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Review

Selected Genotoxic Impurities Profiling During WFI Qualification to Control Carcinogenesis in Large Volume Parenterals

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Abstract: Water For Injections (WFI) which is the main vehicle of Large Volume Parenterals (LVPs) should be free from trace amount of genotoxic impurities. This review gives emphasis on quantification of genotoxic trace metals during qualification of WFI in LVPs manufacturing unit. According to ICH guidelines, impurities related to drug substances are classified into three main categories: Organic impurities, inorganic (elemental) impurities and residual solvents. Within these categories, genotoxic impurities form a special case that poses a significant safety risk, even at low concentrations, because they may be mutagenic and are therefore potentially damaging to DNA. As a result they can lead to mutations or cause cancer. Chemical carcinogens most often directly or after xenobiotic metabolism, act as genotoxic causes to induce DNA damage. The roles of trace metals (some of which are either genotoxic or non-genotoxic) in cancer development and inhibition have a complex character and have raised many questions because of their essential and toxic effects on people's health. Trace metals such as cadmium, nickel, arsenic, beryllium and chromium (VI) have been recognized as human or animal carcinogens by International Agency for Research on Cancer (IARC). There are several genotoxic chemicals like residual solvent, impurities and trace metals present in Pharmaceuticals to form carcinogens. Regulatory body like FDA and EMEA has fixed up specific limits for these elemental impurities. The toxicity of an elemental impurity is related to its extent of exposure (bioavailability). In that sense, parenteral dosage forms has its most possibility to be bioavailable than the other dosage forms, the limit of genotoxic impurities are 10 times lower than that of oral dosage forms by United States Pharmacopoeia (USP). The present article is for the importance of identification and quantification of the trace amount of the metal genotoxic impurities in Water For Injection (WFI) during qualification (IQ, OQ, PQ) as a preventive measure to control the production and distribution of WFI for Large Volume Parenteral (LVP) production.

Keywords: Large Volume Parenterals (LVP), Genotoxic Impurities, Qualification, Water For Injections, Cancer

Introduction

Genotoxic Impurities in Pharmaceuticals

Genotoxicity, as discussed in a consensus report (Vainio *et al*., 1992), is broad and includes both direct and indirect effects in DNA: (1) the induction of mutations (gene, chromosomal, genomial, recombinational) that at the molecular level are similar to events known to be involved in carcinogenesis, (2) indirect surrogate events associated with mutagenesis (e.g., Unscheduled DNA Synthesis (UDS) and Sister Chromatid Exchange (SCE), or (3) DNA damage (e.g., the formation of adducts), which may eventually lead to mutations.

Thus, genotoxic impurities induce genetic mutations, chromosomal rearrangements, chromosomal breaks and act as carcinogenic compounds (McGovern and Jacobson-Kram, 2006).

Carcinogens are classified as either genotoxic or nongenotoxic depending on their modes of action. Genotoxic carcinogens are those which initiate carcinogenesis by direct interaction with DNA, resulting in DNA damage or chromosomal aberrations that can be detected by genotoxicity tests. On the other hand, nongenotoxic carcinogens are agents that indirectly interact with the DNA, causing indirect modification to DNA structure, amount, or function that may result in altered gene expression or signal transduction (Vasseur and Lasne, 2012). The genetic changes are responsible for heritable effects on germ cells and impose significant risk to future generations (Janson, 2009).

The International Agency for Research on Cancer (IARC) (IARC, 2014) has evaluated the cancer-causing potential of more than 900 likely candidates, placing them into one of the following groups:

- Group 1: Carcinogenic to humans
- Group 2A: Probably carcinogenic to humans
- Group 2B: Possibly carcinogenic to humans
- Group3: Unclassifiable as to carcinogenicity in humans
- Group 4: Probably not carcinogenic to humans

According to current regulatory practice it is assumed that (*in vivo*) genotoxic compounds have the potential to damage DNA at any level of exposure and that such damage may lead/contribute to tumour development. Thus for genotoxic carcinogens it is prudent to assume that there is no discernible threshold and that any level of exposure carries a risk (EMAEMHU, 2006).

Genotoxic Impurities Profiling (GIP) in pharmaceuticals are of increasing concerns to both pharmaceutical industries and regulatory agencies due to their carcinogenic potency for humans. Regulatory agencies instructed to do *IN VITRO* and *IN VIVO* tests to detect compounds which induce genetic damage directly or indirectly by various mechanisms. Elemental impurities especially transitional metals are to identify and quantify for the preventive action for carcinogenesis. Elemental impurities include catalysts and environmental contaminants that may be present in drug substances, excipients, or drug products. These impurities may occur naturally, be added intentionally, or be introduced inadvertently (e.g., by interactions with processing equipment) (RBO, 2013).

But all heavy metals are not toxic, Metal ions are required for many critical functions in humans. Scarcity of some metal ions can lead to disease. Well-known examples include pernicious anemia resulting from iron deficiency, growth retardation arising from insufficient dietary zinc and heart disease in infants owing to copper deficiency (Lippard, 2009). It is also known that several essential transition metals, such as zinc, iron, copper, cobalt and manganese participate in the control of various metabolic and signaling pathways (Valko *et al*., 2005). Some metals and metals compounds has its anticancer activity also. The anti-cancer activities of the ten most active metals: Arsenic, antimony, bismuth, gold, vanadium, iron, rhodium, titanium, gallium and platinum have been discovered (Desoize, 2004).

Elemental impurities of the drug substances and drug products will be toxic and genotoxic at the form of Speciation. The determination of the oxidation state, organic complex, or combination is termed as speciation. Oxidative stress induces a cellular redox imbalance which has been found to be present in various cancer cells compared with normal cells; the redox imbalance thus may be related to oncogenic stimulation. Different transitional metals like arsenic, cadmium, beryllium, chromium, nickel, iron etc which can induce oxidative stress and can cause cancer (Valko *et al*., 2006). Table 1 shows the summary of the IARC evaluations of the carcinogenicity of trace elements and other related compounds as detailed in the preamble of the volumes of the IARC monograph series (IARC, 1993a).

Genotoxic Impurities in Large Volume Parenterals

Large volume parenterals are one of the important dosage forms for the patients especially in Intensive Care Unit (ICU), Coronary Care Unit (CCU), in Operation Theater (OT), pre and post operative patient and others for nutrition supply or emergency purpose as well. This is the dosage forms mainly administered on Intra Vein (IV) route which is later directly available to blood circulation of the body. Thus, the manufacturer of LVP products should follow the cGMP strictly to ensure the purity of drug product and drug substances.

Source of genotoxic impurities in the pharmaceutical dosage forms especially for parenteral products is like:

Genotoxic impurities from active pharmaceuticals ingredients:

- Genotoxic impurities during raw material synthesis
- Contaminants from packing material
- Impurities formed by degradation due to aging or during manufacturing
- Residual solvents are organic volatile chemicals used during manufacturing or impurities are formed during production
- Heavy metals: Main source of heavy metals from water which is used in the process and the reactors (if stainless steel reactors are used), where acidification or acid hydrolysis takes place
- Impurities are formed due to side reactions during the synthesis of drugs

Genotoxic Impurities from Water for Injections (WFI)

Table 1. IARC evaluations made of the carcinogenicity of trace elements and related compounds to humans by the international agency of research on cancer

Group 1: Carcinogenic to humans		Group 2A: Probably carcinogenic to humans		Group 2B: Possibly carcinogenic to humans		Group 3: Unclassifiable as to carcinogenicity in humans	
Trace element	Chemical group	Trace element	Chemical group	Trace element	Chemical group	Trace element	Chemical group
As	Arsenic and its compounds	Pl	Cisplatin	Sb	Antimony trioxide	Sb	Antimony trisulfide
Be	Beryllium and its compounds	---	---	Co	Cobalt and its compounds	Cr	Metallic and trivalent chromiumcompounds
Cd	Cadmium and its compounds	---	---	Pb	Inorganic lead compounds	Fl	Inorganic fluorides
Cr	Hexavalent chromium compounds	---	---	Ni	Metallic nickel	Fe	Ferric oxide and hematite
Ni	Nickel compounds	---	---	---	---	Pb	Organic lead compounds
---	---	---	---	---	---	Hg	Metallic and inorganic mercury compounds
---	---	---	---	---	---	Se	Selenium and its compounds
---	---	---	---	---	---	Ti	Titanium dioxide

Water for injections is widely used vehicle in parenteral formulations especially for large volume parenterals. It is also used in Small Volume Parenterals (SVP), powder for injection, lyophilized products, prefilled syringe, injectable suspension or emulsion. But WFI is used in large amount for Large Volume Parenterals (LVP) products. So that LVPs are of great concern for regulatory agencies. When the daily dose of an injection is equal or greater than 100 mL (Large Volume Parenteral (LVP)), the amount of elemental impurities present in the drug product must be controlled through the individual components used to produce the product (RBO, 2013). Regulatory agencies specified a minimum limit of elemental impurities in each component used for LVP (PerkinElmer, Inc, 2013).

Table 2 shows the elementary impurities limit for drug products for United States pharmacopoeia (RBO, 2013).

The purpose of this review is to converse the controlling of genotoxic impurities in large volume parenterals which is taken directly to the blood circulation and readily bio available to blood plasma and water for injection covers the maximum volume of LVP products, so Genotoxic Impurities Profiling (GIP) of WFI should be the initiation of controlling trace elements in LVPs. WFI qualification at the start of the plant for a pharmaceutical company is very important to ensure the supply of elemental impurities free WFI to the production area as well as to the whole plant which can lead finally genotoxic impurities free large volume parenteral products.

Water for Injection in Large Volume Parenterals

Water for injections is widely used vehicle in parenteral formulations especially for large volume parenterals, Water for injections must be free of pyrogens and have a high level of chemical purity especially from genotoxic impurities (Rees *et al*., 2014). Here, I will show some sample manufacturing formula, where we can see that there is few amount of raw materials are used in large amount of Water For Injections (WFI) for production of large volume parenterals. There are several type of water, like drinking water, tap water, distilled water, osmotized water/purified water, Water for injections etc. Among them, Water for injection in bulk is used for each and every formulation of parenteral products. Table 3 shows the formulation of a very common large volume parenterals product name-Dextrose and sodium chloride infusion (B. Braun Medical Inc., 2008). Not only that WFI is used for some other parenteral formulations which are supplied along with sterilized water for injections for dilution of the powder for injection products or some other formulations like Small Volume Parenterals (SVP), eye drops, injectable emulsions etc.

Parenteral preparations may contain excipients such as solvents, suspending agents, buffering agents, substances to make the preparation isotonic with blood, stabilizers, or antimicrobial preservatives. The addition of excipients should be kept to a minimum in LVPs (Ingle *et al*., 2014).

Water for injection is distributed in every Point Of Use (POU) of the state of the large volume parenterals unit (Fig. 1), thus the regulatory body should be more concern about the WFI production, distribution and regeneration unit for a LVP plant. Qualification which includes Installation Qualification (IQ), Operational Qualification (OQ) and Performance Qualification (PQ) here is very much important to cover the controlling of trace elements. As the regulatory body like Food and Drug Administration (FDA) and The European Agency for the Evaluation of Medicinal Products (EMEA) are now very much concern about the large volume parenterals. Water for injection should be the most important item to be qualified for distribution and storage of the elemental impurities free water to be used for injection.

Table 2. Elemental impurities for drug products

Elements	Oral Daily Dose PDE[a] (µg/day)	Parenteral Daily Dose PDE (µg/day)	Inhalational Daily Dose PDE (µg/day)	LVP Component Limit (µg/g)
Cadmium	5	1.5	3.4	0.25
Lead	5	5	5	0.5
Inorganic arsenic	15	15	1.9	1.5
Inorganic mercury	15	1.5	1.2	0.15
Iridium	100	10	1.5	1
Osmium	100	10	1.5	1
Palladium	100	10	1	1
Platinum	100	10	1.5	1
Rhodium	100	10	1.5	1
Ruthenium	100	10	1.5	1
Chromium	Not a safety concern	Not a safety concern	2.9	Not a safety concern
Molybdenum	180	90	7.6	9
Nickel	600	60	6	6
Vanadium	120	12	1.2	1.2
Copper	1300	130	13	13

[a]PDE = Permissible daily exposure based on a 50-kg person

Table 3. The formulation of very common large volume parenterals product name Dextrose and Sodium Chloride infusion by B. Braun Medical Inc

	Composition-Each 100 mL contains:						
Solution	Hydrous dextrose USP	Sodium Chloride USP	Sodium	Chloride	Calories per Liter	Calculated Osmolarity mOsmol/liter	pH
2.5% Dextrose and 0.45% Sodium Chloride Injection USP	2.5 g	0.45 g	77	77	85	280	4.6 (3.5-6.5)
3.3% Dextrose and 0.30% Sodium Chloride Injection USP	3.3 g	0.3 g	51	51	110	270	4.5 (3.5-6.5)
5% Dextrose and 0.9% Sodium Chloride Injection USP	5 g	0.9 g	154	154	170	560	4.4 (3.5-6.5)
5% Dextrose and 0.45% Sodium Chloride Injection USP	5 g	0.45 g	77	77	170	405	4.4 (3.5-6.5)
5% Dextrose and 0.33% Sodium Chloride Injection USP	5 g	0.33 g	56	56	170	365	4.4 (3.5-6.5)
5% Dextrose and 0.20% Sodium Chloride Injection USP	5 g	0.2 g	34	34	170	320	4.4 (3.5-6.5)
10% Dextrose and 0.9% Sodium Chloride Injection USP	10 g	0.9 g	154	154	340	815	4.3 (3.5-6.5)
10% Dextrose and 0.45% Sodium Chloride Injection USP	10 g	0.45 g	77	77	340	660	4.3 (3.5-6.5)
10% Dextrose and 0.20% Sodium Chloride Injection USP	10 g	0.2 g	34	34	340	575	4.3 (3.5-6.5)

Water for Injection USP quantity sufficient to 100 mL

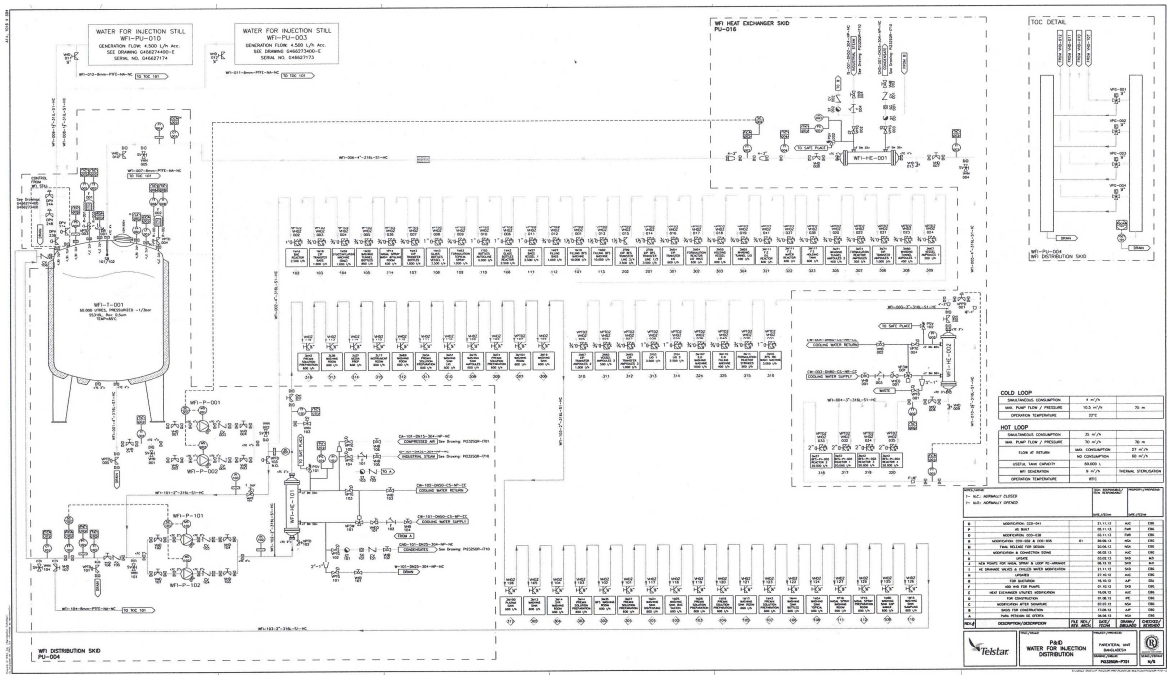

Fig. 1. A sample WFI distribution diagram for a large volume parenteral production unit

Mechanism of Genotoxicity by Trace Metals

Metal-induced toxicity and carcinogenicity, with an emphasis on the generation and role of reactive oxygen and nitrogen species, is reviewed. Metal-mediated formation of free radicals causes various modifications to DNA bases, enhanced lipid peroxidation and altered calcium and sulfhydryl homeostasis. Lipid peroxides, formed by the attack of radicals on polyunsaturated fatty acid residues of phospholipids, can further react with redox metals finally producing mutagenic and carcinogenic malondialdehyde, 4-hydroxynonenal and other exocyclic DNA adducts (etheno and/or propano adducts) (Leonard *et al*., 2004; Stohs and Bagchi, 1995; Pekarkova *et al*., 2001; Valko *et al*., 2001; Halliwell and Guteridge, 1990). Whilst Iron (Fe), Copper (Cu), Chromium (Cr), Vanadium (V) and Cobalt (Co) undergo redox-cycling reactions, for a second group of metals, Mercury (Hg), Cadmium (Cd) and Nickel (Ni), the primary route for their toxicity is depletion of glutathione and bonding to sulfhydryl groups of proteins. Arsenic (As) is thought to bind directly to critical thiols, however, other mechanisms, involving formation of hydrogen peroxide under physiological conditions, have been proposed. The unifying factor in determining toxicity and carcinogenicity for all these metals is the generation of reactive oxygen and nitrogen species (Valko *et al*., 2005).

Overproduction of Reactive Oxygen Species (ROS) through either endogenous or exogenous insults is harmful to living organisms and is termed oxidative stress (Mussali-Galante, 2007). The toxic metals which can be present in the water for injection can cause the following possible mechanisms involved in metal-induced oxidative stress, (Fig. 2) (Ercal *et al*., 2001a).

As, genotoxic impurities are present in large volume parenterals, then WFI is one of the main components to be qualified as genotoxic impurities free for the proactive measure of controlling it in Large Volume Parenterals (LVPs). There are huge possibilities having genotxic impurities in Water For Injections (WFI) which is to be used for LVPs. But, in the current pharmacopoeia (BP and USP), there is no specification and method of identification and quantification of genotoxic metals in 'Water for injections in bulk'.

Toxicity/Genotoxicity of Trace Elements Present in Large Volume Parenterals (LVPs)

As per the guidelines of recent regulatory agencies like Food and Drug Administration (FDA) and The European Agency for the Evaluation of Medicinal Products (EMEA), the following elemental impurities which are gentotoxic should be controlled strictly for LVP components and Parenteral products (RBO, 2013):

Cadmium

Cadmium is the 48th element and a member of group 12 in the Periodic table of elements. The most common oxidation number of cadmium is +2. Cadmium (Cd) is a nonessential, group II_B metal. It is found in foods, Water and tobacco leaves. It has been shown that cadmium is mainly stored in soft tissues, especially in the liver and kidneys and induces lipid peroxidation in the liver, kidneys, brains, lungs and heart. Unless other heavy metals, Cd has a long biological shelf life (10-30 years) and is excreted very slowly from the body (Ercal *et al*., 2001b). Cadmium can cause osteoporosis, anemia, non-hypertrophic emphysema, irreversible renal tubular injury, eosinophilia, anosmia and chronic rhinitis. Cadmium is a potent human carcinogen and has been associated with cancers of the lung, prostate, pancreas and kidney (Valko *et al*., 2005). Cadmium is a cumulative toxicant and carcinogenic that affects kidneys, genrates various toxic effects in the body, disturbs bone metabolism and deforms reproductive tract as well as endocrine system. There are several morphopathological changes in the kidneys due to long-term exposure to cadmium. Increasing intakes of zinc can reduce the renal toxicity of cadmium. An exposure to cadmium increases calcium excretion thus causes skeletal demineralization, probably leading to increases in bone fragility and risk of fractures (Mudgal *et al*., 2010). Because of its carcinogenic properties, cadmium has been classified as a #1 category human carcinogen by the International Agency for Research on Cancer of USA (IARC, 2014).

Lead

Lead has atomic number 82 (symbol Pb) and is one of the heavy metals. Lead is a persistent metal and because of its unusual physical-chemical properties it is used in various industrial applications (Jomova and Valko, 2011). Lead is known to induce a broad range of physiological, biochemical and behavioural dysfunctions in laboratory animals and humans, including central and peripheral nervous systems, haemopoietic system, cardiovascular system, kidneys, liver and male and female reproductive systems (Flora *et al*., 2008). Lead is a toxic metal to humans and animals and its persistency causes prolonged occurrence in the environment-in water, soil, dust and in manufactured products containing lead. Since young organisms bear the heaviest burden of sensitivity to lead exposure, lead-based paint covers represent a serious health threat to children worldwide (Jomova and Valko, 2011). Children under 6 years are especially susceptible to the adverse effects of Pb, as the blood-brain barrier is not yet fully developed in young children, hematological and neurological adverse effects of Pb occur at lower threshold levels than in adults. Pb has effects on erythropoiesis and haem biosynthesis. Chronic Pb intoxication in adults resulted in to anemia, some types of cancer, reproductive harm in males while in young children hormonal imbalance of metabolite of vitamin D, namely 1, 25-dihydroxy-vitamin D, drop in IQ (Mudgal *et al*., 2010).

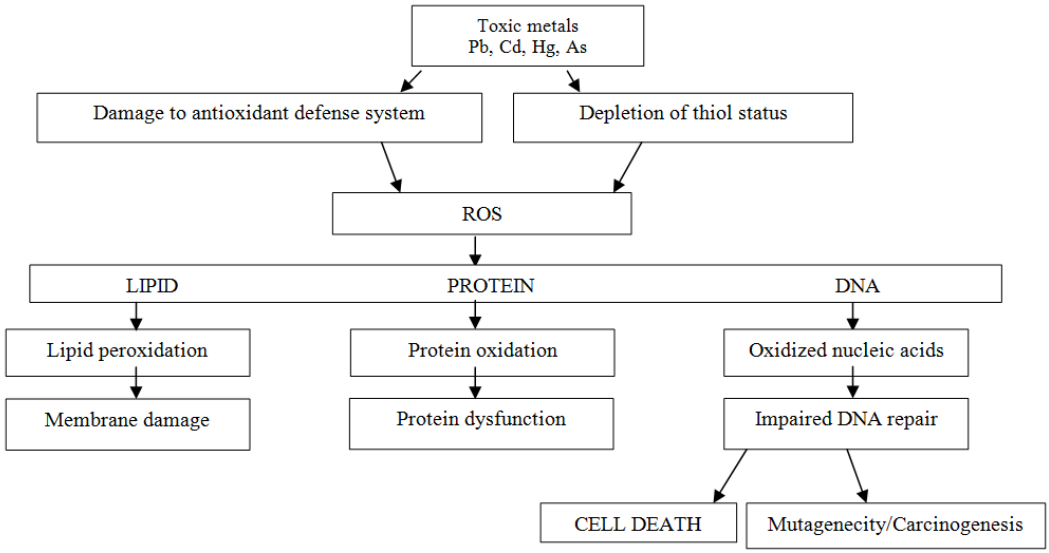

Fig. 2. Possible mechanisms for metal-induced oxidative stress

Inorganic lead salts, with ionic bonds like lead acetate, have different chemical properties and toxicological effects compared to organolead compounds, with covalent bonds like tetraethyl lead. Several epidemiological and animal experimental works suggest that inorganic lead compounds are associated with increased risks of tumorigenesis. Lead acetate, lead sub-acetate and lead phosphate have been found to cause tumors in the kidneys of rats and mice (Mulware, 2013).

Inorganic Arsenic

Arsenic is the 33rd element of the periodic table of elements with the most common oxidation numbers of +5, +3 and -3. Arsenic has the capability to form both inorganic and organic compounds in the environment and human body. Inorganic arsenic includes arsenite (As (III)) and arsenate (As (V)). The inorganic arsenics can be either methylated (Monomethylarsonic Acid, MMA) or Dimethylarsinic Acid (DMA) *in vivo* (Valko *et al*., 2005). Inorganic arsenic is known to be more poisonous than organic one. Arsenic trioxide (As_2O_3) is the most common inorganic arsenical and is well known as a poison and has been discovered to be a carcinogen in humans (Valko *et al*., 2006), while arsenates (AsO_4^{3-}) or arsenites (AsO_2) occur in water, soil, or food (Mudgal *et al*., 2010).

Arsenic is a well-documented carcinogen in a number of studies. Exposure to arsenic is linked with a risk of developing tumours of the lung, skin, liver, bladder and kidney (Jomova and Valko, 2011). As determined by IARC report, arsenic and its compounds are highly carcinogenic to humans. Skin cancer and lung cancer have been reported in patients treated with inorganic trivalent arsenic compounds, drinking water with high levels of arsenic and those with occupational exposures to inorganic arsenic compounds in mining and copper smelting industries, respectively. Other cancers liked to arsenic toxicity include kidney and bladder. While cardiovascular disorders following oral exposure to arsenic are well documented, there is some evidence from epidemiological trials that also inhaled inorganic arsenic can affect the cardiovascular system (Mulware, 2013). It has been reported that arsenic induced oxidative stress also causes DNA strand breaks, alkali-labile sites, which eventually results into DNA adducts (Pu *et al*., 2007). Arsenic mediation can also alter methylation status of oncogenes and tumor suppressor genes and in the processes enhancing carcinogenesis (Reichard and Puga, 2010).

Inorganic Mercury

Mercury is the 80th element of the periodic table of elements. Mercury is unique in that it is found in nature in several chemical and physical forms. At room temperature, elemental (or metallic) mercury exists as a liquid with a high vapor pressure and consequently is released into the environment as mercury vapor. Mercury also exists as a cation with an oxidation state of +1 (mercurous) or 2+ (mercuric). In the environment, humans and animals are exposed to numerous chemical forms of mercury, including elemental mercury vapor (Hg), inorganic mercurous (Hg (I)), mercuric (Hg (II)) and organic mercuric compounds (Flora and Pachauri, 2010). Mercury (Hg) and its compounds are highly toxic, especially methylmercury-a potent neurotoxin. It has caused a significant number of human fatalities in several accidents around the world (Mudgal *et al*., 2010). Mercury has a low excretion rate. A major proportion of absorbed Hg accumulates in the kidneys, neurological

tissue and the liver. All forms of mercury exhibit toxic effects, including neurotoxicity, nephrotoxicity and gastrointestinal toxicity (Tchounwou *et al*., 2012).

Platinum Group Genotoxicity

The platinum-group metals are six metallic elements clustered together in the periodic table. These elements are all transition metals, lying in the d-block (groups 8, 9 and 10, periods 5 and 6). The six platinum-group metals are ruthenium, rhodium, palladium, osmium, iridium and platinum. They have similar physical and chemical properties.

Iridium

Iridium is the 77th element of the periodic table of elements. A radioisotope of iridium, ^{192}Ir, is dangerous like other radioactive isotopes. High-energy gamma radiation from ^{192}Ir can increase the risk of cancer (Audi *et al*., 2003a). External exposure can cause burns, radiation poisoning and death (Bhattacharya, 2010). Ingestion of ^{192}Ir can burn the linings of the stomach and the intestines (MedlinePlus, 2004). ^{192}Ir, ^{192m}Ir and ^{194m}Ir tend to deposit in the liver and can pose health hazards from both gamma and beta radiation (Iridium, 2005). Iridium in bulk metallic form is not biologically important or hazardous to health due to its lack of reactivity with tissues; there are only about 20 parts per trillion of iridium in human tissue. Like most metals, finely divided iridium powder can be hazardous to handle, as it is an irritant and may ignite in air. Very little is known about the toxicity of iridium compounds because they are used in very small amounts, but soluble salts, such as the iridium halides, could be hazardous due to elements other than iridium or due to iridium itself. However, most iridium compounds are insoluble, which makes absorption into the body difficult (Audi *et al*., 2003a).

Osmium

Osmium is the 76th element of the periodic table of elements. It is a hard, brittle, bluish-white transition metal in the platinum group that is found as a trace element in alloys, mostly in platinum ores (Hammond, 2005). Osmium has a blue-gray tint and is the densest stable element, slightly denser than iridium (http://en.wikipedia.org/wiki/Osmium). Finely divided metallic osmium is pyrophoric and reacts with oxygen at room temperature forming volatile osmium tetroxide. Some osmium compounds are also converted to the tetroxide if oxygen is present (http://carpdemo.cloudapp.net/FactFinder/demo/en?source=&subject=osmium&context=osmium). Osmium tetroxide is highly volatile and penetrates skin readily and is very toxic by inhalation, ingestion and skin contact (Luttrell and Giles, 2007). Airborne low concentrations of osmium tetroxide vapor can cause lung congestion and skin or eye damage (Stellman, 1998). Osmium tertroxide is very toxic. Ingestion will lead to the destruction of the tissues of the mouth and throat. OsO_4 is highly poisonous, even at low exposure levels and must be handled with appropriate precautions. In particular, inhalation at concentrations well below those at which a smell can be perceived can lead to pulmonary edema and subsequent death (mirror.chemwatch.net/Bulletin/2005/11/051118-Bulletin.rtf; Ludwig *et al*., 1994). In high (not specified) levels, it may be corrosive to the eyes and the skin and also may cause systemic effects, pneumonia and lethality. Tolerated concentration: 0.001 mg/m^3 for 6 h. Repeated or prolonged contact with skin man causes dermatitis. The substance may cause effect on the kidney (http://www.tedpella.com/msds_html/msds.htm.aspx).

Palladium

Palladium compounds are encountered relatively rarely by most people. All palladium compounds should be regarded as highly toxic and as carcinogenic. Palladium chloride is toxic, harmful if swallowed, inhaled or absorbed through the skin. It causes bone marrow, liver and kidney damage in laboratory animals (Coursey *et al*., 2009; http://www.lenntech.com/periodic/elements/pd.htm). Literature review reveals that the mechanism of Pd or Pt ion-mediated exacerbation of DNA damage by a fenton system is due to the promotion of OH (hydroxyl) production by these metal ions. Another article says “The results indicate that Pd (II) interacts with both the phosphate and bases of DNA. The binding of Pd (II) to DNA brings about considerable conformational changes in DNA (Liu *et al*., 1997; Pillai and Nandi, 1977). Palladium is cytotoxic and kills or damages cells. Palladium also causes considerable damage and degradation of DNA and exacerbates hydroxyl radical damage. Palladium also damages cell mitochondria and inhibits enzyme activity and function. Palladium also causes significant numbers of allergic reactions as well as contacts dermatitis, stomatitis, lichinoid reactions and periodontal gum disease (Reddy *et al*., 2013).

Platinum

Platinum is a chemical element with symbol Pt and atomic number 78. It is a dense, malleable, ductile, highly unreactive, precious, gray-white transition metal. Its name is derived from the spanish term platina, which is literally translated into “little silver”. A very heavy, precious, silver-white metal, platinum is soft and ductile and has a high melting point and good resistance to corrosion and chemical attack (Platinum, 2012; Harper, 2015).

The toxicological effects of platinum in humans are confined to certain of its complex halide salts and to the antitumour agent *cis*-platin and its analogues. The adverse health effects of the halide salt complexes are characterized by sensitization; these compounds are among the most potent sensitizers known. Such sensitization has been reported almost exclusively from occupational environments. An initial report in 1911 described irritation of the nose and throat with difficulty in breathing in workers in a photographic studio handling paper treated with complex platinum salts. In a study in four british refineries, 52 of 91 workers exposed to complex platinum salts exhibited repeated sneezing, rhinorrhoea, chest tightness, wheezing, shortness of breath and cyanosis, while a proportion of these also developed scaly erythematous dermatitis with urticaria (WHO, 2000). We know that platinum is used to produce the nano particles as cisplatin and which are further use for chemotherapy treatment as a cytotoxic elements for carcinogenic cells. It has been found that nanoparticles can induce increased lung toxicity compared to larger particles with the same chemical composition at equivalent mass concentration (Oberdörster *et al*., 2005). In addition, it has been also shown that nanoparticles of different diameters can induce inflammatory reactions in the lungs of experimental animals. Nanoparticles can directly induce cytotoxic morphological changes in human umbilical vein endothelial cells, induction of proinflammatory responses, inhibition of cell growth and reduction of endothelial nitric oxide synthase (Medina *et al*., 2007). Cisplastin which is a platinum compound is known to cause leukemia in rats and lung and skin tumors in mice (IARC, 1993b).

Rhodium

Toxicologic data on the soluble salts of rhodium are meager; solutions of salts splashed in the eye may cause mild irritation. Rhodium trichloride as the salt or soluble form of rhodium was found to have chemotherapeutic action against certain viruses in mice. Rhodium Chloride has caused eye damage in animals. Persons with a pre existing eye disorder may be more susceptible to the effects of this agent. Although rhodium is not known as a kidney toxin in humans, the importance of this organ in the elimination of toxic substances justifies special consideration in those with impaired renal function. Rhodium trichloride has caused central nervous system damage in animals. Individuals with pre existing central nervous system disorders may be more susceptible (DHHS, 1978).

Ruthenium

Ruthenium usually occurs as a minor component of platinum ores (Summary, 2009). Ruthenium can be oxidized to ruthenium (IV) oxide (RuO_2, oxidation state +4) which can in turn be oxidized by sodium metaperiodate to ruthenium tetroxide, RuO_4 (Brown and Butler, 1997). Ruthenium compounds should be regarded as highly toxic and as carcinogenic. Compounds of ruthenium stain the skin very strongly. It seems that ingested ruthenium is retained strongly in bones. Ruthenium oxide, RuO_4, is highly toxic and volatile and to be avoided. It is among the long-lived radionuclides that have produced and will continue to produce increased cancers risk for decades and centuries to come (http://www.lenntech.com/periodic/elements/ru.htm#ixzz3PYcXtv30). A literature review reveals that selected compounds were also tested for their ability to induce the bacterial SOS system in the bacillus subtilis Comptest. In this system, cis-platinum similarly showed greater inducing ability than did the ruthenium complexes. These results also demonstrated that the nature of the sixth ligand in the ruthenium compounds has a significant effect on the mutagenic capacity of these agents (Yasbin *et al*., 1980).

Chromium

Chromium is the 24th element of the periodic table. Chromium exists in a series of oxidation states with a valence from -2 to +6; the most important stable states are 0 (elemental metal), +3 (trivalent) and +6 (hexavalent). Trivalent (Cr [III]) and hexavalent (Cr [VI]) compounds are thought to be the most biologically significant (Valko *et al*., 2005). Chromium has 2 metastates (Audi *et al*., 2003b). Cr (III) is an essential dietary mineral in low doses. It is required to potentiate insulin and for the normal glucose metabolism. Chromium (VI) at high doses is considered to be the greatest health risk (RBO, 2013). According to the International Agency for Research on Cancer (IARC), carcinogenicity of Cr (VI) compounds in the lung and in the nasal cavity has been confirmed. Hexavalent chromium is recognized as a human carcinogen via inhalation and known to cause lung cancer in humans (Jomova and Valko, 2011). Chromium ions is most carcinogenic in the form of CrO^{-2}_4, which enters the body cell by sulfate uptake pathway and is ultimately reduced to Cr(III) through a Cr(IV)-glutathione intermediate species. The hexavalent latter complex then binds with the DNA to produce a kinetically inert and potentially damaging lesion and can cause abnormal phenotype due to the formation of ROS (Mulware, 2013). This occupational exposure to Cr (VI)-containing compounds is known to induce lung toxicity and increased incidence of respiratory-system cancers. Certain extracellulary generated Cr (V) and Cr (III) complexes also have high permeabilities through the cell membrane and therefore such species have to be taken into account when describing the complex model of chromium carcinogenicity (Valko *et al*., 2005).

Molybdenum

Molybdenum is the 42nd element of the periodic table of elements. Molybdenum does not occur naturally as a free metal on earth, but rather in various oxidation states in minerals. Most molybdenum compounds have low solubility in water, but the molybdate ion MoO_4^{2-} is soluble and forms when molybdenum-containing minerals are in contact with oxygen and water. Water-soluble molybdenum compounds are readily taken up through the lungs and gastrointestinal tract; but insoluble compounds are not. Following absorption, molybdenum is distributed throughout the body with the highest levels generally found in the liver, kidneys, spleen and bone (Wennig and Kirsch, 1988). Molybdenum toxicity can result from increased intake of molybdenum (over 10-15 mg/day). Those that were exposed to molybdenum over long periods of time in an occupational setting are also at risk of toxicity, though it has been determined that unlike other metals, a very large amount of Mo exposure is required to elicit toxic effects (MIC, 2012). In humans, it has been shown that those that take more than 10-15 mg/day of molybdenum are at increased risk for gout (HIS, 2012). Chronic exposure to molybdenum in the workplace can cause symptoms of fatigue, headaches and joint pain-if these symptoms are occurring in someone that may have occupational exposure to molybdenum, further investigations may be necessary (Molybdenum, 2012). A case report of acute human molybdenum toxicity revealed as one year after the Mo poisoning, the patient was diagnosed toxic encephalopathy with executive deficiencies, learning disability, major depression and post-traumatic stress disorder (Momcilović, 1999). A research was concluded for the toxicity of molybdenum as, when 0.1% or more molybdenum was fed, gross toxic symptoms were produced. The toxic syndrome was characterized by anorexia, loss of weight, alopecia, dermatosis, anemia and death (Arrington and Davis, 1953).

Nickel

Nickel is the 28th element of the periodic table. It is a silver-white metal found in several oxidation states, ranging from -1 to +4 (Valko *et al*., 2005). Nickel is a human carcinogen that can alter gene expression by enhanced DNA methylation and compaction, rather than via mutagenic mechanisms. The nickel compounds implicated as potential carcinogens are insoluble dusts of nickel subsulphides and nickel oxides, the vapor of nickel carbonyl and soluble aerosols of nickel sulphate, nitrate, or chloride (Valko *et al*., 2006). Occupational exposure to nickel occurs in industries that include: High temperature oxidation of nickel matte and nickel-copper matte, electrolytic refiners, copper plants and during nickel salt extraction in the hydrometallurgical industry. Epidemiological studies among nickel refinery workers exposed to nickel sulfate and a combination of nickel sulfides and oxides showed high risk of nasal cancer, larynx cancer, prostate cancer and lung cancer (Mulware, 2013). Almost all cases of acute nickel toxicity result from exposure to nickel carbonyl. Patients with severe poisoning develop intense pulmonary and gastrointestinal toxicity. The lung is the primary target organ for nickel toxicity in humans (Grimsrud *et al*., 2005). Studies on the direct effect of nickel ions on DNA found that nickel ions could enhance the oxidation, hydroxylation and deglycosylation of DNA bases (deoxynucleosides and deoxynucleotides) induced by active oxygen species. The nickel compounds that have been found to be carcinogenic in experimental animals include metallic nickel, nickel monoxides, nickel hydroxides and crystalline nickel sulfides (Valko *et al*., 2006).

Vanadium

Vanadium is the 23rd element of the periodic table. Vanadium may be beneficial and possibly essential in humans, but certainly essential for some living organisms. All vanadium compounds should be considered toxic. Tetravalent $VOSO_4$ has been reported to be over 5 times more toxic than trivalent V_2O_3 (http://75.82.149.74:10007/modules/olpc/wikislice-en/files/articles/Vanadium.htm). Vanadium oxides are usually more toxic than vanadium salts and Vanadium (V) is usually more toxic than vanadium (IV) compounds. Animal studies revealed various toxic effects induced by vanadium compounds. The most affected organs, as documented by histopathological alterations, were liver and kidney. Intraperitoneal injections of rats with orthovanadate revealed nephrotoxicity (Valko *et al*., 2005). There is little evidence that vanadium or vanadium compounds are reproductive toxins or teratogens. Vanadium pentoxide was reported to be carcinogenic in male rats and male and female mice by inhalation in an NTP study (Ress *et al*., 2003). Intravenous infusions of cumulative doses of vanadate produced significant increase in arterial blood pressure. Potent vasoconstrictor effects *in vitro* as well as in animals were demonstrated in several laboratories. Vasoconstrictor effects of vanadate are due to inhibition of Ca-ATPase and reduction in calcium efflux (Venkataraman and Sudha, 2005). High level acute exposures may result in CNS effects including paralysis, respiratory depression, convulsions and death (Dabney, 1994).

Copper

Copper (cuprum) is the 29th element of the periodic table. Its electronic configuration is $3d^{10}4s^{1}$. Copper toxicity, also called copperiedus, refers to the

consequences of an excess of copper in the body. Acute symptoms of copper poisoning by ingestion include vomiting, hematemesis (vomiting of blood), hypotension (low blood pressure), melena, coma, jaundice and gastrointestinal distress (Klaassen, 2007). Chronic effects of copper exposure can damage the liver and kidneys (EFS, 2005). One study of two strains of mice fed copper compounds found a varying increased incidence of reticulum cell sarcoma in males of one strain (http://www.epa.gov/iris/subst/0368.htm). The studies of reproductive toxicity and the carcinogenicity of metals, performed on human protamine HP2 (1-15) a peptide modeling the *N*-terminal amino acid sequence of human protamine HP2, were presented by (Liang *et al*., 1999; Bal *et al*., 1997; Valko *et al*., 2005) and they ended their research as The results revealed that HP2 is capable of binding Ni(II) and Cu(II) and, in this way, attenuating the mediation of oxidative DNA damage by copper(II), (but not nickel(II)). These effects may be mechanistically involved in the reproductive toxicity and carcinogenicity of metals. Copper is believed to be the switch that turns on the angiogenesis process in tumor cells. Abnormally high serum copper levels are found in patients with many types of progressive tumors, making copper an obligatory cofactor in angiogenesis process. Copper-binding molecules (ceruloplasmin, heparin and tripeptide glycly-histadyl-lysine) are non-angiogenic when free of copper, but they become angiogenic when bound to copper. As such, anti-copper drugs have been used cancer treatment (Mulware, 2013).

Suggestion and Discussion

Preventive Action for Genotoxic Impurities Controlling in LVPs

Regulatory body suggested and implemented, the trace elements or gentoxic impurities in a very lower range (Table 2: Elemental impurities for drug products) after risk assessment for LVP component.

Water for injections is the common component for all LVPs (see Table 3 as an example). So, there should have taken some precaution of manufacturing and distribution of water for injections in a parenteral manufacturing unit.

To control the genotoxic impurities in large volume parenterals, Water for injections should be genotoxic Impurities free first which might ensure the final genotoxic impurities free finished Products. Water for injections is continuously produced and supplied from the generation unit to the end users of manufacturing unit; so, it is to be confirmed as genotoxic impurities free at the very beginning of the production of water for injection for the manufacturing of large volume parenterals. WFI qualification (OQ and PQ) should be done under considering these genotoxic impurities which might help to ensure a qualitative WFI. The protocol of WFI qualification (OQ, PQ) should cover all those specification which will ensure the controlling of genotoxic impurities in the final production of WFI.

According to the current pharmacopoeia (BP and USP) monograph for water for injections, there is only limit of aluminum test, but there is no specification against the trace metals in WFI. But during water for injections qualification, the validation protocol should cover the wide range of limit tests as per current general chapter for elemental impurities-limit test for cadmium, lead, arsenic, mercury, iridium, osmium, palladium, platinum, rhodium, ruthenium and molybdenum instead of heavy metal test. Because heavy metal test which is conducted by a color change method is not sufficient enough to identify and quantify the specific elemental impurities present. Each and every genotoxic impurity should be tested individually during water qualification (OQ and PQ) which will ensure a genotoxic impurities free WFI for the manufacturing unit of Large Volume Parenterals (LVPs).

Example of WFI Production, Storage and Distribution System

The feed water of WFI Generator System is the purified water. WFI is produced by a two twin plants with a combined production capacity. The WFI generation system includes three U-type double tube shell heat exchangers built in stainless steel: Two pre-heaters for inlet water; one heats the purified water by means of the pure steam and distillate water coming from the last column and the other by means of the plant steam condensates of the first column. The cooling water flows inside the tubes and the WFI and pure steam in the shell side. The distillation column which is being used for the generation of WFI from purified water is a vertical column with two parts. The lower part consisting in a shell tube heat exchanger which is joined to another empty shell in the upper part which constitutes the separation chamber, designed to prevent carryover of water droplets and impurities. All the piping, fittings and valves those come contact with WFI should be made of specific grade stainless still otherwise the carcinogenic trace metals like chromium, nickel can be generate from stainless steel (no specific grade) and could be available in water for injections also (Santonen *et al*., 2010).

The storage system includes the following equipment:

- WFI storage tank including its accessories (installed out of the skid frame and equipped with a vent filter of specific nominal pore size)
- Support frame made in modular basis in stainless steel

The WFI distribution system includes the following equipment:

- Two different WFI loops according to temperature
- One cold loop at 20°C re-circulating on its self and with a cooling/heating heat exchanger
- One hot loop at 85°C re-circulating to the tank with 1 heat exchanger cooling the last 4 points to 60°C and one heat exchanger at the end of the loop to heat it up to 85°C
- Double sanitary centrifugal distribution pumps
- Double Tube-Sheet (DTS), for cold loop to allow thermal control at the WFI distribution cold loop
- Double Tube-Sheet (DTS) for hot loop
- Instrumentation and control system for monitoring the relevant and critical water loop variables for both loops (cold and hot WFI loop)

Proposed Protocol to Qualify WFI to Supply in Large Scale

From the very beginning of the production of WFI, the manufacturers should take action for controlling the genotoxic impurities or elemental impurities of WFI. To do that, there should have a protocol for qualifying the production, storage and supply of Water for injection to every Point Of Use (POU) for the production of large volume parenterals.

A three-phase approach should be followed to satisfy the objectives of proving the reliability and robustness of the system in service over an extended period.

Phase-1

A two weeks daily sampling and testing of each and every Point Of Use (POU) and other defined sample points (Generation plant) for extensive monitoring of the WFI system. The following should be included in this phase:

- Chemical tests including all Genotoxic Impurities Profiling (GIP) will be performed in accordance with a defined plan
- Microbiological tests will be performed in accordance with a defined plan
- Finalize sanitizing and maintenance procedures
- Demonstrate production and delivery of product water of required quality and quantity
- Use and refine the Standard Operating Procedures (SOPs) for operation, maintenance, sanitization and troubleshooting

Phase-2

A further test period of two weeks with the same sampling scheme as in Phase 1 (Perform the chemical including GIP and microbiological test) will be conducted for further intensive monitoring.

Water can be used for manufacturing purposes during this phase which has the following features:

- Demonstrate consistent operation within established ranges
- Demonstrate consistent production and delivery of water of the required quantity and quality when the system is operated in accordance with the SOPs

Phase-3

Phase 3 will run up to 1 year after the satisfactory completion of Phase-2. Water can be used for manufacturing purposes during this phase which has the following objectives and features:

- Demonstrate extended reliable performance
- Ensure that seasonal variations are evaluated
- Sampling and testing of WFI from each and every Point Of Use (POU) from Generation, storage and distribution loops will be conducted on a weekly basis where chemical including GIP and microbiological test will be conducted

Conclusion

The above discussion provides an insight to the role of analysis of speciation of the transitional trace elements present in water for injections during qualification in a pharmaceutical factory to ensure the supply and storage of pyrogen free, genotoxic impurities free water for the production of LVPs in large scale. Apart from this, this review also provides a view of toxicity of the trace metals which are enlisted into the "Elemental impurities-limit" chapter of United States Pharmacopoeia (USP). This is an insight of the toxicities of those trace metals which could be present in LVP products also. Genotoxicity of these trace metals are incorporated by the production of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) which is harmful to living organisms and is termed as oxidative and nitrosative stress which can attack DNA and is mutagenic and therefore is a potential biomarker of carcinogenesis (Huang *et al*., 2010). This ROS and RNS is the oxidation state of the transitional metals which is termed as speciation. Each of the elemental impurities has the potential to be present in differing oxidation or complexation states (RBO, 2013). However, arsenic and mercury are of particular concern because of the differing toxicities of their inorganic and complexed organic forms where inorganic arsenic and inorganic (2+) oxi-dation state of mercury is most toxic. Other examples of oxidation state are $Cr^{III} \leftrightarrow Cr^{VI}$, $V^{IV} \leftrightarrow V^{V}$ and Mo $^{IV/V} \leftrightarrow Mo^{VI}$. This trace metals are very toxic and sometimes genotoxic to human body which is summarized in this article, still there are other examples of toxicicties remainining to disclose for these metals, which

are demonstarted with some other aricles. There are some scopes to research on these elemental impurities to investigate more about the genotoxicity and other toxic effects. But in this review, it is illustrated that these genotoxic impurities are to be identified and qauntified during qualification of the water for injections production and distribution prior to use to ensure elemental impurities limit. If we include this in the protocol of the qualification program and do the same in a routine way by Standard Operating Procedure (SOP), then we can only assure a genotoxic impurities free WFI supply. Elementary impurities generally amenable to detection by inductively coupled plasma-atomic (optical) emission spectroscopy (ICP-AES or ICP-OES) or by ICP-MS. Voltammetry or Polarographic analyzer might also be used to develop alternative cost effective, accurate, precise and validate method especially for detection in speciation state of trace metals to implement the methods in the Large Volume Parenterals (LVPs) manufacturing unit.

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Abbreviations

WFI	=	Water for injections
LVPs	=	Large volume parenterals
DNA	=	Deoxyribonucleic acid
IARC	=	International Agency for Research on Cancer
FDA	=	Food and Drug Administration
EMEA	=	European Agency for the Evaluation of Medicinal Products
USP	=	United States pharmacopoeia
BP	=	British Pharmacopoeia
UDS	=	Unscheduled DNA synthesis
SCE	=	Sister chromatid exchange
GIP	=	Genotoxic impurities profiling
IN VITRO	=	within the glass
IN VIVO	=	within the living
SVP	=	Small volume parenterals
ROS	=	Reactive oxygen species
RNS	=	Reactive nitrogen species
DTS	=	Double tube-sheet
POU	=	Point of use
SOP	=	Standard operating Procedure
ICP-AES	=	Inductively coupled plasma-atomic emission spectroscopy
ICP–OES	=	Inductively coupled plasma optical emission spectrometry
ICP–MS	=	Inductively coupled plasma mass spectrometry