosation more readily than the amine.

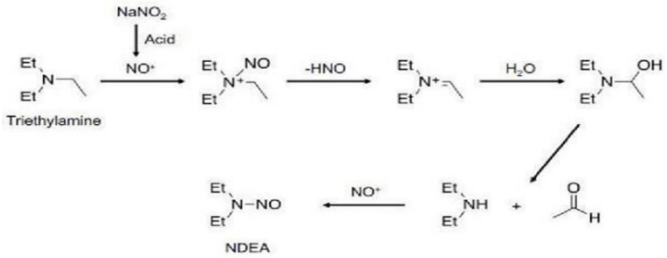

Figure 3 Nitrosamine formation from tertiary amine

DESTRUCTION OF NITROSAMINES

The destruction of nitrosamines requires strong reaction conditions. Strong acids like hydrochloric acid can transfer nitrosamines into the related amines and nitrous acid, which, if trapped with a nucleophile like a thiol, may resulting in to an irreversible reaction. Nitrosamines are often reduced by metals such as aluminium (with potassium hydroxide) and zinc (with acetic acid). Hydrogenation of nitrosamines is feasible within the presence of palladium, iron and nickel, as catalysts. Organometallic reagents like, phenyllithium, Grignard's, and tert-butyl lithium can eliminate nitrosamines. Finally, nitrosamines can be oxidized by strong oxidants like hydrogen peroxide and potassium permanganate in sulfuric acid.

NITROSAMINE TOXICITY

The primary toxicity problem associated with nitrosamines found within the human diet, environment, cosmetics, tobacco products, and as an impurity in marketed drug products has been that these structures are genotoxic chemical carcinogens.

In particular, N-nitrosodimethylamine (NDMA) produced tumours in a many of nonclinical species starting from fish, rodents, amphibians, and other mammals after exposure via various different routes. delicate and malignant tumours after exposure to NDMA were identified within the respiratory tract, digestive tract, kidney, hematopoietic system, liver and bile duct, and female reproductive tract. Also, tumours were identified after exposure of N-nitrosodiethylamine (NDEA) in numerous nonclinical species including dogs and pigs in liver, kidney, respiratory tract, and also the digestive tract.

Although number of epidemiological studies analysing the relationship of exposure to NDEA and human cancer have been conducted, various population-based case-control studies and ecological studies were conducted in order to assess the relationship between dietary sources of NDMA

and cancer. Dose-related associations ofcolorectal, stomach, esophageal, and oropharyngeal cancers with estimated NDMA exposure were identified in various case-control studies. Also, an increased risk related to lung cancer was identified with dose-related increases in estimated dietary exposure to NDMA.

Therefore, NDMA, NDEA, and other structurally-related nitrosamines are suspected by regulatory authorities to act as carcinogens in humans based on the confirmation of carcinogenicity in a different nonclinical species and a small number of case-control studies with humans.

DIETARY CONSTITUENTS WHICH ARE KNOWN TO INHIBIT THE FORMATION OF NITROSAMINES

natural constituents: antioxidant ascorbic acid (vitamin C), a-tocopherol, caffeic acid, caffeine and tannic acid. The latter three compounds are found in considerable quantities in common beverages like cola drinks, coffee, tea, beer, and wine.

Synthetic food additives: phenolic antioxidants (e.g. t-butylhydroquinone, propyl gallate, and butylated hydroxyanisole and hydroxy toluene), and isoascorbic acid, sorbic acid and erythorbic acids. Also, cysteine and histidine, amino acids, a tripeptide and glutathione, which is present in our body similarly as in normal diet, are all known as inhibitors of nitrosamine formation. These inhibitors are believed to act as scavengers of nitrite via various oxidation-reduction pathways.

HOW IMPURITIES CAME TO BE PRESENT IN VARIOUS PRODUCT

HOW IMPURITIES CAME TO BE PRESENT IN SARTANS [17-22]

Before June 2018, NDMA and NDEA were not among the impurities estimated in sartan medicines and hence, detected by routine tests.

USFDA first became aware of the NDMA and MDEA impurities present in valsartan in July 2018, also Medicine Regulatory Authorities got an awareness of the problem. Then various Regulatory authorities like USFDA, EMA, WHO and many more has taken serious action to battle the nitrosamine impurities reaching the public.

In July 2018 contaminations of sartan containing drugs led to various batch recalls due to their content of N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA).

N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) is believed to have been introduced into the sartan products as a result of change in the manufacturing process of the active pharmaceutical ingredient (API). The contamination is in all possibility related to a change within the manufacturing process in Zhejiang Huahai Pharmaceuticals in 2012.

It is now known that these NDMA and NDEA impurities can form during the production of sartan that contain a specific ring structure referred to as a tetrazole ring and formation of this tetrazole ring employs the utilization of sodium nitrite, under certain conditions and when certain solvents, reagents like as dimethylamine within the common solvent dimethyl formamide (DMF) and other raw materials are used.

Sodium azide and N, N-dimethylformamide (DMF) were used for the formation of the tetrazole ring in sartan drugs. Subsequently, more amount of sodium azide, remaining after the formation of the tetrazole ring, was quenched with sodiumnitrite under acidic conditions, leads to the formation of nitrous acid. One potential source of NDMA and NDEA might be the degradation of DMF under the acidic conditions and reaction with nitrous acid. The formation of nitrosamines is only feasible when secondary or tertiary amines react with nitrous acid. Nitrous acid itself is unstable but it can be formed in situ from nitrites (NO2) under acid conditions.

Solvents and Materials can become cross-contaminated with nitrosamines or with impurities that might react downstream to make nitrosamines, if equipment is not adequately cleaned.

In addition, it is feasible that impurities were present in several sartans because manufacturers had inadvertently used contaminated reagents or equipment within the manufacturing process.

These larger concerns have prompted the European Medicines Agency(EMA) to request that marketing Authorisation Holders(MAHs) of all finished pharmaceuticals products(FPPs) conduct risk analysis to identification of the risk of nitrosamine content.

HOW THE IMPURITY COME IN RANITIDINE [23-25]

The U.S food and drug administration has requested a manufacture's market withdrawal of ranitidine, known commonly by the name of Zantac. This Implies ranitidine product will not be available for new or existing prescriptions or over the counter (OTC) use within the U.S.

This means that ranitidine will not be available to be used within the U.S. The agency is taking the action because FDA laboratory testing results show that the levels of NDMA in ranitidine may increase to unacceptable levels over time.

The tests also show NDMA levels increase in some ranitidine products when the drug is exposed to more than room temperatures. On the Basis of this findings, FDA has determined that several currently marketed ranitidine drug products might expose consumers to unacceptable health risks. All the ranitidine drug products, including the oral liquid or syrup, are withdrawn by their manufacturers and cannot be available on the U.S. market. This different from the past actions because this is often the first time FDA is requesting market withdrawal of all ranitidine products.

In September 2019, the US Food and Drug Administration (USFDA) observed that several ranitidine drugs, including various products sold under the brand name of Zantac, contained a nitrosamine impurity called N nitroso dimethylamine (NDMA), classified as a possible human carcinogen.

On13, September 2019, the USFDA first warned consumers that several ranitidine products also contain the unacceptable high amount of NDMA levels and were are being recalled.

Many manufactures, including Sandoz, Apotex corporation, Aurobindo, Sanofi, and Dr Reddy labs, have had to recall products containing ranitidine because of the high amount of NDMA.

In October 2019, Sanofi replaced all over-the- counter drug by the brand name of zantac within the united states and Canada, perrigo issued a word wide ranitidine recall, Dr Reddy issued a recall of all ranitidine drug product withinthe united states market, and Novitium pharma displace all ranitidine hydrochloride capsules in the united states.

In November 2019 Amneal pharmaceuticals, American health packaging,Aurbindo pharma ,Golden state medical supply and precision dose recalled various batches of ranitidine tablet, syrup and capsule.

In December 2019 Glenmark pharmaceutical Inc., USA, withdrew several batches of ranitidine tablets. In December 2019, the FDA asked ranitidine and nizatidine product manufacture to expand their NDMA trails to include all drug batches before making them available to consumers.

In January 2020, appco pharma LLC and northwind Pharmaceuticals withdrew some batches of ranitidine capsule.

In April 2020, the FDA requested all prescription and over-the-counter ranitidine products to be removed from the market immediately.

HOW IMPURITIES CAME TO BE PRESENT IN METFORMINE [25,26]

Dimethyl amine (DMA), which is uses as a precursor of NDMA, and Dimethyl amine (DMA), is used in the synthesis of Metformin. Thus, there is always a probability of NDMA present in lots of Metformin. The FDA has prior to found that the source of NDMA can be related to change in drug's manufacturing process or its chemical structure, during its testing process or even in the conditions in which they are stored or packaged. The presence of NDMA in metformin Drug could be primarily due to contamination during manufacturing as opposed to a fundamental instability of the drug molecule. But, feasible role of the drug itself cannot be ruled out entirely now. There may be more than one contamination point by which NDMA is introduced in to the drug product. NDMA may be formed due to contact with oxidants in the process of synthesis of metformin. As the metformin manufacturing process should also be checked first.

In addition, in December 2019, the FDA released a statement that NDMA had been found in some metformin products outside of the United States.

In February 2020, the FDA determined that levels of NDMA in United Statessamples of metformin were not detectable or below acceptable limits and no medication recalls were issued. However, in May 2020, additional FDA laboratory testing on extended- release metformin products yielded NDMA levels above the acceptable limit, triggering a voluntary recall of extended release metformin products in the United States. Unlike the ARBs, neither metformin nor ranitidine contains a tetrazole ring. confirming that a tetrazole ring is not a prerequisite for the development of nitrosamine contamination.

REVIEW OF THE MANUFACTURING PROCESS [5,27]

The European Medicines Agency (EMA) has issued templates for marketing authorization holders to use when filing results of product testing for Nitrosamine contamination. The following steps are required by the manufacturer to control the review of Nitrosamine in human medicinal products.

Step I. Risk evaluation

According to ICH Q9 and ICH M7 guidelines the marketing authorisation holder along with drug substance and drug product manufacturer should perform a risk evaluation of Nitrosamine within six months. The risk assessment could be conducted in a priority manner that is the highest probability of contamination should be evaluated first. Authorities should be informed about the risk evaluation results. If a risk of potential contamination has been identified, the marketing authorisation holder should proceed to go to the 2 steps as below.

Step II. Confirmatory testing

After risk evaluation, confirmatory testing activity should be start instantly. By validated analytical methods that having greater sensitivity that used for the product with higher-risk must be analytically tested first as soon as possible for Nitrosamine impurities. furthermore, confirmatory testing of all the related drug product should be determine at the latest within three years of the publication of the notification or otherwise justified. If Nitrosamine is identified, the competent authorities are to be informed instantly, irrespective of the amount detected.

Step III. Changes to the marketing authorisation

Changes to the marketing authorisation like as a change in the manufacturing process of a drug substance or drug product specifications are to be applied in a reliable manner. If there is a presence of risk to public health the competent authorities must be informed immediately. All steps should be completed within three years in a prioritised manner.

IMPACT OF VALSARTAN RECALL IN INDIA [28]

On July 20, 2018, After the news of contamination of valsartan with NDMA, the Drug Controller General of India (DCGI) has initiated a survey into all companiesimporting raw material of valsartan from Zhejiang Huahai Pharmaceuticals. The Central Drugs Standard Control Organisation (CDSCO) has taken to action and all the imported products are required to be tested before they are taken up for manufacturing. The health care professionals have also been put on high alert. Novartis is India's one of the companies that manufactures the product has initiated recall of specific batches of valsartan that contains NDMA. In spite of the starting of investigation into the issue, no stringent action should be taken to recall drugs containing valsartan in the Indian market. It to be mentioned Valsartan manufactured by two Indian Pharmaceuticals Hetero Pharma and Torrent pharmaceuticals in banned in USA due to impurity.

REGULATORY PERSPECTIVE [5,11]

US FOOD DRUG ADMINISTRATION (USFDA)

Housed within the US Department of Health and Human Services, the FDA is responsible for "protecting the public health by ensuring the safety, efficacy, and assurance of human and veterinary drugs products, biological products, and medical devices...". The FDA's responsibilities contain the regulating brand-name prescription drugs, generic drugs, as well as non-prescription (over the counter) drugs products. FDA approval of a drug significantly that the FDA has considered a medication effective and safe. however, problems influencing the safety of approved products do occur. When problems influencing the safety of drugs products occur, these may result in a drug recall. A drug recall is defined as "a voluntary action taken by the company at any time to remove a deficient drug product from the market". A recall is introduced either by the FDA or by the drug company when a pharmaceutical product is considered to be unsafe to patients or when FDA rules have not been obeyed. Among the various reasons for drug recalls that are mislabelling, contamination, presence of impurities, and lack of sterility. By one way of counting, 226 drug recalls were announced by the FDA within the past 2 years. The total number of drug recalls is likely even more, since "...not all recalls are announced on FDA.gov or in the news media."In June 2018, FDA was aware by one of valsartan drug substances manufacture about the presence of an impurity, detected as N-Nitrosodimethylamine (NDMA). Further research by the FDA found out that other Nitrosamine impurities, that is, N- Nitrosodiethylamine (NDEA) were also present at unacceptable levels in drug substances from multiple drug substances manufacturer of valsartan and other drugs in

the ARB class. As there was no acceptable limit in the specification for Nitrosamines, as a primary measure, the FDA published “interim acceptable limits” for these Nitrosamine impurities in ARB drugs. Drug substances and drug product beyond these limit levels were recommended to recall the drug product from the market. The FDA recommended that the drug product manufacturers, that they should test the samples of each drug product batch or drug substances lot used for the drug product manufacture for the US market to determine whether it has contained a detectable amount of Nitrosamine impurities. FDA has also published validated methods to identification and quantification of NDMA and NDEA impurities in all ARB drug substances and some drug products.

EUROPEAN MEDICINES AGENCY (EMA)

EMA first initiated Nitrosamine impurities analysis in July, 2018 and found out that sartan containing medicines are contaminated with Nitrosamine impurities, which leads to recall of several drug products and come under review by the European Union (EU), thus it set strict new manufacturing requirements for these medicines.Earlier in January 2019 the EMA advised that companies making sartan medicines review their manufacturing processes so that Nitrosamine impurities cannot be formed. Companies were given a transition period to make crucial changes during which strict temporary limits on levels of these impurities were applied. After this transition period companies will have to demonstrate that their sartan products have a safe level for these impurities before they can be used in the EU. The EMA noted that in the vast majority of sartan medicines, impurities were either not found or were present at very less amount. The EMAs Committee for Medicinal Products for Human Use (CHMP) will provide guideline on avoiding the presence of Nitrosamine impurities to marketing authorization holders, which they should consider alongside their understanding of the manufacturing processes of their products.

OTHER REGULATORY AGENCY

Therapeutic Goods Administration (TGA) of Australia prepared guidance in a public notice that it has introduced requirements for sponsors of 'sartan' blood pressure medicines to take measures to avoid the presence of Nitrosamine impurities in medicines and implement strict testing of their medicines to identify the presence of any Nitrosamine impurities. The TGA provides a two-year transition period (2019- 2021) to allow sponsors to review and if required, make changes to their manufacturing processes and to implement adequate testing methods. During the transition period sponsors must inform the TGA if they identify the presence of Nitrosamine compounds in their medicines. Changes in to manufacturing processes and/or controls if required should be lodged as a 'category 3' request under section 9D (3) of the Therapeutic Goods Act 1989 (the Act). The TGA Laboratories has adapted a publically available USFDA test method.

Health Canada continues to hold manufacturers responsible for the safety and effectiveness of drugs sold on the Canadian market and has take various actions to mitigate the risk to Canadian. Health Canada proceed to work closely with international regulatory partners including the FDA and the EMA to share information and coordinate efforts on inspections, risk assessments and public communications. Health Canada has provided a method that has been developed to identification and quantification of the Nitrosamine impurities NDMA and NDEA in angiotensin II receptor blockers (ARBs).

ANALYTICAL METHOD

ANALYTICAL METHOD DEVELOPMENT[5]

The basic task for the development of an analytical method for Nitrosamine impurities is to develop the analytical method which can identify these impurities at very less amount and well below the TTC. The developed method could have less variability by conducting a series of controlled experiments thus to make quality and safety of drug products.

ANALYTICAL METHODS [5,6,29]

The development of analytical methods is to identification of Nitrosamines impurities is the challenging task due to which the developed method can identified the genotoxic impurity at very less amount and below the TTC present within the complex matrices. Identification of nitrosamines in each drug is that the application of appropriate measurement technology focused on identifying very less amount (nanogram)of nitrosamines in solvents, intermediates, APIs and finished dosage forms. Regulatory agencies and pharmaceutical manufacturing firms around the world have developed and validated analytical method focused on nitrosamine detection.

The developed analytical methods also required to be validated to conform to GMP requirements. Several

methods are published by the FDA to cover determining nitrosamine content in various active pharmaceutical ingredient (API)and finished product. The control strategy for nitrosamine impurity involves different process parameters like temperature, and extra purification, humidity, additional time cycles and such, during API manufacturing and similar stringent controls during formulation manufacturing. Most manufacturers have used LCMS systems to identified NDMA, to ensure that every batch is tested for NDMA before releasing the batch to the market so that the quality of the final product reaching patient is assured, so that recalls should not overly burden the healthcare system. The USFDA has suggest that the use of an LC-HRMS method for testing ranitidine due to lower temperature conditions method, higher temperature conditions of some test method may cause to generate NDMA.

Most of the methods used for testing of Nitrosamines in drug substance and drug product utilize the chromatographic techniques such as reversed-phase liquid chromatography (LC) or gas chromatography (GC) combined with various detectors such as mass spectrometry (MS), Ultraviolet spectrophotometry (UV) or nitrogen chemiluminescence (NCD) etc.

- **HPLC (high-pressure liquid chromatography)**

Liquid chromatography is a very important separation technique that has a considerable impact in the area of pharmaceuticals and chemistry. HPLC consists of following parts that include the solvent reservoirs, degasser, low- or high- pressure gradient pump, guard column, sampling port, main column, detector, and computer display. Different stationary phases such as C18, SuperC18, C8, C5, C4, C4- 300, phenyl hexyl, HILIC, PFP, CSH, DAB, RP-Amide, SCX, CN-300, normal silica is available in the market for good selectivity and sensitivity.C18/Phenylhexyl are the most remarkable used for nitrosamine impurities detection. Initially, NDMA detection using diode array detector (DAD) in the wavelength range 230–233 nm was reported. In recently, NDMA identification using UV detector at 228 nm in valsartan drug was reported by French National Agency for Medicines and Health Products Safety, and Official Medicines Control Laboratories (OMCLs) of the General European OMCL Network (GEON). It is mentioning that reproducible analysis of nitrosamine using HPLC should be achieved by post-column photolysis and chemiluminescence detector (LC-PR-CLD). French National Agency for Medicines and Health Products Safety Laboratory Controls Division reported that NDMA content should be detected using the HPLC method. Reported the NDMA detection limit in valsartan drug 0.0085µg/mL and quantification limit 0.0285 µg/mL, respectively.

- **LC-MS/MS**

LC-MS is sophisticated technique, which separates and quantify the components from a complex mixture with the help of mass spectrometer. Mass spectrometer separates and identified the charged components. LC-MS is a technique that use to analyse large, ionic, polar, non-volatile, and unstable organic compounds. LCMS/MS analysis can be done via soft ionization and impurity analysis using various types of modern ionization sources including electrospray ionization (ESI) and matrix- assisted laser desorption ionization (MALDI), APCI, APPI, CI, EI, FAB, SIM5, Z Spray, and TSP.ESI and APCI are widely used for analysis of nitrosamine impurities present in various samples. several methods are being developed and reported for routine QAQC for nitrosamine impurities using LC-MS/MS.

- **GC-MS, GC-MS/MS, GC-MS-Head space, and GC-QTOF**

GC-MS is a destructive and hard ionization technique for a qualitative and quantitative estimation of volatile organic compound or APIs. Although GC with various detectors can be used for nitrosamine detection, however, nitrogen-phosphorous detector (NPD) and nitrogen chemiluminescence detector (NCD) are the most suitable for the nitrosamine detection.

On 24th September 2018 FDA has released a gas chromatography-mass spectrometry (GC/MS) headspace method for manufacturers and regulators to detect and quantify NDMA in valsartan API and finished drug products. As per that NDMA impurity should be less than 0.3 ppm. Various methods reported for identify/quantify nitrosamine impurities in sartans, and ranitidine using GC-MS, GC-MS/MS, GCMS-Head space, and GC- QTOF.

USFDA quantified/ detected the presence of four toxic nitrosamine impurities, namely NDMA, NDEA, NEIPA, and NDIPA in valsartan drug using GC-MS/MS-Head space (HS) technique.

ANALYTICAL METHOD VALIDATION [5]

A general definition of validation is establishing documented evidence which provides a high degree of

assurance that a specific procedure, process, equipment, activity or system will consistently produce a product meeting its predetermined specifications and quality attributes. Validation is an important characteristic after the development of any analytical method because it is closely related to the quality of the results of product. All analytical methods, whether qualitative or quantitative are necessary to be validated. The degree of validation is various for the type of method and its application. Validation is an essential activity in the process of impurities profiling where the developed analytical method used for the detection of genotoxic impurities in drug substances is validated in order to establish that the method is suitable for its aimed purpose. The analytical methods are validated with accuracy, precision, linearity specificity, ruggedness, robustness and forced degradation parameters in accordance with ICH Harmonized Tripartite Guidelines.

CONCLUSION

The present review describes the details regarding genotoxic impurities in pharmaceuticals. Nitrosamines are genotoxic impurities, and due to their carcinogenic behavior, they pose an alarming warning to all creatures of earth. Nitrosamine impurity formation can be avoided by selecting proper reagent, catalyst and solvents in the manufacturing of drug substances. To alleviate this global issue, regulatory agencies such as CDSCO, US-FDA, and the European Medicines Agency (EMA) have given their continuous effort for quantitative determination of amine impurities present in food stuffs, and in various intermediates in organic synthesis. However, it is a challenging task for researchers, and industrialists to explore innovative techniques, and methods for precise estimation of nitrosamine impurities from various pharmaceutical APIs. We report various modern analytical methods, for smooth quantification of nitrosamines from complex mixtures. This review revealed broad analytical quantification, and detection of nitrosamine from a series of Valsartan drug, and ranitidine drugs and metformin drugs.

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