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Review

Genotoxic Impurities From Pharmaceutical Drugs – A Review

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Abstract

Genotoxic impurities are the substances that can potentially damage to an organism's genetic material that may lead to mutations. Genotoxic impurities (GTIs) in pharmaceuticals at trace levels are of increasing concerns to both pharmaceutical industries and regulatory agencies due to their potentials for human carcinogenesis. The impurities formed may be intermediate, through are material, chemical reaction, as by products due to catalyst excipients or in storage. They are unavoidable hence they are kept under threshold limit. The criteria for these genotoxic impurities are mentioned. As per ICH M7 Guidelines acceptable daily exposure is 5.0 mg/ml/day. Other guidelines like EMA, USFDA provide specific guidelines for these genotoxic impurities. Some of these impurities are detected and the methods of the detections include HPLC, GC, and ion chromatography. The genotoxic impurities are classified as organic impurities and inorganic impurities.

Keywords: Genotoxic, impurities, dravet syndrome, lennox gastaut syndrome, valproic acid.

INTRODUCTION

Pharmacy and the pharmaceutical industry have progressed at a dizzying pace over the last century, from small pharmacies and dispensaries to multi-billion-dollar global corporations. Apart from the well-known R&D (research and development) of novel pharmaceutical products, the safety of medicines is becoming increasingly important. There has been a plethora of pharmaceutical scandals in recent decades, ranging from unsafe chemicals and wrong dosage forms to purposely fortified drugs and unintentional contaminations.

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. The presence of those undesirable chemicals even in the smallest amounts may produce an effect on the safety and efficacy of the pharmaceutical products. International Council for Harmonisation (ICH) 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” [1,2].

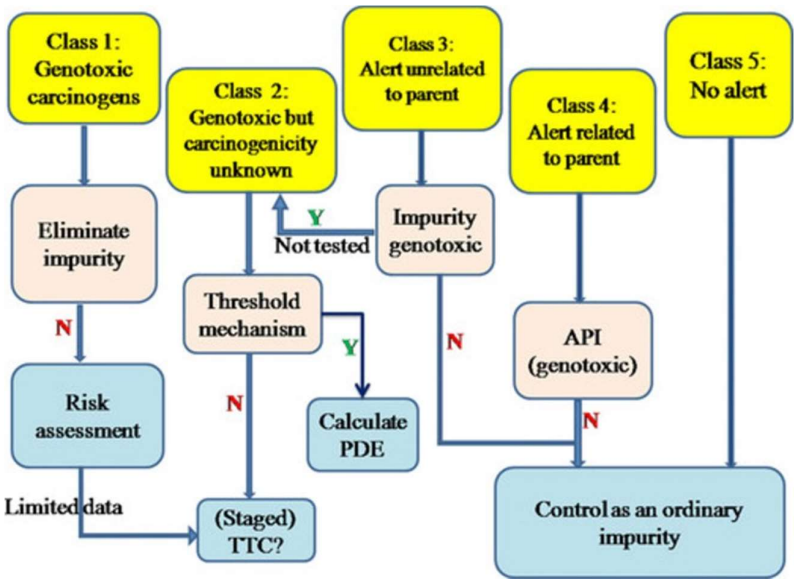


Fig 1: Classification of genotoxic agents

Genotoxic impurities can get incorporated through various sources, mostly starting material used in drug synthesis and its impurities in the form of genotoxic intermediate or process related by-products in the synthesis process. Furthermore, synthesis components such as solvents, catalyst, and reagent used in drug synthesis takes part as genotoxic impurities in drug substances. Drug degradation on storage, exposure to light, air oxidation, or hydrolysis results in generation of impurities in drug substances. Synthesis of stereoselective drug also may contain stereoisomers of raw material and intermediate also contribute to the generation of chiral impurities in drug substances [3].

Regulatory aspects

Several regulatory recommendations and position papers have been developed to manage the amount of PGIs in pharmaceuticals using specified limits. Various industry and regulatory bodies have created rules especially addressing genotoxic contaminants. Pharmaceutical regulatory authorities such as the USFDA, ICH, and EMA have expressed concern about the presence of PGIs in APIs and have provided suggestions to restrict their prevalence in APIs. To address their concerns, R&D scientists must discover PGIs early in the process development process, establish appropriate analytical tools, and illustrate the control strategies of synthetic processes leading to PGIs. To safeguard patients, the acceptance threshold for contaminants in drug substances should be set lower than the qualified level.

ICH guidelines

To the ICH guidelines, contaminants in drug substances and drug products are potentially harmful and provide no value to patients. For example, ICH Q3A15 regulates impurities in novel medicinal substances by establishing thresholds for reporting, identification, and qualification of impurities.

ICH Q3B16 and ICH Q3C17 are guidelines for contaminants in new pharmaceuticals that manage residual solvents. Residual solvents are classified into three categories based on their potential danger to human health. Class I solvents should be avoided, class II solvents have authorized daily exposure limits, and class III solvents have no health-based exposure limitations if the daily exposure is less than 50 mg/day. ICH Q3D is currently being developed and will include elements and limitations for heavy metal impurities.

USFDA draft guidance

In December 2008, the USFDA issued a draft regulation titled “Genotoxic and Carcinogenic Impurities in Drug Substances and Products-Recommended Approaches”. It was recently replaced by the ICH M7 guideline on “Assessment and Control of DNA Reactive Impurities (PGIs) in Pharmaceuticals to Limit Potential Carcinogenic Risk”. The USFDA guidelines have provided specific recommendations for the safety certification of impurities with known/suspected genotoxicity. These guidelines outline various methods for characterizing and

reducing the potential cancer risk associated with patient exposure to genotoxic and carcinogenic contaminants. The recommended procedures are:

(i) Prevention of genotoxic and carcinogenic impurities generation,
(ii) Reduction of genotoxic and carcinogenic impurities levels (maximum daily permissible target of 1.5 g/day),
(iii) Enhanced characterization of genotoxic and carcinogenic risk.

EMA guidelines

The EMA was among the first regulatory agencies to impose precise rules for dealing with genotoxic contaminants. The EMA's CHMP published the final guideline on the limits of genotoxic contaminants in June 2006. The document addresses genotoxic contaminants in novel medicinal compounds. It also applies to new applications for existing active chemicals where evaluation of the synthesis method, process control, and impurity profile does not provide reasonable assurance. The EMA guidelines on PGI limits are divided into two categories. (i) PGIs having appropriate (experimental) evidence for a threshold-related mechanism are to be regulated in accordance with ICH Q3C (R4) for class 2 solvents. PGIs without sufficient (experimental) evidence for a threshold-related mechanism are to be eliminated.

PhRMA approach

In 2004, the Pharmaceutical Research and Manufacturing Association (PhRMA) established a task force to address testing, categorization, qualification, and toxicological risk assessment of PGIs in medicines. It states that various structurally signaling functional groupings have been implicated in DNA reactions. These DNA-affecting functional groupings were divided into three categories: Aromatic groups, such as N-hydroxyaryls, Nacylated aminoaryls, aza-aryl N-oxides, aminoacyls and alkylated aminoacyls, purines or pyrimidines, intercalators, and so on, are included in group 1.

Alkyl and aryl groups, such as aldehydes, N-methylols, N-nitrosamines, nitro compounds, carbamates, epoxides, aziridines, propiolactones, propiosulfones, beta-halo ethyl hydrazines, and azo compounds are examples of group 2. Michael-reactive acceptors, alkyl esters of phosphates or sulfonates, haloalkenes, and primary halides (alkyl and aryl-CH_2) are examples of hetero aromatic groups. The impurities were classified into five categories by PhRMA [4-7].

Analytical methods

Analysis of genotoxic impurities

Because genotoxic impurities must be lower than 0.01–0.03%, their analysis can be extremely difficult. The method of analysis is supposed to permit detection limits between 1 and 5 ppm (0.0001–0.0005% w/w). Such low levels necessitate sophisticated instruments for analysis. Some of the genotoxic impurities are low molecular weight compounds and are highly volatile. Some highly used analytical methods are given in the below Figure 2. HPLC (with UV/Vis detectors) and GC (with FID detectors) are highly used techniques for analyzing genotoxic impurities. Mass spectrometers have been used to achieve higher sensitivity and selectivity [8].

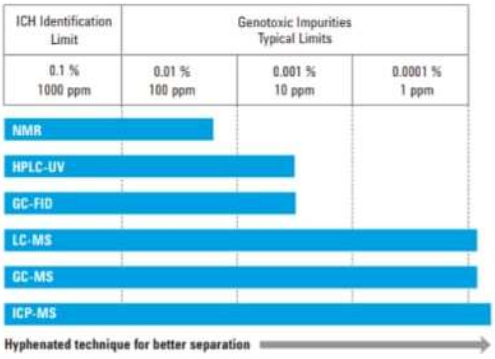


Fig 2: Analytical methods

HPLC

HPLC is mostly commonly used technique for detecting non-volatile genotoxic impurities. The ultra-sensitive detection of Potential Genotoxic Impurities can be optimized using the revolutionary Agilent Max-Light cartridge cell, with a 60 mm optical path length for even lower detection limits and higher sensitivity. A recent advancement. But to detect trace amount of genotoxic impurities advanced methods in HPLC are being used some of these techniques include the following:

- Hydrophilic interaction chromatography (HILIC) is a unique method of separating contaminants from API utilizing different polar stationary phases. The technique demonstrated good linearity with a 3 ppm limit of detection (LOD) and a 10 ppm limit of quantification (LOQ) for impurity II.
- Ultra-High Performance Liquid Chromatography (UHPLC): When compared to traditional HPLC systems, UHPLC systems provide higher resolution, faster analysis times, and enhanced sensitivity. This may result in faster and more accurate genotoxic impurity detection and quantification.
- Column Technologies: By improving chromatographic resolution and peak form, advances in stationary phase chemistry and column design have made it possible to separate closely eluting contaminants more effectively.
- Advanced Detectors: The development of more selective and sensitive detectors, including mass spectrometry (MS) detectors used in conjunction with high-performance liquid chromatography (HPLC-MS), can improve the ability to detect genotoxic contaminants, particularly at trace levels.
- Miniaturization and Microfluidics: Miniaturized HPLC systems and microfluidic devices provide advantages like less sample and solvent consumption, faster analysis, and portability, that is helpful for on-site testing for genotoxic impurities.

Mass spectrometry (MS)

It is employed in detecting genotoxic impurities (GTIs) in pharmaceutical products due to its high sensitivity, selectivity, and capability for structural elucidation. By ionizing analytes and separating them based on their mass-to-charge ratio, MS can detect GTIs at trace levels in complex matrices. Tandem MS techniques like multiple reaction monitoring (MRM) enhance specificity, facilitating targeted analysis of GTIs. MS provides structural information through fragmentation analysis, aiding in the identification of unknown impurities. This technique can be coupled with chromatographic methods like liquid chromatography (LC-MS) or gas chromatography (GC-MS) for improved separation and detection. Recent advancements include high-resolution MS for enhanced mass accuracy and resolution, portable MS systems for on-site analysis, and ambient ionization techniques for rapid screening. Overall, MS plays a crucial role in ensuring pharmaceutical product safety by accurately detecting and characterizing genotoxic impurities, thereby contributing to regulatory compliance and patient health. Recently, many advancements have been made in mass spectroscopy. They include the following methods:

- ✓ High Resolution Mass Spectrometry (HRMS): HRMS instruments offer superior mass resolution and mass accuracy compared to traditional MS systems. This ensures better discrimination of GTIs and matrix interferences, giving more reliable detection of impurities. Tandem Mass Spectrometry (MS/MS): Advances in MS/MS techniques such as multiple reaction monitoring (MRM) and selected reaction. monitoring (SRM), allows targeted analysis of specific GTIs with improved sensitivity and selectivity. MS/MS can also provide structural information through fragmentation analysis to help identify unknown impurities.
- ✓ Ion Mobility Spectrometry (IMS): Coupling MS with IMS allows ions to be separated based on their size, shape and charge. gas phase IMS improves the specificity of MS analysis by providing additional resolution, which is particularly useful for complex samples containing multiple isobaric species.
- ✓ Miniaturization and portable mass spectrometers: Advances in miniaturization have led to the development of portable mass spectrometers that are smaller, more affordable, and easier to use than traditional tabletop instruments. These portable MS systems enable on-site or field analysis of pharmaceutical samples, reducing the time and cost associated with sample transport and laboratory analysis.
- ✓ Ambient ionization techniques: Ambient ionization techniques such as desorption electrospray ionization (DESI) and direct analysis in real time (DART) allows direct analysis of samples. without extensive sample preparation or chromatographic separation. These techniques offer rapid analytical capability and are compatible with MS detection, making them attractive for high-throughput screening of GTIs in pharmaceutical samples.

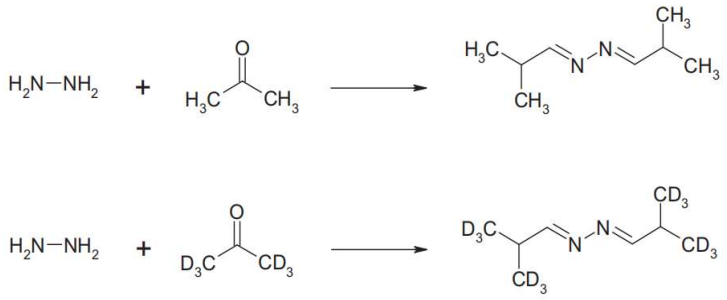
Chromatography (GC)

Hydrogen atoms in hydrazine are unsuitable for normal Flame Ionization Detection (FID) analysis, leading to the use of alternative detection techniques as shown in scheme 1. A GC procedure involving the formation of a benzalazine derivative was developed to monitor residual levels of hydrazine in hydralazine and isoniazid API, tablets, combination tablets, syrups, and injectable products. This method utilized nitrogen selective detection and had a LOD of ≤3 ppm of hydrazine. A modified previously published method involving the formation of a benzalazine derivative and monitoring this derivative using capillary gas chromatography with nitrogen selective detection. The method gave recoveries of between 86 and 94% and a precision of 7.7%.

This method, using a benzalazine derivative and monitoring this using GC with electron capture (EC) detection. The inter-day residual standard deviation (RSD) improved slightly at increased analyte concentrations. Carlin et al. also found elevated levels of hydrazine in isoniazid syrup when stored at ambient temperatures for 4-months. The Canadian Health Protection Board reported on levels of residual hydrazine in formulations of

isoniazid, hydralazine, and phenelzine over a 2-year period. The levels of hydrazine in phenelzine tablets remained unchanged but showed significant tablet to tablet variability. The levels of hydrazine in hydralazine tablets at ambient storage conditions remained unchanged, but a corresponding injectable formulation doubled from 4.5 g/ml to 10.0 g/ml.

The generic method was successfully applied to determine residual hydrazine levels in 5 APIs, using acetone-d6 as a stable isotope alternative. The method was validated at higher levels (25 and 100 ppm hydrazine) due to higher control limits. Instrument contamination was minimized, and the method could be automated using autosamplers with hating, stirring, and reagent addition facilities [9-13].



Scheme 1: Derivatization with acetone to form acetone-azine or corresponding acetone-azine-d 12 derivatives

The EMA and FDA guidelines on genotoxic and carcinogenic impurities

The EMA guideline on the limits of genotoxic impurities

The European Medicines Agency (EMA) guidelines outline how to manage genotoxic impurities (GTI) in new drug substances, only applicable to new applications of already commercialized products and manufacturing process changes. The guidelines do not apply retrospectively to authorized products unless there is a specific cause for concern. The EMA guidelines focus on toxicological assessment and establishing limits for new drug substances.

Classification of genotoxic impurities

The guideline categorizes genotoxic compounds into two groups: those directly interacting with DNA and those acting through other mechanisms. The first group is concerned as they cannot be identified by a threshold-related mechanism, while the second group can be demonstrated. To determine acceptable exposure levels, it is crucial to determine if there is sufficient experimental evidence for a threshold-related mechanism. However, this approach often lacks information for most substances used in synthetic processes. While some well-known GTIs can be recognized, it can be challenging to determine if an unstudied compound acts through a threshold-related mechanism.

Toxicological assessment

A complete risk assessment involves hazard identification, hazard characterization, and exposure assessment. Hazard identification evaluates chemicals based on chemical-specific knowledge, leading to a qualitative assessment of safety impacts. Hazard characterization involves a dose-response and a quantitative estimate of a safe intake. However, for GTIs with limited or no data, this approach cannot be applied. Ames and in vitro chromosomal aberration tests are used to confirm PGIs as GTIs, but they are not suitable for dose extrapolation. Therefore, a method for establishing exposure levels related to negligible risk becomes necessary when a compound is positive.

The threshold of toxicological concern

The threshold of toxicological concern (TTC) is a concept used to estimate the daily exposure level of most carcinogens, which would cause less than one in a 100,000 life-time risk of cancer. This exposure level corresponds to 1.5lg/person/day. Limiting the GTI level at or below the TTC obviates the need for further qualification to support marketing authorization. If the concentration of the impurity does not exceed the TTC limit, the ALARP principle is not necessary, unless the impurity belongs to the class of highly genotoxic compounds. The TTC concept was first introduced as ToR (Threshold of Regulation) and was applied by the FDA to manage low doses of exposure in the control of potential carcinogenic substances in food packing materials. It was further elaborated in subsequent years and adopted by WHO for the control of flavouring substances and cosmetics. The TTC concept has evolved as a pragmatic risk assessment tool, based on the principle of establishing a human exposure threshold value for all chemicals.

Limits for multiple genotoxic impurities

The European Medicines Agency (EMA) has established guidelines for managing additive risks in genotoxicity in drug substances. The TTC limit applies to individual impurities when they are structurally unrelated, but when they are structurally related, the limit is recommended at 1.5 lg/person/day. This requirement is based on the possibility of increased cancer risk due to concurrent exposure. However, assessing multiple impurities in the low ppm range can be a significant analytical problem. Key factors to consider include the daily dose of the drug, the therapeutic indication, the process to remove these impurities, and analytical control issues. The management of additive risks in genotoxicity remains a challenge due to limited information about toxicological synergies

The FDA guidance on genotoxic and carcinogenic impurities

The FDA has issued guidance supplementing the ICH Q3 guidelines for the qualification of impurities with known or suspected genotoxic or carcinogenic potential. The guidance applies to clinical development, marketing, biologics license, and ANDA applications. It is applicable to already approved products only if a safety concern exists, and to supplemental applications involving increased carcinogenic risk.

Assessment of genotoxic potential

The FDA guidance considers impurities exceeding the ICH Q3A qualification threshold for determining genotoxic potential. If toxicological data is unavailable, isolated impurities in standard in vitro batteries are screened for toxicity. Spiking the drug substance with the impurity is acceptable. Impurities exceeding the identification threshold should be evaluated for genotoxic potential using available data or computational toxicology assessment. Structural alerts can be confirmed using the Ames test.

GTI prevention of formation and reduction of the level

The guidance emphasizes the importance of preventing genotoxicity and carcinogenic potential in drug substances, and suggests characterizing genotoxic and carcinogenic properties if limits are not reached during clinical development, and developing analytical methods for identifying and monitoring GTI at appropriate threshold levels.

Limits for genotoxic and carcinogenic impurities

The TTC limit (1.5 lg/person/day) is acceptable for marketing applications, except for highly potent compounds. The guidance considers paediatrics' higher cancer susceptibility and applies a correction factor of 10 for children up to 2 years and 3 for those aged 2-16. Non-oral administration routes like dermal or ophthalmic can be addressed, and the staged TTC approach is suitable for controlling GTI during clinical development [14-17].

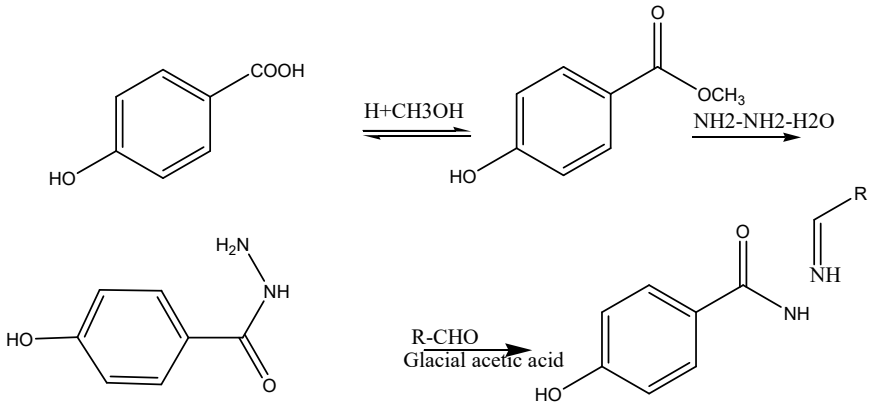
Nifuroxazide

It is a nitro furan derivate. The IUPAC name is (E)-4-hydroxy N-[5-(nitrofuran-2-yl) methylidene] benzohydrazide. It is an intestinal anti infectious drug. The mechanism involves inhibition of STAT-3(signal transducer and activator of transcription factor-3) through JAK family kinases. JAK2 and TYK2 cause anti-proliferative activity. It inhibits dehydrogenase enzyme which leads to results in inhibition of protein synthesis and further leads to damage of DNA. The impurities obtained are hydrazide and amide families. It is an intestinal anti infectious agent, used in treating tumour growth and attack breast cancer cells. A and D are the synthesis intermediates related to hydrazide and amide families. An impurity is [4-hydroxy benzohydrazide (P-hydroxy benzohydrazide)].

Nifuroxazide undergo hydrolytic degradation and leads to formation of impurity-A. It is a principle degradation product and a metabolite. It gives rise to DNA damage and gene mutations. B impurity is methyl 4-hydroxy benzoate [methyl para-hydroxy benzoate] it includes methylparaben. Methylparaben is an antibacterial preservative used in food and cosmetic preparations. Some research hypothesized application of methylparaben on skin may get exposure to the UV-B rays which results damaging of DNA. Impurity C as shown in scheme 2, [(5-nitrofuran-2-yl) methylidene diacetate]. The nitro group present in this impurity reduces and undergo redox cycling which results in the formation of reactive species[14]. This reactive species acts as intermediate and binds to the protein and DNA. This drug is administered for short period of time. It is consumed for quarter of the death at a dose of 200mg for 10 days. The acceptance criteria for this drug are 120microgram hydrazine in 800mg of drug.

Analysis

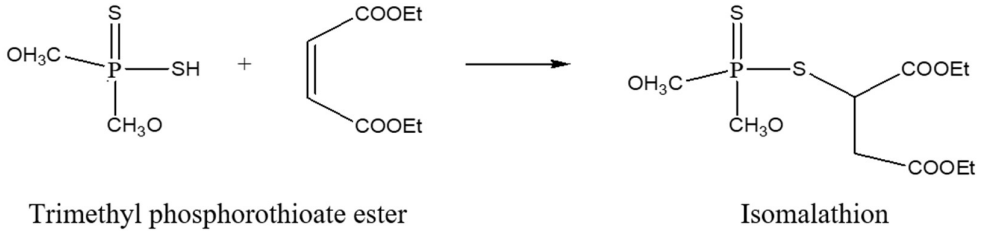
Analysis can be done by using various methods such as spectrophotometry, HPLC, colorimetry, voltammetry, differential pulse photometry.



Scheme 2: Synthesis of nifuroxazide

Isomalathion

Isomalathion is diethyl 2-[(dimethoxy phosphorothioyl)-sulfanyl] butanedioate as shown in scheme 3 is the major genotoxic impurity obtained from the drug called malathion. The drug Malathion is used as an insecticide in the agriculture fields. Trimethyl phosphorothioate esters are the impurities found in the Malathion as per scheme 4. According to the history, in the year 1976, isomalathion caused several deaths and many other problems majorly in Pakistan. The mechanism of action involves the non-competitive inhibition of the choline esterase. The use of isomalathion has been tremendously reduced due to its effect on the environment.



Scheme 3: Synthesis of isomalathion using trimethyl phosphorothioate ester

Cefotaxime

IUPAC name of the compound is 3-(acetoxymethyl)-7-(2-(2-aminothiazol-4-yl)-2-(methoxyimino) acetamido)-8-oxo-5-thia-1-azabicyclo-oct-2-ene-2-carboxylic acid shown in Figure 3. It is a third-generation intravenous cephalosporin. They act on gram positive and gram-negative bacteria and treat urinary tract infections and respiratory tract infections. It acts by binding to penicillin binding proteins with the help of beta lactam rings and it inhibits transpeptidation in the cell wall, thus inhibits cell wall formation. Impurities formed are dimeric impurity and other 6 qualified impurities. Liquid chromatography and Mass spectroscopy are the methods for recognising the impurities present in the compound named cefotaxime.

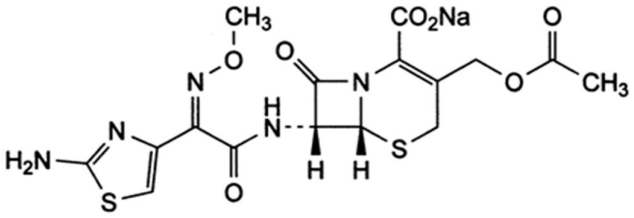


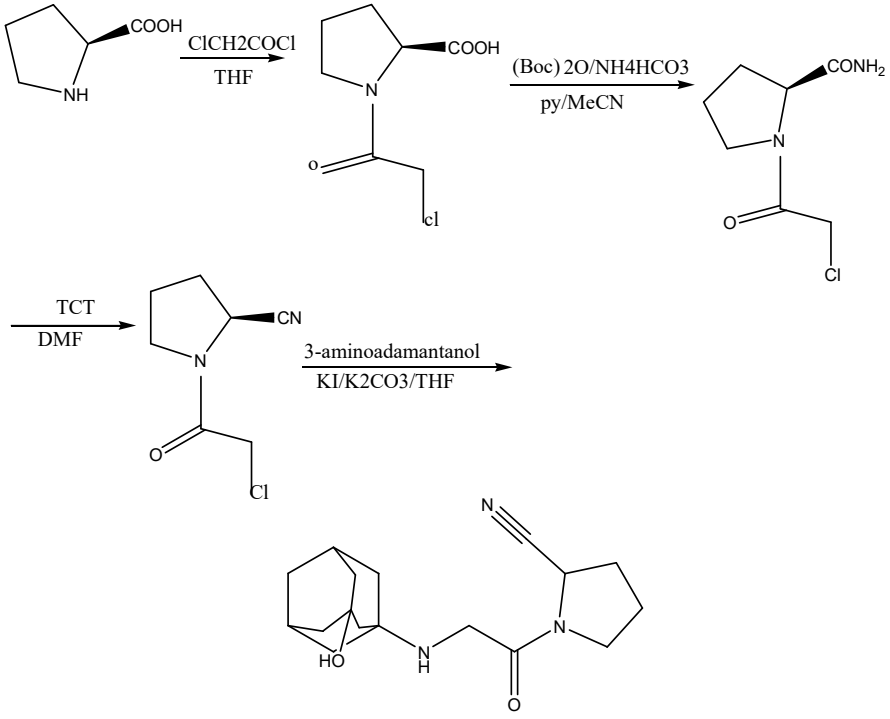
Fig 3: Cefotaxime structure

Vildagliptin

is used in the treatment of diabetes. As it is a pharmaceutical drug it generally has impurities. There are three major genotoxic impurities in the vildagliptin. They are-

1. Pyridine
2. N, N-dimethylaniline
3. 4-Dimethyl aminopyridine

These three are used as raw materials for the synthesis of drugs. They are considered as genotoxic because of reactive bases and due to presence of the electrophilic functional group in the structural moiety as shown in scheme 4. As they are free radicals, they may cause radiations and thus lead to mutations in the DNA. They are considered as potential genotoxic substances as per International Agency for Research on Cancer (IARC). The drug vildagliptin is an oral-hypoglycaemic drug which helps in reduction in glucose levels in blood. Vildagliptin bind covalently at the catalytic site of dipeptidyl peptidase-4 and prolong the glycan like peptide-1 level in blood stream due to which insulin release from B cells in pancreatic cells increases. It is used to treat Type 2 diabetes. The methods used in the determination of the impurities are LC-MS and HPLC and many others. The synthesis as shown in scheme 5 involves impurities as reactants and finally forms the product that is the drug named vildagliptin [18,19].



1-[2-[(3-hydroxy-1-adamantyl)amino]acetyl]pyrrolidine-2-carbonitrile

Scheme 4: Synthesis of vildagliptin

Tetrazole groups

like Losartan, Candesartan, Irbesartan, Valsartan, Olmesartan have a specific structural moiety called tetrazole which is written as 2'-(2H tetrazol-5-yl)-[1, 1'-biphenyl]-4-yl) methyl as shown in Figure 4. For the synthesis of sartan, nitrile and azide reactions are required but as a result the impurity 4-(azidomethyl)-[1, 1'-biphenyl]-2-carbonitrile as shown in Figure 5, is formed in subsequent synthesis step. It may also form 5-(4'-(azidom ethyl)-[1,1-biphenyl]-2yl)-1H tetrazole which is suspected to have potential mutagenic property as shown in Figure. 6. The mechanism of action involves a long pathway. Angiotensin-2 causes constriction of blood vessels that leads to hypertension. Sartans block these Angiotensin-2 receptors due to which Angiotensin production is inhibited. This leads to vasodilation of blood vessels. It helps in the treatment of hypertension, myocardial infarction, congestive heart failure.

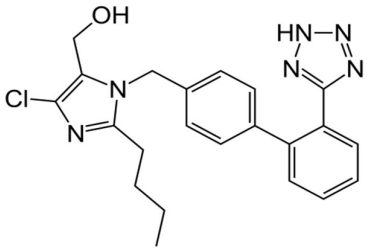
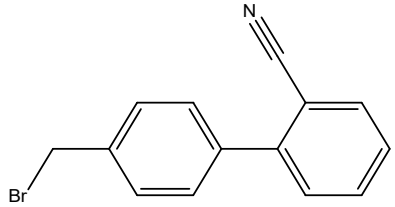
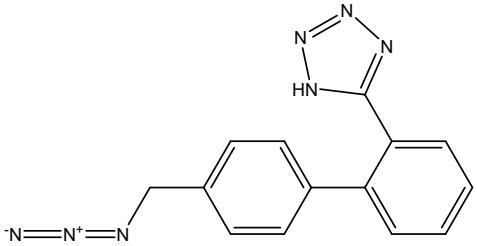


Fig 4: Losartan structure



4'-(bromomethyl)-[1,1'-biphenyl]-2-carbonitrile

Fig 5: Structure of 4-(bromo methyl)-[1,1'-biphenyl]-2-carbonitrile



5-(4'-azidomethyl)-[1,1'-biphenyl]-2yl tetrazole

Fig 6: Structure of 5-(4-azido methyl)-[1,1'-biphenyl]-2-yl tetrazole

Valproic acid

Valproic acid is a pharmaceutical drug which is used in the treatment of bipolar disorder and sometimes epilepsy too. The mechanism of action includes inhibition of voltage – gated sodium ion channels. It prevents the entry of sodium ions into the neurons and this leads to a reduction in the neuron's firing rate. The IUPAC name of valproic acid is generally 2-propylpentanoic acid. Valproic acid is generally named as a mood stabilising drug. It contains organic and inorganic impurities which are formed during the process or synthesis. Cd, Pb, As, Hg, Co, v, Ni, Fe, Al and Sn are the 10 elements used in the evaluation of the impurities. Different methods were used in the determination of impurities. They are TLC, UPLC-MC/MS, HPLC, LC-MS, GC, GC-MS, capillary electrophoresis. Impurities are also formed due to valproic acid, formic acid, butyric acid. The recent and majorly used method is ICP-MS.

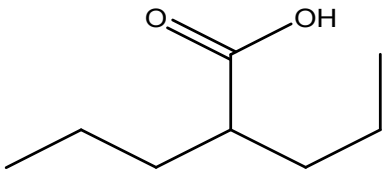


Fig 7: Structure of valproic acid

Zolmitriptan

(S)-4-[(3-(2(Dimethyl amino) ethyl)-1H-indol-5-yl) methyl]-1,3-oxazolidine-2-one is the IUPAC name of the drug. The mechanism of action includes inhibition of 5HT 1D receptors. It blocks selectively. 5HT1D receptors cause constriction of blood vessels and thus relive migraine pain, hence they are used in the treatment of migraine. Zolmitriptan has some impurities and they are formed at the intermediate state as shown in scheme 5. The impurities are hence referred to as intermediate impurities. The compound (s)-4-[(3-(2-(dimethyl amino)

ethyl-1H-indol-5yl] methyl] the most prevalent contaminant identified in the medication is 2-oxazolidine. Other impurities created during the manufacture of zolmitriptan include (2s)-2-amino-3-(4-aminophenyl) propan-1-ol (APP) as in Figure 8, (S)-methyl-4-nitrophenylalanine hydrate as in Figure 9, (S)-2-amino-3-(4-nitrophenyl) propanol (NPP) as shown in Figure 10, and (S)-methyl-4-nitrophenylalanate hydrochloride (MNA) as in Figure 11. These contaminants are determined using the techniques HPLC and LC-MS.

Impurities of zolmitriptan

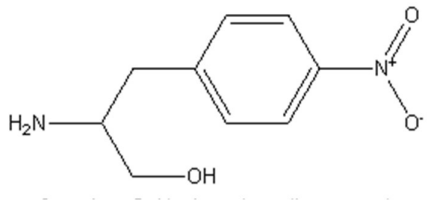


Fig 8: Amino-3-(4-aminophenyl) propan-1-ol

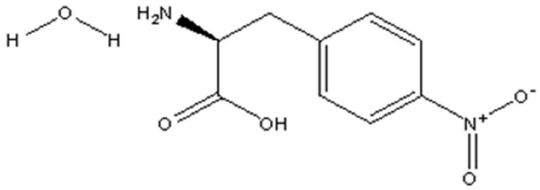


Fig 9: (S)-Methyl-4-nitrophenyl alanine hydrate

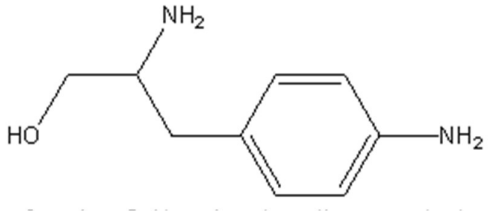


Fig 10: (S)-2-amino-3-(4-nitrophenyl) propanol

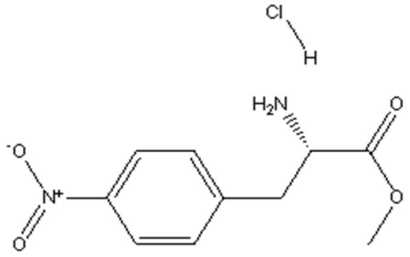
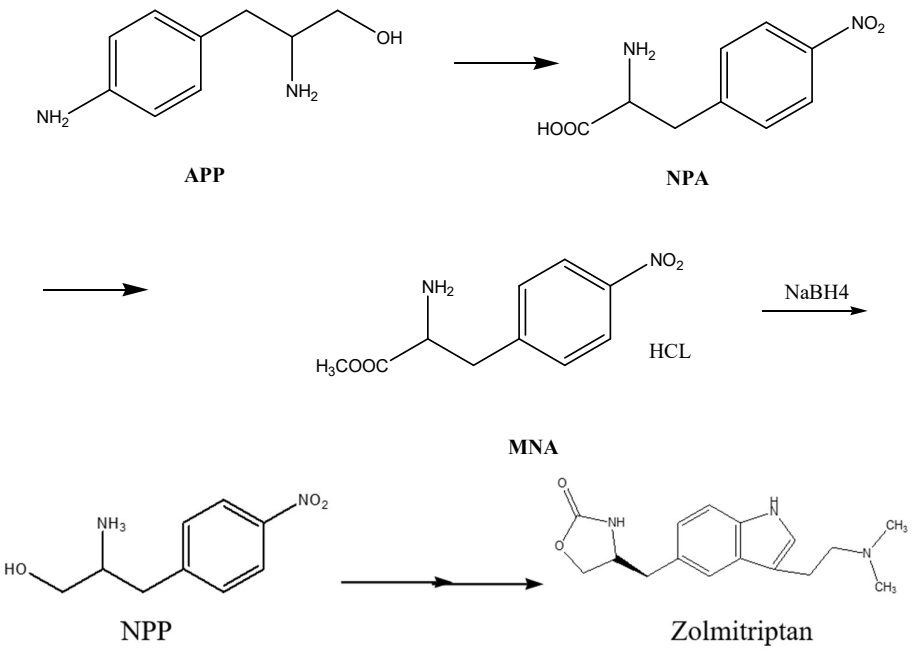


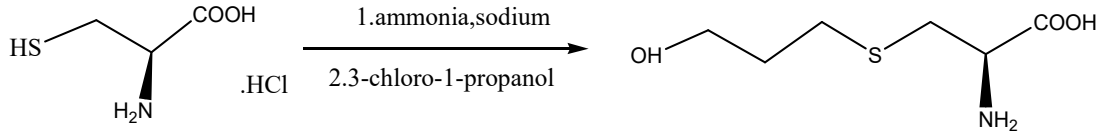
Fig 11: (S)-Methyl-4-nitrophenyl alanate hydrochloride



Scheme 5: Synthesis of zolmitriptan

Fudosteine

Fudosteine is a pharmaceutical drug which is termed as S-(-3-hydroxy propyl)-L-cysteine. Fudosteine gives process impurities such as chloropropyl hydroxy propyl ether, 3-chloro propyl acetate, 1,3-dichloro propane. The reagent used is an impurity 3-chloro-1-propanol as shown in scheme 6. The impurities which are obtained are the organic residual impurities. The mechanism of action of this drug is that it helps in the airway clearance. It decreases the number of goblet cells formation in airways and hyper secretion of mucus. Fudosteine is used in the treatment of chronic respiratory diseases. It is also used as an expectorant and it decreases the inflammation in the airways [20,21].



Scheme 6: Synthesis of fudosteine

Cannabidiol

Cannabidiol is a natural drug as shown in Figure 12. Extracted from cannabis sativa. It contains less than 0.3% of tetrahydrocannabidiol. It is found that it contains potential genotoxic impurities that leads to the mutagenicity in the genes. Cannabidiol bind to two types of G protein coupled receptors. These receptors are endogenously present in the mammalians. Hence, they are also called as endocannabidiods. The drug goes and binds to these receptors, thus helping in the release of the neurotransmitters. Cannabidiol is used as anti-convulsive agent, anti-anxiety, analgesic, neuroprotective, and anti-oxidant, anti-microbial. It is also used to treat dravent syndrome in paediatric patient and lennox gastaut syndrome.

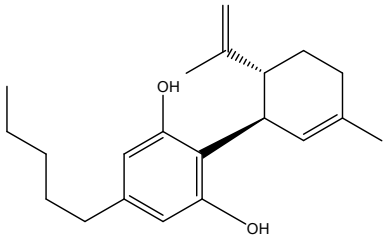


Fig 12: Structure of cannabidiol

Molnupiravir

Molnupiravir is the pharmaceutical drug which is used in the treatment of corona virus SARS-COV2. It is a prodrug, when administered in the body it undergoes hydrolysis with the help of host esterase in the plasma to form beta-D-N4-hydroxy cytidine (NHC). It forms NHC triphosphate by the action of host kinase. This blocks replication of the RNA virus. It also has the genotoxic impurities such as 4-amino-1-((2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxyl methyl) tetrahydrofuran-2-yl) pyridine-2-(1H)-one as in the Figure 13, and also the other impurity formed is 1-(2R,3R,4S,5R)-3,4-dihydroxy-5-(hydro methyl) tetrahydro furan-2-yl) pyrimidin-2,4(1H,3H)-one. These are considered as class 3 genotoxic impurities as per EMA guidelines. As per ICH guidelines the limit of these impurities ranges from a concentration of 0.5-10ppm for both the genotoxic impurities. The evaluation methods used to detect the impurities are highly sensitive ultra-performance liquid chromatography-mass spectrometry.

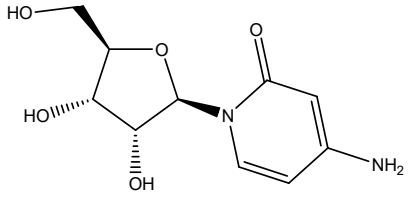


Fig 13: Genotoxic impurity in molnupiravir 4-amino-1-((2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxyl methyl) tetrahydrofuran-2-yl) pyridine-2(1H) one

Impurity removal methods

Test model for acetamide removal

Scavenging studies were carried out with cartridges from Isolute SPE Accessories, Biotage (Sweden), and each containing 25 mg of the PA3 polymer or the matching non-imprinted polymer PAN3. The cartridges were thoroughly cleansed with methanol before being equilibrated with acetonitrile. On the cartridges, 0.5 mL of a todolac (5 g L1) and acetamide (250 ppm) solution in acetonitrile was added. The sample was pulled through the packed polymers using a SPE-cartridge manifold and collected in a vial. After washing the SPE cartridges with 1 mL of acetonitrile, the washing elutants were collected in separate vials. The loading and washing eluents were evaluated using HPLC on Hewlett-Packard HP 1050 or 1100 equipment.

Test model of aryisulfonate removal:

The rebinding test stock solution was 10,000 ppm Halobetasole propionate pharmacological material in dichloromethane supplemented with 1000 ppm MeTS. In 1.5 mL glass vials, 50 mg of each polymer, PS1, PS2, PS3, PS4, PSN1 and PSN2 were inserted, and 1 mL stock solution was added. The vials were closed and the liquids were shaken for 24 h. The supernatants were filtered with Millipore syringe filters after sedimentation to remove any floating particles before analysis. Before analysis, the samples were diluted 100 times with acetonitrile. The control experiments were carried out in the same manner, but with no analytes in the loading solution. GCMS was used to evaluate the supernatants using capillary column DB-1 [22-25].

CONCLUSION

Genotoxic impurities in drug are bound to occur these genotoxic impurities are highly harmful. Some of the most highly occurring genotoxic impurities are hydrazine, amine, and nitrile with azide with tetrazole ring are sartan, nifuroazide pyridine, vildagliptin. Alkyl halides in fudosteine, pyrimidine in molnupiravir, aromatic amines are used in most of the above drugs. The present review article describes what is genotoxic impurity (GTI), its sources, its classification, and approaches to control genotoxic impurities in pharmaceuticals, also this review article gives brief information regarding different types of GTI and covers examples of each class.

Conflicts of interest

The authors have declared that no competing interests exist.

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REFERENCES

1. Reddy AV, Jaafar J, Umar K, Majid ZA, Aris AB, Talib J, Madhavi G. Identification, control strategies, and analytical approaches for the determination of potential genotoxic impurities in pharmaceuticals: a comprehensive review. J Sep Sci. 2015; 38(5):764-79. https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/abs/10.1002/jssc.201401143
2. Hmelnickis J, Pugovics O, Kazoka H, Viksna A, Susinskis I, Kokums K. Application of hydrophilic interaction chromatography for simultaneous separation of six impurities of mildronate substance. J Pharm Biomed Anal. 2008: 48(3):649-656. https://www.sciencedirect.com/science/article/pii/S0731708508003567
3. Al Azzam KM, Aboul-Enein HY. Recent advances in analysis of hazardous genotoxic impurities in pharmaceuticals by HPLC, GC, and CE. Journal of Liquid Chromatography & Related Technologies. 2016; 39(1):1-7. https://www.tandfonline.com/doi/abs/10.1080/10826076.2015.1111794
4. Ho TD, Yehl PM, Chetwyn NP, Wang J, Anderson JL, Zhong Q. Determination of trace level genotoxic impurities in small molecule drug substances using conventional headspace gas chromatography with contemporary ionic liquid diluents and electron capture detection. Journal of Chromatography A. 2014; 1361:217-228. https://www.sciencedirect.com/science/article/pii/S0021967314012047
5. Hernandez F, Sancho JV, Ibáñez M, Abad E, Portolés T, Mattioli L. Current use of high-resolution mass spectrometry in the environmental sciences. Analytical and bioanalytical chemistry. 2012; 403:1251-1264. https://link.springer.com/article/10.1007/s00216-012-5844-7
6. Collins D, Lee M. Developments in ion mobility spectrometry–mass spectrometry. Analytical and Bioanalytical Chemistry. 2002; 372:66-73. https://link.springer.com/article/10.1007/s00216-001-1195-5
7. E.G. Lovering, F. Matsui, D.L. Robertson, N.M. Curran, Determination of hydrazine in pharmaceuticals IV: hydrazine and benzyl hydrazine in isocarboxazid. J. Pharm. Sci. 1984; 74:105–107. https://onlinelibrary.wiley.com/doi/abs/10.1002/jps.2600740130
8. Bailly C. Toward a repositioning of the antibacterial drug nifuroxazide for cancer treatment. Drug Discov Today. 2019; 24(9):1930-1936. https://www.sciencedirect.com/science/article/pii/S1359644619301485
9. Abdelwahab NS, Ali NW, Zaki MM, Abdelkawy M, El-Saadi MT. Quantitative Determination of Synthesized Genotoxic Impurities in Nifuroxazide Capsules by Validated Chromatographic Methods. Journal of AOAC International. 2018; 101(2):385-393. https://academic.oup.com/jaoac/article-abstract/101/2/385/5653953
10. Kean T, Miller JH, Skellern GG, Snodin D. Acceptance criteria for levels of hydrazine in substances for pharmaceutical use and analytical methods for its determination. Pharmeur Sci Notes. 2006; 2006(2):23-33. https://www.academia.edu/download/42550755/Acceptance_criteria_for_levels_of_hydraz20160210-3699-en8kzg.pdf
11. Ahrén B, Schweizer A, Dejager S, Villhauer EB, Dunning BE, Foley JE. Mechanisms of action of the dipeptidyl peptidase-4 inhibitor vildagliptin in humans. Diabetes Obes Metab. 2011; 13(9):775-783. https://dom-pubs.onlinelibrary.wiley.com/doi/abs/10.1111/j.1463-1326.2011.01414.x
12. Al-Sabti B, Harbali J. Development and validation of an analytical method for quantitative determination of three potentially genotoxic impurities in vildagliptin drug material using HPLC-MS. Journal of Separation Science. 2021; 44(13):2587-2595. https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/abs/10.1002/jssc.202100136
13. Satish Ganta, T. Siva Rao, K. Rama Srinivas, Pallapati Suman. Determination of elemental impurities in valproic acid an epilepsy drug by using ICP-MS. Journal of Trace Elements and Minerals. 2022; 2:100036. https://www.sciencedirect.com/science/article/pii/S2773050622000350
14. Vijaya Bhaskar Reddy A, Venugopal N, Madhavi G, Gangadhara Reddy K, Madhavi V. A selective and sensitive UPLC-MS/MS approach for trace level quantification of four potential genotoxic impurities in zolmitriptan drug substance. J Pharm Biomed Anal. 2013; 84:84-89. https://www.sciencedirect.com/science/article/pii/S0731708513002483
15. Amarnatha Reddy Gooty, Hussain Reddy Katreddi, Raghavender Reddy S, Raveendra K. Hunnur, Hemant Kumar Sharma, Narendra Kumar Masani, Simultaneous Determination of Genotoxic Impurities in Fudosteine Drugs by GC–MS. Journal of Chromatographic Science. 2016; 54(8):1277–1281. https://academic.oup.com/chromsci/article-abstract/54/8/1277/2196929
16. Narender Boggula, Ridhika M Patel, Akhil Rapolu, Pramod Kumar Bandari, Krishna Mohan Chinnala. Synthesis, characterization and anti bacterial activity of novel 2-mercaptobenzoxazole derivatives. Indo American Journal of Pharmaceutical Sciences. 2016; 3(7):738-749.
17. Henderson RG, Welsh BT, Trexler KR, Bonn-Miller MO, Lefever TW. Genotoxicity evaluation of cannabidiol. Regul Toxicol Pharmacol. 2023; 142:105425.
18. Narender Boggula. Anti fungal and anti oxidant activities of novel 2-mercaptobenzoxazole derivatives. International Journal of Pharmaceutical, Chemical and Biological Sciences. 2016; 6(4):365-375.

19. Liu DQ, Sun M, Kord AS. Recent advances in trace analysis of pharmaceutical genotoxic impurities. J Pharm Biomed Anal. 2010; 51(5):999-1014. https://www.sciencedirect.com/science/article/pii/S0731708509007043
20. Santhoshi Priya, Narender Boggula et. al. Estimation of heavy metals in different brands of Amlodipine Besylate tablet dosage forms by inductively coupled plasma-mass spectrometry (ICP-MS). Int. Res. J. Pharm. 2019; 10(9):98-104.
21. Parajuli RR et al. Impurity Profiling : An Emerging Approach for Pharmaceuticals. World Journal of Pharmacy and Pharmaceutical Sciences. 2018; 7(4):1670–1683.
22. S Selvaraju, M Vasanth, R Rajarajan, R Muralidharan, V Raghupathy. Genotoxic Effects of Carbendezim (fungicide) on the Root Apicalmeristems of *Allium cepa* L. Res. J. Pharmacognosy & Phytochem. 2015; 7(1):29-33. https://www.indianjournals.com/ijor.aspx?target=ijor:rjpp&volume=7&issue=1&article=007
23. Mahmood Kahtan, Ali Faris Hassan, Nada Naje Al-Shawi, Intesar Tareq Numan, Ghaith Abbas Bahader. The Genoprotective Activity of Aqueous Green Tea extract against Metronidazole and Tinidazole Genotoxic Effect. Research J. Pharm. and Tech 2020; 13(2):889-894. https://www.indianjournals.com/ijor.aspx?target=ijor:rjpt&volume=13&issue=2&article=072
24. Reddy AVB et al. Identification, control strategies, and analytical approaches for the determination of potential genotoxic impurities in pharmaceuticals: A comprehensive review. The Journal of Separation Science. 2015; 38(5):764–779. https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/abs/10.1002/jssc.201401143
25. Shaikh T, Gosar A, Sayyed H. Nitrosamine Impurities in Drug Substances and Drug Products. Journal of Advances in Pharmacy Practices. 2019; 2(1):48–57. https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3958595