SCOPE OF FINDING GENETOXIC IMPURITIES IN PHARMACEUTICALS

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Abstract

Reliable quality is one of the key attributes of drugs nowdays.Patients deserve the highest quality and are expecting to not be put at a risk for health effects especially related to impurities in drug susbstance or drug products.While ICH guidelines for "ordinary" impurities have been available for many years, a harmonized guidelines on how to assess, limits and controls potential health effects of low levels of genotoxic/carcinogenic impurities was lacking and only regional (draft) guidelines exised.With the ICH M7 guidelines entitled "Assessment and control of DNA-reactive (mutagenic) impurities in pharmaceuticals to limits potential carcinogenic risk",this gap in the internationally harmonized regulatory framework has been filled.This review deals with the history of the guidelines and concepts of risk assessment with a focus on the Threshold of Toxicological Concern (TTC) principle.Furthur, a few example are given on how to deal with potential impurities with mutagenic/carcinogenic potential or for compounds for such a potential is assumed but not demonstrated.Hence, in the following review, the reader can expects some background information about the ICH M7 guideines and tips how to use it in practice.Regulatory precedence of the use of evidence of non –linear dose –response for genotoxic carcinogenic such as EMS is also referred.

- **Keywords** Amen test , Cancer risk assessment, DNA –reactive impurities, Genotoxic impurities,Impurities in pharmaceutical, QSAR, Threshold of toxicology concern,TTC.

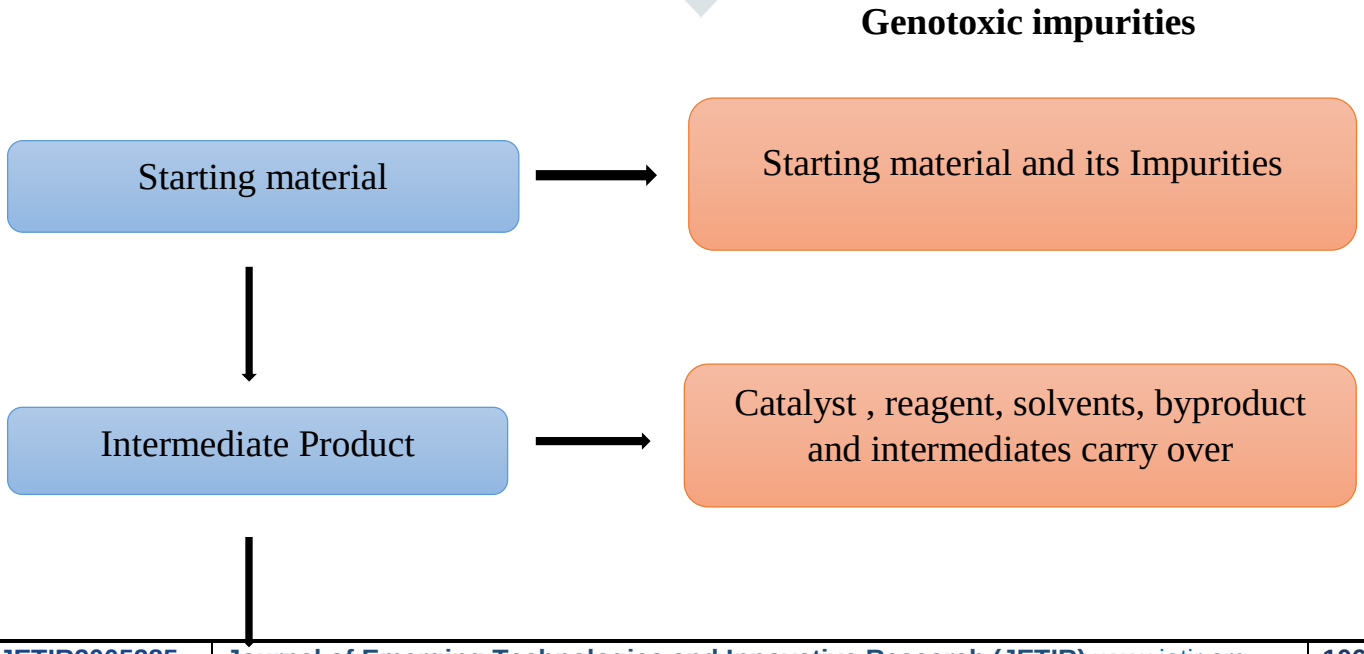
Introduction

Pharmaceutical drugs are always related with some unwanted chemicals alongside with lively components. This undesirable chemical compounds is referred as an impurity. Thus, the frequent definition of impurity is any substance coexisting with the drug substance, such as beginning material, reagents, catalyst, raw material or intermediates bobbing up at some stage in the synthesis or enhance in the course of storage or shipment of the drug. Impurity furnish no benefit to the patient whilst it may additionally have the plausible to cause the destructive effect. International council for Harmonisation in its tenet ICH S2 (R1) defines genotoxicity as "a vast term that refers to any deleterious changes in the genetic material, regardless of the mechanism by using which the change is induced." While genotoxic impurities have been defined as "Impurity that has been verified to be genotoxic in an gorgeous genotoxicity check model, e.g.,bacterial gene mutation. (Ames) test".The manageable genotoxic impurity(PGI)

has been described as an "Impurity that shows a structural alert for genotoxicity however that has not been examined in an experimental take a look at model. Here potentially relates to genotoxicity, now not to the presence or absence of this impurity".Genotoxic impurity have an effect on the genetic fabric through skill of mutations via chromosomal breaks, rearrangement, covalent binding or insertion into the DNA during replication. These modifications in the genetic material, triggered by the publicity to very low stages of a genotoxic chemical, can lead to cancer. Thus, it is very important to perceive genotoxic impurities in capsules followed with the aid of monitoring and manage at very low ranges to ensure safety to the public.ICH and EMEA suggestions grant the limits for impurities in drug supplies and drug products. These limits are not acceptable for GTIs due to their damaging affects and hence it is integral to set up limits based totally on day by day dose of the drug substance. Even although this is applicable in high-quality point of view, it deploys the resources in system development. To overcome this, scientists have to become aware of GTIs early in technique development, develop analytical methods and exhibits the artificial process controls. However the applicable strategies are now not with ease available to all the drug substance or active pharmaceutical ingredients (APIs) manufacturers. Hence ,we have made an efforts to presents an overview on GTI identification, manipulation and determination strategies in drug supplies.

Sources of Genotoxic Impurities

Genotoxic impurities can get integrated into drug element through the a variety of sources, the essential sources is the beginning material used in the synthesis of drug resources and its impurirties. Similarly, genotoxic intermediate and by-product shaped in the synthesis method may also get be carried forward to the dtug components as genotoxic impurities. Besides these,solvents, catalyst and reagents used in the synthesis technique can additionally be a workable supply of genotoxic impurirties in drug substances. Degradation products generated on storage and cargo or on exposure to light,air oxidation or hydrolysis contribute to the ers of impurities in drug substance. If drug substance required ia a particular isomer then stereoisomers of raw material and intermediate additionally contribute to the generation of chiral impurities in drug substance.The following figure indicates the formation of impurities in a various ranges of drug synthesis.Beside these excipients and their impurities, extractables and leachable can also contribute to genotoxic impurities in drug products.



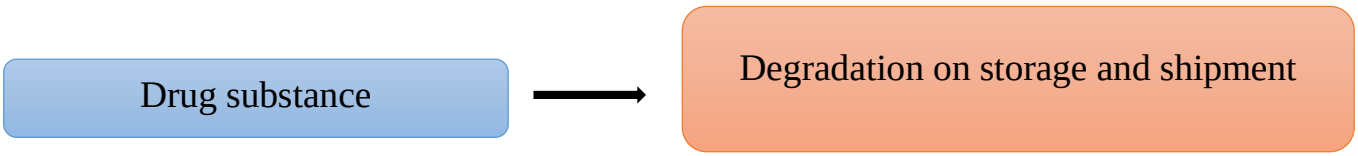


Figure 1: Sources of genotoxic Impurities.

Category/stages	Compounds
Starting material	Hydrazine,Nitroso,acrylonitrile compounds
Intermediate	Benzaldehyde ,Nitro compounds
By-product	Sulphonated esters,phosgene
Reagent	Formaldehyde, epoxides,esters oof phosphate & sulphonates
Solvent	Benezene,1,2-dichloroethane
Catalyst	Toxic heavy metals,metal phosphates
Degradation product	N-oxides,aldehydes

Table 1: Genotoxic compounds in drug substance

Regulatory Guidelines

Pharmaceutical Research and Manufacturers Association (PhRMA)

The PhRMA posted a manner for the testing, classification, qualification ,and toxicological hazard evaluation of genotoxic impurities in 2006.They listed genotoxic impurities in the one-of-a-kind group based totally on their useful corporations involved which react with DNA.These useful groups have been categorized into three companies such as Aromatic,Alkyl /Aryl and Hetero atomic.

European Medicine Agency (EMA)

EMA delivered the guideline on the limits of Genetoxic Impurities after the draft releases for consulation in 2002 and 2004 with the remaining model launched in 2006.The toxicity statistics available for genotoxic impurities is quite variable and it is the only main thing that governs the technique used for the assessment of acceptable limits.In the absence of data, thereis normally the need for one mounted risk evaluation method.Therefore ,EMA firstly introduced the idea and values for the threshold of toxicological concern(TTC) to manipulate the genotoxic impurities .EMA additionally introduced question and answer on the guidelines on

the limits of genotoxic Impurities which was once published in 2010 for classification and harmonization of genotoxicity guideline.

Food Drug Administration (FDA)

USFDA posted the draft education for the enterprise entitled Genotoxic and Carcinogenic Impurities in Drug Substance and Product in Dec.2008.The FDA preparation presents specific recommendations concering the safety qualification of impurities with recognised or suspected genotoxic or carcinogenic potential. The guidance describes a range of approaches to characterize and reduce the doable cancer risk with affected person publicity to genotoxins and carcinogenic impurities.The approaches were similar as that of EMA guidelines.

Step	Action
1	Change the synthesis or purification manner to limit the formation & maximum the removal of the relevant impurity.
2	Allow a most daily publicity target of 1.5µg per day of the relevant impurity as a typical target.
3	Further represent the genotoxic and carcinogenic danger to better guide fantastic impurity specification,both for higher or lower values.

Table 2:FDA guidance steps risk minimization.

International Council for Harmonisation (ICH)

The first tenet associated to genotoxicity was once delivered by way of ICH in July 1995 as S2A:Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceutical; this tenet furnished specific preparation and tips for in vitro and in vivo test and on the comparison of test and on the comparison of test results.The second guidelines was in 1997 as S2B:Genotoxicity: A Standard Battery for Genotoxicty Testing For Pharmaceutical;In 2013 M7 guiding principal used to be posted which provide education on the evaluation of Structure-Activity Relationships (SAR) for genotoxicity.After which M7 (R1) tenet was released in steps 2 in june 2015 and step 4 in May 2017.The M7 (R2) is undertaken for the revision. This guiding principal accommodates acceptable limits (Acceptable Intake (AIs) or Permitted Daily Exposure (PDEs) for new DNA reactive (mutagenic) impurities and revising appropriate limits for impurities already listed in the Addendum as new records will become available,which will results in the future ICH M7 (R2) version.

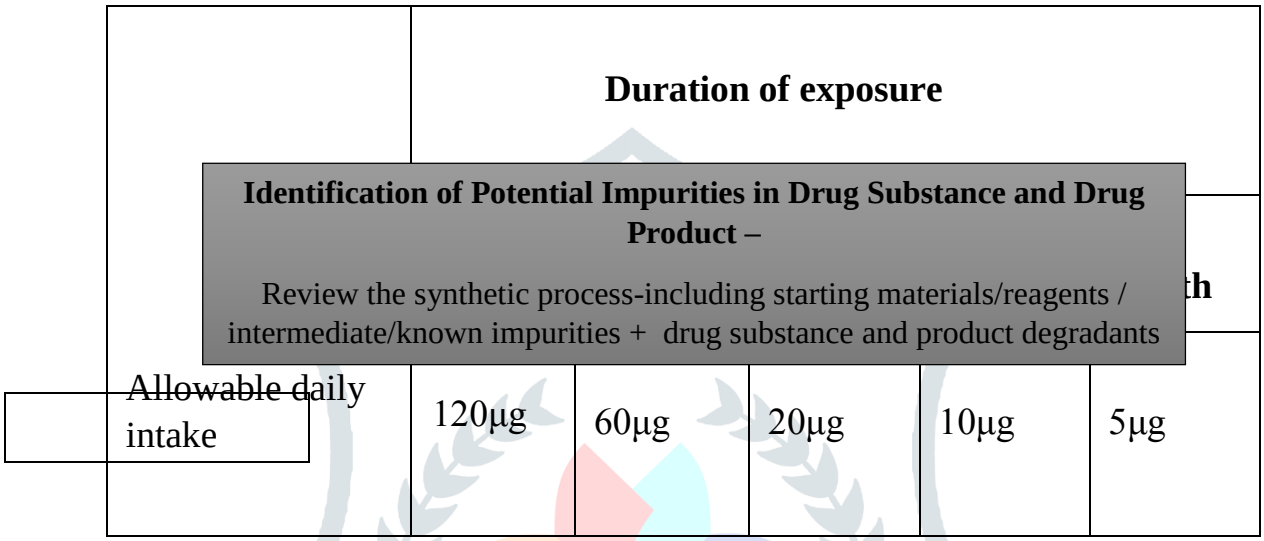
Threshold of Toxicological Concern (TTC) Approach

As toxicological assessment of all the impurities is very difficult, therefore the threshold of toxicological subject (TTC) was proposed by EMEA (2006) to manage the genotoxic impurities .Where acceptable intake of a mutagenic impurity of 1.5µg per person per day is regarded to be associated with a negligible threat (theoretical excess most cancern chance of <1 in 100,000 over a lifetime of exposure).This approach would normally be used for mutagenic impurities present in prescribed f=drug for long-term remedy larger than 10 years

and where no carcinogenicity information are accessible.The limits for individual genotoxic impurities can be calculated by the formula given below.

Limit (ppm) = 1.5(µg/day) / maximum daily dose (g/day)

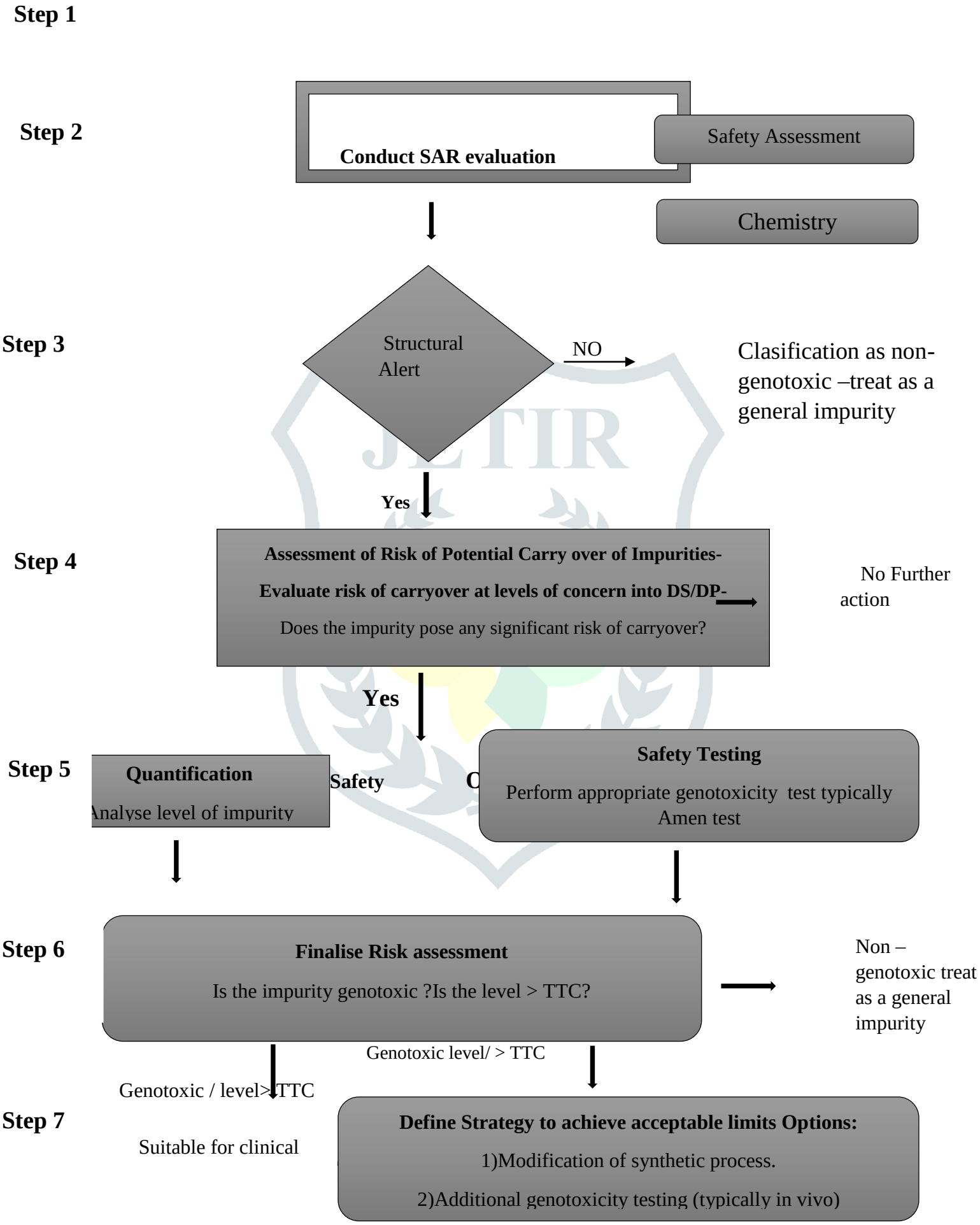
Considering all the drug are now not used for long-term treatment, the new thought staged TTC used to be introduced by Mueller.This concept takes into account the fact that the length of exposure is a key factor impacting on the chance of a carcinogenic response. The endorsed limits for daily consumption of genotoxic impurities are given in Table.



GI Risk assessment process

The method starts with the evaluation of the artificial route for postulated or known impurities. This is followed by means of structural assessment of agreed "likely" impurities, along with different route materials and reagents the place appropriate, hazard classification, quantification and safety testing, threat evaluation and finally establishment of manipulate measures the place required. The system ought to be flexible, each API/DP synthesis has its own specific features, and where appropriate, the ordering of the steps described may additionally be changed, however the standard technique should generally continue to be the same. There is a clear link between the assessment have to therefore take into account the section of development, the intended dose and probable medical trial study duration. Permissible limits are primarly based on the "staged TTC" principle. The EMEA guiding principle additionally states that values greater than the TTC may additionally be acceptable underneath positive condition, inclusive of quick-time period exposure, for therapy of a existence – threatening condition, when life expectancy is much less than 5 years or when there is higher publicity from other sources such as food. It is recommended that a accredited limit, for example staged TTC, is established in boost of instigating the formal evaluation, with the caveat that this restrication will alternate dependant on both time (duration of scientific phase) and dose (absolute level of exposure).

Fig**:**GI risk assessment –Process flow diagram


Step 1
Step 2
Conduct SAR evaluation
Safety Assessment
Chemistry
Step 3
Structural Alert
NO
Clasification as non-genotoxic –treat as a general impurity
Yes
Step 4
Assessment of Risk of Potential Carry over of Impurities-
Evaluate risk of carryover at levels of concern into DS/DP-
Does the impurity pose any significant risk of carryover?
No Further action
Yes
Step 5
Quantification
Analyse level of impurity
Safety
Safety Testing
Perform appropriate genotoxicity test typically Amen test
Step 6
Finalise Risk assessment
Is the impurity genotoxic ?Is the level > TTC?
Non – genotoxic treat as a general impurity
Genotoxic level/ > TTC
Genotoxic / level>TTC
Step 7
Suitable for clinical
Define Strategy to achieve acceptable limits Options:
1)Modification of synthetic process.
2)Additional genotoxicity testing (typically in vivo)

for GIs during clinical development, a staged TTC approach depending on duration of exposure

	1 day		2 day to 1 month	>1-3 month	>3-6 month	>6-12 month	>12 month
ADI* (µg/day) for distinctive duration of exposure(as normally used in medical development)		120a or 0.5%c whichever is lower	60a or 0.5%c whichever is lower	20a or 0.5% whichever is lower	10a Or 0.5% whichever is lower	5a,c	1.5b

a - Probability is not exceeding a 10^{-6} risk is 93%.

b - Probability is not exceeding a 10^{-5} risk is 93%, which considers a 70- year exposure.

C- Other limits (higher or lower) can be appropriate and the approaches used to identify,quality and control ordinary impurities during developed should be applied.

Step 1: Evaluation of Drug Substance and Drug Product Processes for Sources of Potentially Genotoxic Impurities.

The duty for this step is probable to fall to the chemistry and analysts responsible for the plan and development of the API synthetic process with additional input from pharmaceutical development corporation who can remark on problems bobbing up from balance and degradation studies, as well as excipient compability. An comparison of the artificial route, targeted on beginning material, intermediates, reagents, catalyst and solvents is carried out to identify materials which could possibly survive the process and present in the API as impurities. It could also include consideration of different attainable impurities that may additionally arise from the artificial route, mainly in the final stages. These could include related components of the API or intermediates, through to materials derived from interaction between reagents and solvents. However, care must be taken when considering the scope of impurities to be included in assessment. The EMEA guidelines contains the following advice:

As stated in the Q3A Guidelines, authentic and workable impurities most probable to arise during the synthetsis, purification and storage of the new drug substance should be identified, primarily based on sound scientific appraisal of the chemical reaction worried in synthesis,impurities associated with raw substance that could make a contribution to the impurity profile of the new drug substance,and feasible degradation products.The discussion can be restricted to those impurities that might moderately be expected based totally on scientific understanding of the chemical reaction and prerequistics involved.

Such impurities should include , for example, a regioisomer of an alerting intermediate that does no longer react as per the important element in a cyclization reaction,leaving the regioisomer to potentially ignore unreacted into the API.Other example involved side reaction between sulfonic acids and alcohols to yields sulfonate esters.

Potentially genotoxic impurities that can be present the API generally falls into following categories:

- Unreacted contributory substance or intermediate with alerting substructures that have survived processing(e.g. unreacted nitroaromatic in a nitrogen heterocycle API, due to incomplete hydrogenation or a positional isomer unable to cyclize).

- Substances closely associated to contibutaory materials, intermediate,or the API itself that comprise an alerting structural motif(e.g. a chloroalkyl analogue of a hydroxyalkyl analogue of a hydroxyalkyl containing API following therapy with hydrochloric acid during processing.

- Unrelated resources formed by mixture of solvent and reagent with each other or with contributory substances or intermediates (e.g. isopropyl tosylate formed as a end result of isopropanol hydroxylated intermediate being used in a reaction with tosyl chloride).

This is aligned with the tenets of ICH Q9,7 which focuses on the likelihood of an event occurring, mixed with an comparison of the affect of the match occurring, leading to a considering of the threat posed. The magnitude of the hazard is therefore related to the likelihood of the PGI being present. Hence the greatest risk is posed by these marketers used in the tiers of the API synthsis that posses nicely- mounted alerting structural motifs and these need to be the essential focus of the evaluation.

Step 2: Structural Assessment

Both EMEA and FDA draft guidelines recognize the utilization of structural assessment as a valid means by which an assessment of genotoxic potentiallyare often made.The use of in silico system is usually recommended; the are most commonly applied are the business application DEREK and MCase and frequently used in tandem.Evaluation are exceptionally centered on mutagenicity (Amen positive) due to this being recogniszed as the most appropriate indicator for direct interaction with DNA.

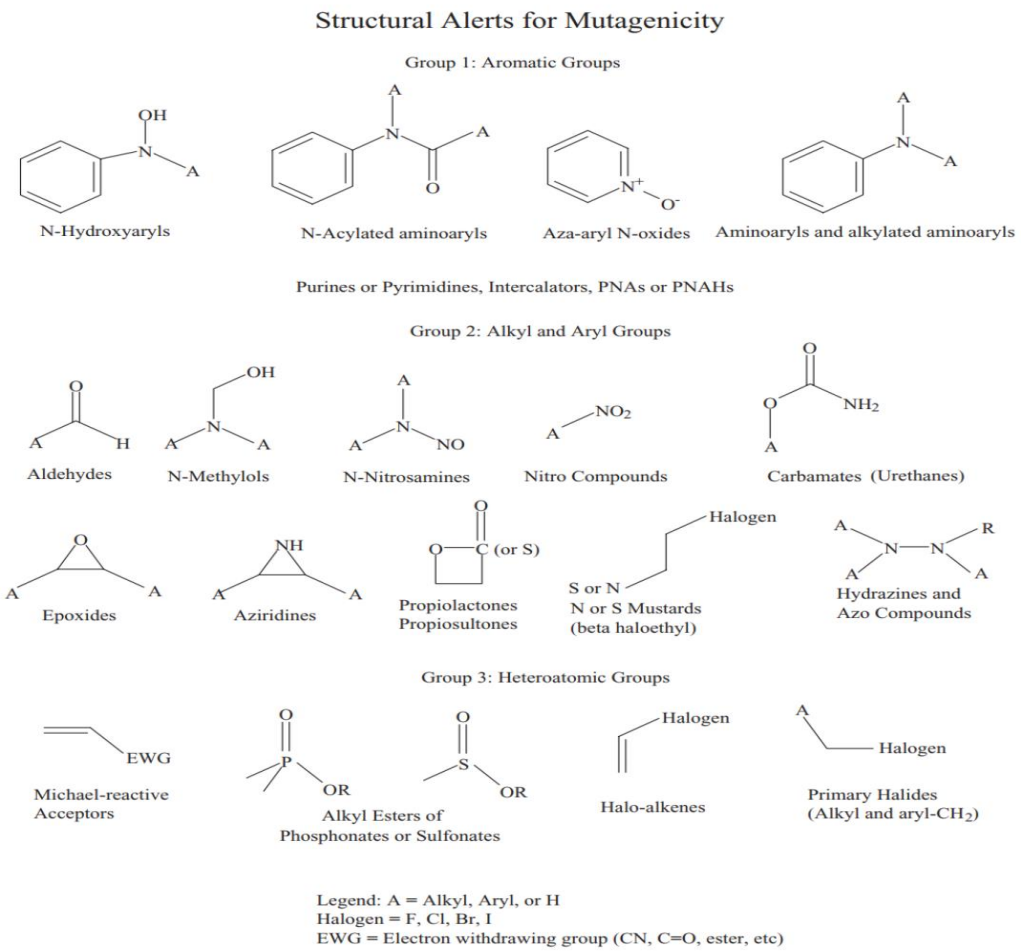


Figure 9.2 Structural alerts.

Step 3:Classification

Once a structural evaluation has been completed, every impurity must be categorised according to its genotoxic hazards.It is vital to be conscious that the SAR assessment approaches can solely be as good as the database and rule units that underpin the system. It is regarded that there are deficiencies in the fashion for some compounds classes, for instance those pertaining to the anilines and heteroaromatic amines.It might also be really useful to threat these cases individually, with the alternative to think about protective checking out (Amen test) the place this is deemed necessary.

Evaluation of genotoxic chance can also be augmented by records from within the public domain.Included within these is TOXNET, a searchable database provided by way of the U.S. Library of Medicine.This gives get entry to a series of databases via a frequent portal:

- HSDB : Hazardaous Substances Databank;
- CCRIS :Chemical Carcinogenesis Research Information System; and
- IRIS :Integrated Risk Information System

Which provides an excellent sources of a safety data for many common chemicals.It is often viable with common reagents to stumble on sufficient safety information to permit genotoxic risk to be assessed on a compound specific foundation rather than surely applying the TTC.this is definied as an allowable approach inside the recent FDA guideline.

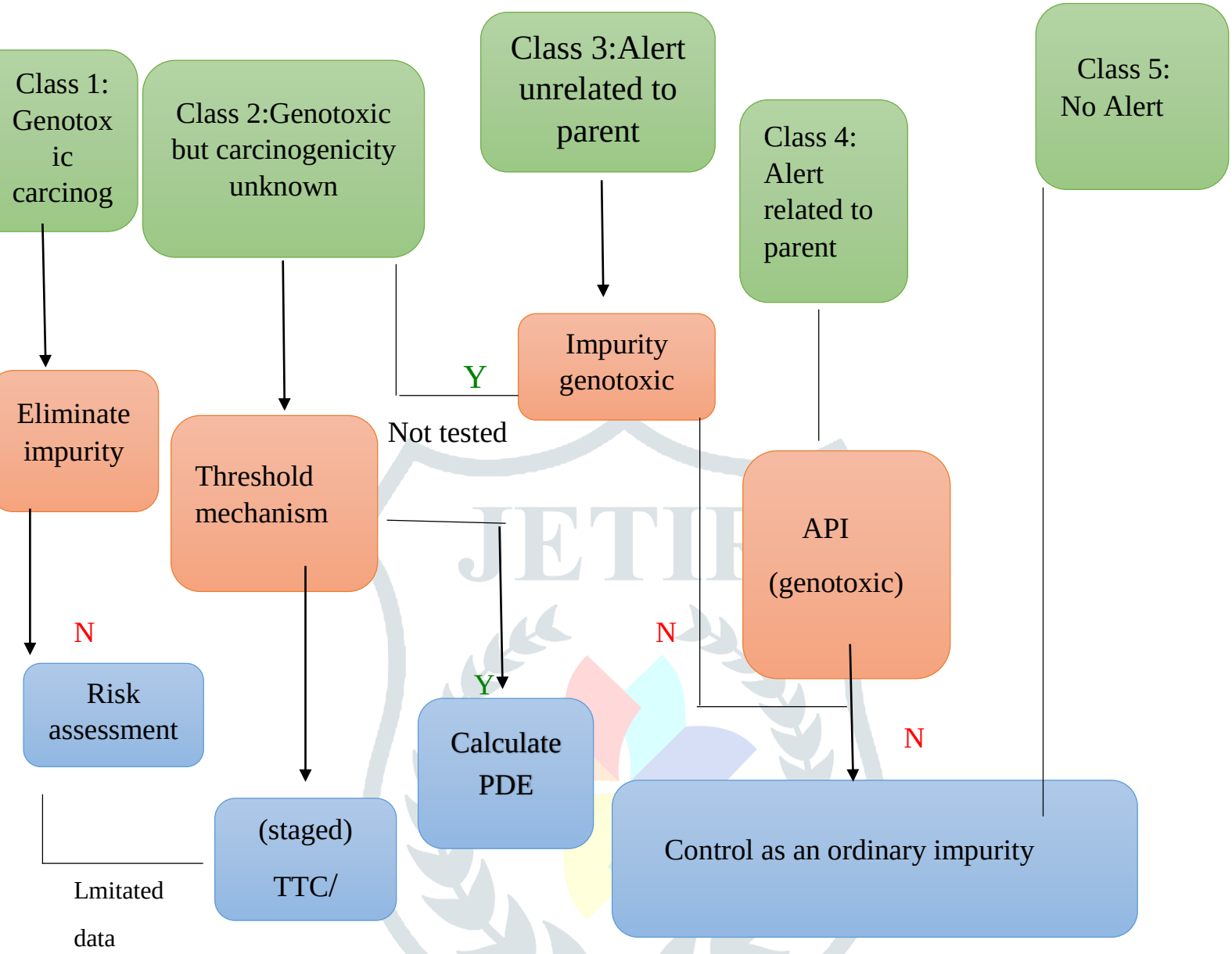


Table 4:Impurity Clasification

Step 4:Assessment of Risk of Potential Carry over of Impurities.

Once impurities with a achievable genotoxic safety difficulty have been identified by way of the SAR assessment process,the subsequent step is to reflect on consideration probability of a specific impurity being current in the isolated API, frequently referred to as impurity destiny mapping. The impurities underneath consideration are via nature often exceedingly reactive, hence their removal throughout downstream processing is facilitated by way of this intrinsic activity.Acidic and basic work-up conditions regularly encountered in manufacturing processes can also lead to decomposition and removal of the fabric of concern.Similarly, different reagents used in downstream processing may additionally react with material, rendering it less reactive and thereby "safe".Such impurity destiny assessment have largely been primarly based on the theroretical knowledge and trip of the evaluating chemist. Unfortunately ,on the other hand compelling the argument developed, they have regularly been viewed as nonquqntitative from a regulatory perspective. Thus , in manycases, there is a want

to supply similarly analytical facts to substantiate the impurity fate assessment.Hence , a high-quantity by testing (QbT) method is adopted alternatively than a satisfactory by using layout (QbD) method. There would be large cost from an enterprise and regulatory perspective in defining a standardised approach to such assessments.It need to be feasible to investigate destiny at least semi-quantitatively primarily based on factors linked to the impurity's physicochemical homes (and taking in to account those of the API and intermediates) and the technique prerequisites employed in the route of manufacture to the API.Resreacher have counseled that an assumption may want to be made of a 10 – fold reduction per artificial stage.In many cases,this would sufficient and certainly may even be a cautions estimate of the danger.However , in certain circumstances, for instance an unreactive genotoxic reagent or intermediate used in a "telescoped" method (no isolation between stages),this may also be too simplistic. For this reason, a more quantitative approach , based on proper method conditions and the physicochemical properties of the genotoxic impurity in question, has been sought.

Genotoxic Impurities (GIs)

Sulfonates

Salfonates salts are the most frequently used compounds in pharmaceutical development.Salt formation is a beneficial method for optimizing the physicochemical processing (formulation), biopharmaceutical or therapeutic houses of active pharmaceutical components (APIs),and sulfonate salts are widely used for this motive. In addition to the advantages of sulfonates salts posses some advantages over different salts such as producting greater melting point of the sulfonated API. This helps to decorate the balance and provide exact solubility and can also have positive in vivo benefits as well.For instance,in contrast to other salts of strong acids,mesylates do now not have a tendency to structure hydrates, which makes them an appealing salt structure for secondary processing, mainly moist granulation.Another advantages of these salts is their high melting point due to the fact APIs with low melting points often show off plastic deformation at some stage in processing which can cause both caking and aggregation. Typically,an increase in the melting point has an unfavourable impact on aqueous solubility owing to an increase in the crystal lattice energies.Sulfonic acid salts tend to be an exception to this rule,since they exhibits both excessive melting points as properly as desirable solubility.In addition,as mentioned in the literature,the high solubility and excessive floor area of haloperidol mesylate result in more desirable dissolution costs (<2 min in pH 2 simulated gastric media) which are more fast than the competing frequent ion formation.

On the other hand,sulfonic acid can react with low molecular weight alcohols such as methanol,ethanol,or sulfonates esters. In general, sulfonic acid esters are viewed as considered as alkylating marketers that might also exert genotoxic effects in bacterialand mammalian telephone structures and perchance carcinogenic results in vivo; thus, these copmounds have raised security concern in current instances

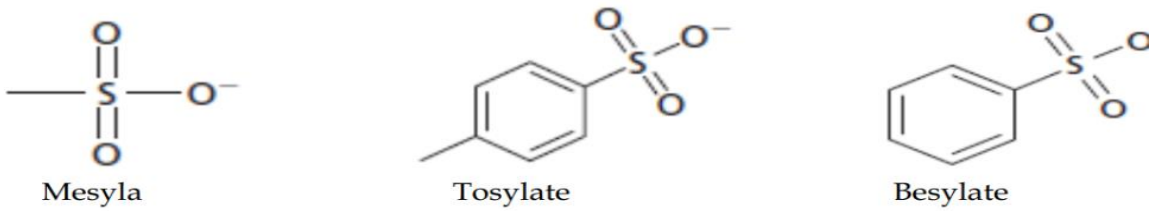


Fig :Structure of common sulfonate salts

Genotoxicity Profile

Sulfonate impurities contain the most investigated crew of genotoxic impurities (GIs).Initially in 2007,sulfonate impurities raised primary subject when over a length of there month (March to May 2007), quite a few thousand HIV sufferers in Europe had been uncovered to viraceptR (nelfinavir mesylate) capsule containing the contaminant ethyl methane sulfonate (EMS).However, the available in vitro and animal records indicate that the range at which HIV patients had been exposed to EMS (maximal dose of 0.055 mg/kg/d) did no longer result in any risk; nevertheless ,any further level used to be of great challenge to their protection.Since 2007 other pills have been mentioned for infection via sulfonate impurities, such as alkyl benzene sulfonate in amlodipine besylate dimethyl sulfate (DMS) in pazopanib hydrochloride ,EMS and methyl methane sulfonate (MMS) in imatinib mesylate,EMS in zugrastat, alkyl sulfonates in flouroaryl –amine, and ethyl besylate.

EMS is a well-established genotoxic agebt in this crew which reacts with DNA producing alkylated (specifically ethylated) nucleotide.MMS, an analog of EMS, is a genotoxic compound each in vitro and in vivo. The global business enterprise for lookup on cancer (IRAC) has classified EMS and MMS in crew and 2A, respectively reviewed both in vivo and in vitro genotoxicity,carcinogenic, ordinary and the consequences on reproductive and embryo fetal improvement of EMS.They reported that the genotoxic effects triggered through EMS have been discovered in viruses/phages, bacteria,fungi,plant,insect ,and mammalian cells.In another study,the induction of gene mutations at the hprt locus and the induction of cgromosomal harm were examined as evidence bt the formation of micronuclei in human lymphoblastoid cells.It was located that the lowest dose inducing a fantastic response used to be 1.40 g/ml, and a no observed impact level (NOEL) could be described at 1.2 g/ml.Also, no toxicity used to be determined at doses up to 2.5 g/plate.This observation is in robust contrast to the generally linear dose-response discovered in thw previous studies.As a result of in vivo assay for the physique and induces comparable tiers of DNA damage in the a number organs.Also,EMS is clastogenic in all take a look at system. The minimal dose of EMS applied in these research was both 50mg/kg or a hundred mg/kg.In the majority of research the dose-response relationship regarded sub linear and a threshold under 50 mg/kg regarded possible validated that EMS in quite a number gene mutation checks such as induction of hprt,lacZ, and dlb-1 mutations in mice used to be mutagenic.The carcinogenic of EMS was validated in a number of animal models.In every other study,three methanesulfonates and three benzenesulfonates were tested by micronucleus and yeast deletion recombination (DEL) assays.It used to be determined that all six material produced high quality response in the exams.

Epoxides

Epoxides are considered as electrophilic compounds owing to the strained epoxide ring. These alkylating operators specifically respond with DNA. Alkene oxides are more responsive than arene oxides and symmetrically substituted epoxides are less responsive than lopsidedly substituted compounds. A few illustrations for APIs with epoxide pollutions are betamethasone acetic acid derivation, atenolol, and a few home grown cures. Carbamazepine, cyproheptadine, and protriptyline have steady epoxide metabolites. In expansion, phenytoin,lamotrigine, amitryptiline, and diclofenac tend to make responsive arene oxide metabolic intermediates. The digestion system of epoxides basically includes epoxide hydrolase (EH) and glutathione S transferase (GST), which leads to either detoxification or generation of epoxides. These pathways play a key part within the genotoxic activity of epoxides.

Genotoxicity Impurity

As demonstrated in in vitro studies, epoxides are genotoxic in bacterial turn around transformation tests; however, other studies have appeared diverse comes about. Hude et al. (1990) detailed that 12/51 epoxides were nongenotoxic within the Ames Salmonella measure. In this study, 51 epoxides were assessed with the SOS-Chromo test utilizing Escherichia coli PQ37 taken after by a comparison with the comes about of the Ames test. All compounds were tried with and without S9 blend up to cytotoxicity. In tests without S9 blend the SOS-repair acceptance of each explore was controlled by the reaction to 4-nitroquinoline-N-oxide, and in tests with S9 blend, it was controlled with benzo[a]pyrene. Within the Ames test, 20 epoxides were tried for mutagenic action with the Salmonella typhimurium strains TA100, TA1535, TA98, and TA1537. By comparing the comes about of the Ames test and the SOS-Chromo test, it was found that among 51 epoxide-bearing chemicals 39 initiated base-pair changes in at slightest one Salmonella strain. The mutagenicity of 17 aliphatic epoxides utilizing the extraordinarily constructed mutants of Salmonella typhimurium that were created by Ames. It was found that all the compounds within the think about, with the special case of 2-methyl-3,3,3-trichloropropylene oxide, cis-stilbene oxide, and cyclohexene oxide that were mutagenic in strain TA100 were also mutagenic, but-with diminished affectability, within the moment strain TA1535. Be that as it may, none of the epoxides in this ponder were found to be mutagenic in strains TA1537 and TA98 which detect frame-shift mutagens. The comes about demonstrate that the monosubstituted epoxides are the most powerful mutagens which the expansion of a single methyl bunch to the oxirane ring could decrease or dispose of mutagenicity. Examined 35 epoxides for mutagenicity, utilizing inversion of his Salmonella typhimurium TA98 and TA100 as the natural end-point. The comes about gotten were negative with the anti-microbials oleandomycin, anticapsin and asperlin, the cardiotonic drug resibufogenin, the broadly utilized parasympatholytic drugs butylscopolamine and scopolamine, the narcotics valtratum, didovaltratum and acevaltratum, the tranquilizer oxanamide as well as the sedate metabolites carbamazepine 10,11-oxide and diethylstilbestrol ┘ and ┘ oxide. It was found that among the drugs and sedate metabolites, as it were the

cytostatic ethoglucide was extraordinarily mutagenic. Three barbiturate epoxides appeared exceptionally powerless mutagenicity as it were at greatly tall concentrations such that the impacts were likely of low viable pertinence.In vivo rat bioassays on epoxides are not continuously positive and a few epoxides are carcinogenic as it were at the point of organization. For illustration, it was found that when given by verbal gavage, both ethylene oxide and propylene oxide caused late-onset tumors as it were in the rodent fore-stomach. Once more, when managed by inward breath, propylene oxide may be a nasal carcinogen. On the other hand, in vivo considers in rodent have appeared that carbamazepine epoxide have the potential to start cellular harm in case not satisfactorily detoxified through conjugation with glutathione.It was watched that owing to the part of digestion system, epoxides that are shaped in vivo, such as those created by epoxidation of alkenes and arenes, have a more prominent potential to cause adverse impacts than preformed epoxides. This can be since they are frequently delivered at near proximity to their location of activity and can hence reach their target very promptly. In this manner, this mechanism can clarify the constrained prove of creature carcinogenicity tests for a few epoxide compounds.

Aromatic compounds

Aromatic compounds **i**nclude different debasements; a few impurities, such as fentanyl impurities, tremogenic pollutions, p-nitrophenol (PNP) that have fragrant structure and aromatic amines.

Aromatic Amines

Primary and auxiliary fragrant amines (by and large after digestion system) create an electrophilic species and in this way deliver a positive result within the Ames test when S9 blend exists. 2, 4-Diaminotoluene, 2, 4-diaminoethylbenzene and many amines containing a nitrogroup are coordinate mutagens. Concurring to the in vivo carcinogenicity test, Ames positive compounds deliver positive result about, in spite of the fact that p-anisidine and p-chloroaniline are noncarcinogenic in rodant bioassays.

p- Nitrophenol

This synthetic chemical possesses fungicidal activity and is used as a starting material for the synthesis of some drugs. PNP and other substituted nitro benzenes after reduction produce arylhydroxylamines or hydroxamic esters which contain electrophilic nitrogen atoms. Thus, the electrophilic atoms might show genotoxic property for these compounds. It ought to be specified that negative comes about were gotten for Ames tests with the different strains of Salmonella typhimurium within the nonappearance and nearness of metabolic actuation with rat liver S9. Another in vitro test, the hprt change test in Chinese hamster ovary (CHO) cells displayed the same result as the Ames test for PNP. In any case, it was seen that PNP could initiate chromosomal distortions in mammalian cells, especially within the presence of metabolic enactment. Too, PNP was negative within the bone marrow micronucleus test in mice at dosages extending from small poisonous quality to the most extreme endured measurements. In expansion, PNP was cytotoxic to the bone marrow of male mice at tried measurements.

Fentanyl Impurities

The constrained corruption of fentanyl created seven fragrant degradants. Among these, propionanilide (PRP), N-phenyl-1-(2-phenylethyl)-piperidin-4-amine (PPA), 1-phenethyl-1H-pyridin-2-one (1-PPO), fentanyl N-oxide, and 1-styryl-1H-pyridin-2-one (1-SPO) possibly demonstrate security concerns. PPA was recommended as a potential genotoxic compound and the DNA harm in unscheduled DNA blend (UDS); the comes about were positive for PRP when in vitro rodent hepatocytes were checked. Within the ACD/Tox suite, 1-PPO and 1-SPO were distinguished as Ames dangers. These compounds were too anticipated to have higher probabilities of being Ames positive.

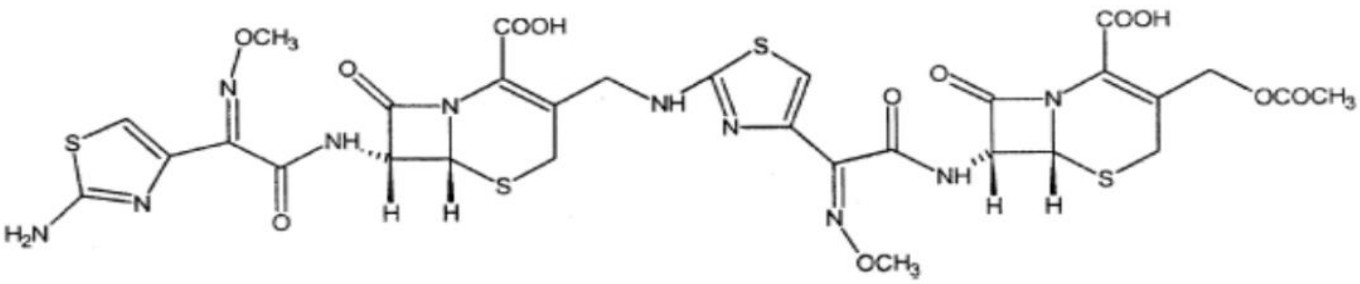
Tremogenic impurities

Tremogenic impurities include another sub-class of profoundly harmful pollutions in APIs. Two pharmacopoeial APIs are known to have the potential to be sullied with tremogenic impurities; pethidine and paroxetine (3-[(1, 3-benzodioxol-5-yloxy) methyl]-4-(4-fluorophenyl) piperidine). Pethidine can contain follow sums of 1-methyl-4- phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) determined from the hydrolytic corruption of side chain. 4-(4-Fluorophenyl)-1-methyl-1,2,3,6-tetrahydropyridine (FMTP) can be a potential reactant/intermediate within the union of paroxetine. Owing to their toxicity to cells within the Substantia nigra, these exceedingly strong debasements can initiate Parkinsonism in people. In this way, these compounds are known poisonous debasements; be that as it may their genotoxicity remains hazy.

β-lactam related impurities

The following two impurities relate to the well known antibiotic cefotaxime and piperacillin.

Dimeric impurity of cefotaxime



The manufacturing and storage processes of cefotaxime produce various impurities such as dimeric impurity.

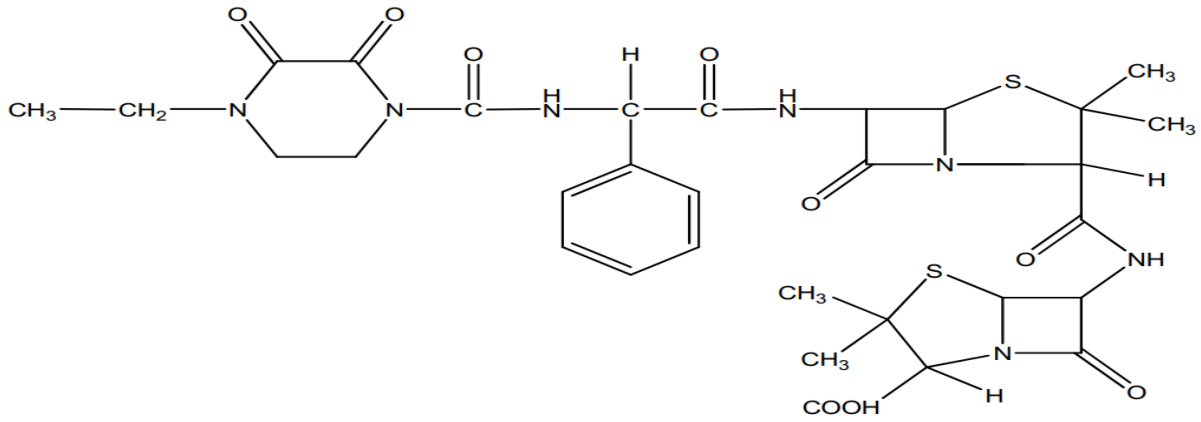
The results of the mutagenesis measure demonstrate that the dimeric impurity is nonmutagenic to any test strains utilized within the nearness and nonappearance of S9 division. The results of the in vitro chromosomal test appear a few chromosomal distortions in refined mammalian cells up to the greatest suggested concentration of 45 mg per culture, and no clastogenicity in mammalian cells in vitro.

Piperacillin impurity-A

The piperacillin impurity-A may be a noticeable corruption item of piperacillin that shows up during fabricating and capacity forms . In all the strains of S. typhimurium; TA 97a, TA 98, TA 100, TA 102, and TA 1535, piperacillin impurity within the nearness and nonappearance of metabolic enactment was found to be non-mutagenic. Too, in vitro chromosomal distortion test did not uncover any critical alterations.

Fig: structure of piperacillin impurity

It is found that piperacillin impurity-A up to 5 mg/ml is nonclastogenic to CHO cell lines within the nearness and nonappearance of metabolic activation.



Analytical approaches

As a discussed above, GIs possess unwanted effects and their contamination levels should be controlled.To achieve this, pharmaceutical R&D should employ robust and sensitive analytical methods for supporting drug development and monitoring the levels of GIs. In addition, analytical methods that are capable of measuring trace GIs must be employed to monitor the outcomes of GIs during chemical synthesis.In recent years, manufacturers have developed sensitive methods for analysing various GIs .In this context,conventional HPLC/UV methods are the first option for GIs analysis; however ,these method are often inadequate for the accurate determination of analysis trace levels,depending on the properties of the analysis and sample matrices.Some of the challenges in the analytical determination of GIs in pharmaceuticals at trace levels include the diverse structural types of GIs ,the unstable or chemically reactive nature of GIs ,and extremely high level of API as contaminant.

HPLC methods

In general, non-volatile GIs are analysed by HPLC separation techniques, among which reversed phase HPLC (RPLC) is the most widely used separation mode. A simple isocratic RPLC method has been employed for the determination of four genotoxic alkyl benzenesulfonates (ABSs) viz,methyl,ethyl,n-propyl,and isopropyl benzenesulfonates (MBS,EBS,NPBS, and IPBS)in amlodipine besylate(ADB).The RPLC isalsp applicable for sulfonate impurities with phenyl moiety such as methyl (MTs),ethyl (ETs) and isopropyl tosylate (ITs), methyl (MBs),ethyl (EBs),butyl (BBs) and isopropyl besylates

(IBs).Epoxides/hydroperoxides were analysed using HPLC,and simple RPLC methods employing direct analysis (no sample preparation) were used for some of them.As described an HPLC method for the determination of loteprednol impurities including a minor for the determination of related subdtances,including the epoxides impurity of nadalol.A rapid resolution HPLC method was used for separation and quantifying the related impurities of atorvastatin, including two epoxide impurities atorvastine epoxy dihydroxy and atorvastatin epoxy diketone.The limit of detection (LOD) and limitof quantitation(LOQ) for atorvastatine epoxy dihydroxy and atorvastatin epoxy diketone were 0.025 and 0.075 g/ml ,and 0.026 and 0.077g/ml, respectively.The researcher determined two epoxide terpenoid impurities in a traditional Chinese herbal preparation.Subsquently, they compared the HPLC results with evaporative light scattering detection(ELSD) with UV detection and found that the ELSD was significantly more sensitive.Sample pretreatment was performed prior to analysis owing to the complexity of the matrix.For 2 epoxides the on-coulmn sensitivity using UV detection was found to be 606 and 880 ng,respectively, whereas the sensitivity using ESLD was 40 and 33ng,respectively. Using the optimised extraction procedure(methanol/water,80/20 v/v) the level of the two analytes were detected to be 3.44 ±0.02 and 1.42±0.01%, respectively.

A more common method for the analysis of alkylating impurities is by RPLC and MS detection;however,HPLC/UV methods are also carried out successfully for alkylating impurities,researcher reported an HPLC/UV method for the separation of 13 impurities of verapamil; this method is claimed to be superior to both the existing pharmacopoeial methods for verapamil.Using this method was found to be sensitive to pH and mobile phase composition;however, it was in contrast to the findings pf previous studies insensitive to stationary phase changes.

Hydrophilic interaction liquid chromatography(HILIC) seems complementary to RPLC for the retention and separation of small molecular polar analaytes , and has thus gained increasing attention recently.Good retention can be achieved for more polar analytes,which is not possible on RPLC /UV and HPLC/MS method.An Indian research group reported the development and validation of a stability indicating HPLC method for the determination of the anti-tuberculosis drug,rizatriptan, and its degradation products,including a hydrazine impurity used a HILIC method with different polar stationary phases (silica,cyano,amino and the zwitterionic sulfobetaine) to separate six polar impurities ,including 1,1,1-trimethylhydrazinium bromide,and demonstrated that HPLC was a useful alternative to reserve phase or ion chromatography(IC).Researcher has reported a table summarizing the various HPLC methods that were used in the literature for a wide range of drugs.

Active Potential ingredient (API)	Impurities	Method details
Allopurinol	Hydrazine	Derivative using benzaldehyde, following by LLE.HPLC with a 5µm cyanosilyl stationary phase (R type) at 30°C.Mobile phase; 2- propanol/ hexane (5/95,v/v). Flow rate 1.5 ml/ min; detection at 310nm
API (general method)	Hydrazine	HPLC with(1) 5µm ZIC HILIC (seQuant),(2) 5µm Develosil 100 Diol-5(Nomura),(3) 5µm TSK-Gel Amide-80(Tosoh Bioscience) and (4)5µm Zorbax NH_2 (Agilent) at different column temperature (10-60°C).Mobile phase.
API (general method)	Hydrazine	(1) Derivative using benzaldehyde .HPLC with no operating conditions reported.(2) LSE, followed by derivatization using benzaldehyde at lower temperatures.HPLC with no operating condition reported.Detection at 190nm.
Azelastine	Impurity A: Benzohydrazide, impurity B:1- benzoyl -2-(4RS)-1-methylhexahydro- 1 Hazepin -4yl) diazane	HPLC with a 10µm cyanosilyl stationary phase (R)at 30°C.Mobile phase;Ph 3.0 phospahte buffer and sodium octane sulphonicacid in water/acetonitrile (740/260,v/v).Flow rate 2.0 ml/min detection at 210 nm.
Aryl hydrazones	E- Aryl hydrazones	HPLC with a 5µm ODS stationary phase (Merck Lichrospher)at 25°C.Mobile phase :1 ml PH 6.0 Phosphate buffer with 2Mm EDTA and

		methanol (40/60,v/v).flow rate 1.0ml/min;detection at 200-400 nm (DAD). HPLC with a 5µm phenyl hexyl stationary phase at 25℃.Mobile phase; water and acetonitrile (50/50,v/v0.flow rate 0.3ml/min.Positive and negative ion model ESI with ion trap analyser in SIM mode (M = H ion).Range 50-1000m/z.Voltage 4 kV,capillary temperature 250℃.
Carbidopa	Hydrazine	Derivative using benzaldehyde,followed by LLE.HPLC with a 5µm ODS stationary phase .mobile phase ;aqueous phase 0.03%EDTA and acetonitrile (300/700,v/v). (

Ebifuramin	Impurity 3:(+) 5- morpholino methyl-3 –(5-nitrofurfurylidene amino)-oxazolidin-2-one	HPLC with a 5 µm ODS stationary phase (hypersil ODS).Moobile phase:aceronitrile /THF/ph 2.6 10Mm dibutyl aminephosphate (15/5/80,v/v).flow rate 1.5ml/min; detection at 254 nm.
Hydralazine	Hydrazine	Derivative using benzaldehyde,followed by LLE HPLC with a 5µm ODS stationary phase.Mobile phase aqueous 0.03%EDTA and acetonitrile (300/700,v/v) flow rate 1.0ml/min:detection at 305nm.

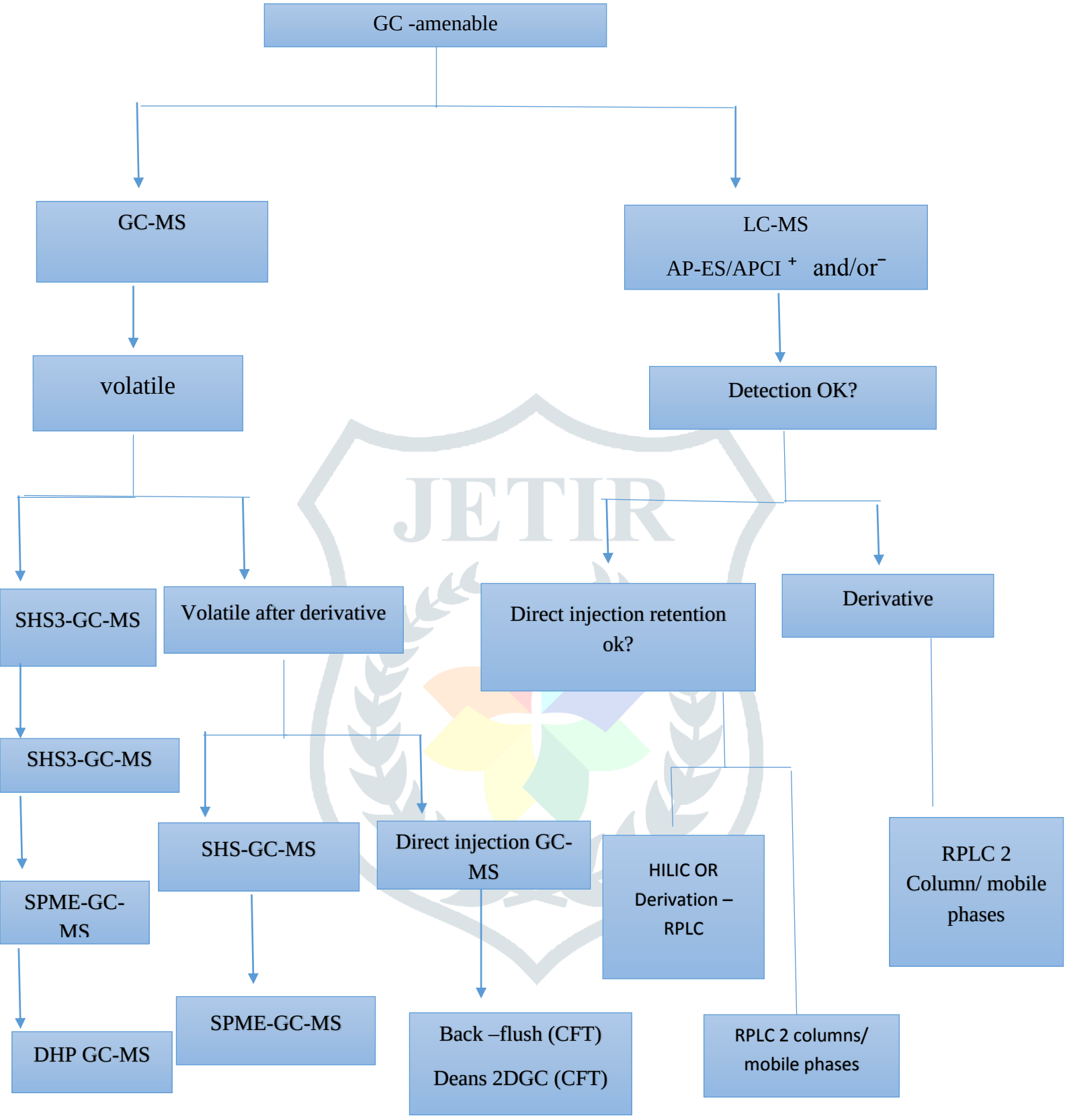
Hydralazine tablet	Hydralazine hydrazone	HPLC with a 10µm ODS stationary phase at room temp.Mobile phase acetonitrile /5Mm SDS/phosphoric acid (150/850/0.45,v/v/v).flow rate 2.0ml/min;detection at 220nm.
Isoniazide	Impurity 1: 1- nicotinyl-2-lactosyl hydrazine	HPLC with a 10µm cyanopropyl stationary phase and a mobile phase consisting of a mixture of ph 3.5 10 mm acetate buffer and acetonitrile (95/5,v/v).flow rwte and detection wavelength not specified.
Nitrofural, nitrofurazone nitrofuroxazide	Hydrazine	Derivatization using benzaldehyde ,followed by LLE. HPLCwith a 5µm ODS stationary phase .mobile phase aqueous 0.03% EDTA and acetonitrile (300/700,v/v)flow rate 1.0ml/min;detection at 350nm.
Nitrofurazone	Impurity A: Bis-(5-nitrofuran-2-yl) methylene) diazane	HPLC with a 5µm ODS stationary phase (R TYPE) Mobile phase acetonitrile/water (400/600,v/v0.flow rate 1.0 ml/min;detectin at 310 nm.

Povidone	Hydrazine	Derivatization using benzaldehyde,followed by LLE.HPLC with a 5µm ODS stationary phase.Mobile phase aqueous 0.03%EDTA and acetonitrile (300/700,v/v).flow rate 1.0ml/min;detection at 305nm.
Pyridoxal isonicotinyl hydrazine	Hydrazine, isoniazide	HPLC with 5µm ODS (Nucleosil C18) and an isocratic mobile phase consisting of a mixtureof methanol5mm 1-heptane sulphonic acid and 2mm EDTA (B) in a ratio of 49/51,v/v.flow rate 0.9ml/min;detection at 279 qnd 254nm.
Rifampicin	Hydrazones; rifampicin quinone and 25-desacetyl rifampicin	HPTLC with a silica gel 60 TLC plate with a chloroform/methanol/water (80/20/2.5,v/v/v)mobile phase.Examined using scanner 2 at 330nm for 25-desacetyl rifampicin and 490nm for rifampicin quinone.
Vindesine sulphate	Impurity C (desactyl vinblastine hydrazide)	HPLC with a 5µm ODS stationary phase (R type)and a gradient mobile phase consisting of varying mixture of ph 7.5 diethyl aminephosphate buffer and methanol. Flow rate2.0ml/min;detection at 270nm.

Table:Various HPLC method used for a wide range of drugs: Abbreviation: DAD:diode array detection; EC:electrochemical detection;ESI:electrospray ionization;FDC:Fixed Dose combination; HILIC: hydrophobic interaction liquid chromatography ; LLE:liquid liquid extracrtion ;LSE: liquid solid extraction;MS;Mass spectroscopy ;ODS octadecyl silyl; SDS: sodium dodecyl sulphate;SIM ;single ion monitoring;TOF :Time of flight.

GC Methods

GC methods are commonly used for the analysis of several volatile small molecular GIs. Some example include the liquid injection technique and the headspace sampling technique.Liquid injection is prone to contamination in which injection of a large amount of non-volatile API can accumulate in the injection linear or on the head of the GC column,which can cause a sudden deterioration in method performance.headspace infusion,on the other hand, is desirable since it minimizes potential defilement of the injection or column by avoiding the presentation of a expansive amount of API.Analyst proposed a strategy determination chart containing GC or LC methods,both in combination with a single quadrupole counting sulphonates,alkyl halides,and epoxides.Analyst created a GC/MS strategy for remaining levels of EMS in a mesylate salt of an API crystallized from ethanol.The strategy was able of identifying EMS down to levels of 50-200 ppb.Hence,extraction procedures were created for disposing of or reducing lattice related impedencen.Hence analyst overviewed liquid-liquid extraction 9LLE),fluid stage micro-extraction (LPME),strong stage extraction (SPE), and solid stage micro-extraction (SPME)coupled with GC/MS and single ion-monitoring (SIM).Using these approaches,they created restrain tests (5ppm) for a few alkyl aryl esters of sulfonic acids.Comparative attempts were made for lessening or disposing of the lattice impact for alkylating agents as well.In all these methods,e.g: low boiling point and within the close of halide atom.GC strategies were rarely utilized for the investigation of epoxides/ hydroperoxides, as compared to other impurities in almolkalant.Other literature grant an account of GC-MS startegies for the investigation of unstable components in traditional Chinese home grown medications.



Method selection chart for analysing genotoxic impurities with GC/LC ;AP-ES/APCI:atmospheric pressure electrospray ionization/atmospheric

pressure chemical ionization,If the analyte has sufficient vapour pressure in water or other low volatile solvent,SHS:static headspace;SPME;solid phase micro-extraction :DHS;Dynamic headspace:HILIC:hydrophobic interaction liquid chromatography;derivative-RPLC reserved

phase HPLC with precolumn derivatization ;back-flush(CFT):capillary flow technology based back-flushing ,Dean 2DGC (CFT):capillary flow technology based two-dimensional GC.

For the hydrazine group the normal flame ionization detection (FID) in GC analysis is not appropriate because these compounds possess no carbon atoms.A GC procedure involving the formulation of a benzalazine derivative was developed for monitoring the residual levels of hydrazine in hydralazine and isoniazide APIs,tablets,combined tablets,syrups,and injectables products in which nitrogen selective detection was used.

In addition researcher adopted a previously published method for monitoring a benalazine derivative using GC with electron capture (EC) detection.The LOQ was 10ppm and the method was linear over the range of 10-100ppm.The inter-day residual standard deviation (RSD) based on su=ix measurement at analyste levels of 10ppm was 15%, however this improved slightly at increased analyte concentration of 25 and 100ppm to 9.5% and 11.3% ,respectively.

Nevertheless,non-volatile API does not partition into the headspace and therefore does not enter the GC system,as a result,headspace injection becomes the preferred choice whenever possible.

TLC/HPTLC methods

In general practice,thin layer chromatography (TLC) is not preffered for the accurate determination of very loe residual analyte level.However this technique is still used for the determination of related substance in the pharmacopoeial monographs for amiodarone,bromazepam,carmustine,ifosamide,indoramine,and tolnaftate.Neverthless, there are several examples of its use in association with determining levels of the epoxyl alkaloids, including scopolamine in extracts of datura stramonium.Saas and Stutz (1981)used TLC to determine residual sulphur and nitrogen mustrads (beta haloethyl compounds)in a variety of substrates in which the sensitivities in the microgram range were typically achievable .High performance liquid chromatography was used for monitoring the degradation products of rifampicine quinone (RQU).Finally, it was concluded that the method is suitable for routine quality control and stability analyses, especially in the developing world

Capillary electrophoresis methods

Researcher reviewed the applicability of capillary electrophoresis(CE) methods for the analysis of pharmaceutical impurities.In addition ,they discussed the applicator of these methods in various groups of compounds such as chemotherapeutic agents,central nervous system(CNS) drugs,histamine receptor and cardiovascular drugs.The main advantages of CE tecniques is their selectivity ,thus they are suitable for the analysis of complex herbal products.Reseacher reported the separation of 13-cis and all-trans retinoic acid and their photo-degradation products (including all-trans -5,6- epoxy retinoic acid,13 –cis-5,6-epoxy retionoic acid)using both capillary zone electrophoresis (CZE) and miceller electokinetic chromatography (MEKC) methods.A Chinese research group reported the development of CE methods for the simultaneous determination of some hydrazine related impurities.Researcher evaluated a series of electrically driven separation techniques,(CZE,MEKC and microemulsion eletrokinetic chromatography (MEEKC) for the determination of residual alkylating impurities in bromazepam API.Hoowever,the poor sensitivity of the techniques posted a problem even when specialized detection cells(e.g. bubble or Z-cells) were used. Researcher demonstrated the poor sensitivity of CE based on methods, in comparison to other separation

methods.The problem of limited sensitivity of CE methods can be solved either by the use of detection methods with sensitivity higher than UV absorpation or by pre-concentartion of the analytes.

Conclusion

The need to adequately assess the risk posed by GIs, and to limit the level present in API/DP is clearly established in the EMEA 7 and draft FDA 8 guidelines. It is the opinion that the most effective way to achieve this is to establish control strategies based on a combination of semi - quantitative assessment, allied to analytical results and data from appropriate purging studies. Such an approach should ensure that any actual GI related risk is clearly identifi ed and managed. It also provides valuable information regarding the classification of impurities and regulatory guidelines to control these impurities. Various techniques used in the isolation of impurities and analytical techniques require for their determination and quantification along with analytical method development and its validation.In addition to the challenge of setting a more pragmatic limit for GIs, the development of extremely sensitive and robust analytical methods that can adequately monitor GIs at very low levels is very difficult. Also, the pharmaceutical industry has no long-term experience in the use of these methodologies within the factory settingThus,analysts make attempts to determine a way for analyzing various GIs by using unique robust methods as far as possible. In this way, simple HPLC/UV or GC/FID methods are usually performed at the first stage, while more complicated LC/MS or LC/MS/MS methods are used as alternatives.

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