ISSN: 2279 - 0594

Available Online at www.jbpr.in

Journal of Biomedical and Pharmaceutical Research 2 (5) 2013, 39-47

REVIEW ARTICLE

GENOTOXIC IMPURITIES - AN OVERVIEW

***Usha Yadav[1], Priyanka Dhiman[1], Neelam Malik[1], Anurag Khatkar[1], Neelam Redhu[2], D.P. Singh[3]**

[1]Faculty of Pharmaceutical Sciences, M.D.University, Rohtak, India

[2]Department of Microbiology, Punjab Agriculture University, Ludhiana, India

[3]Bhagwant Institute of Pharmacy, Muzaffarnagar, Bijnor, India

Received 05 September 2013; Revised 10 September 2013; Accepted 15 September 2013

ABSTRACT

Genotoxic impurities (GTIs) in pharmaceuticals are of increasing concerns to both pharmaceutical industries and regulatory agencies due to their carcinogenic potency for humans. Practical guidance with respect to the analytical determination of diverse classes of GTIs is currently lacking in the literature. This article provides an industrial perspective with regard to the analysis of GTIs that are commonly encountered during drug development. Determination of these impurities at ppm levels requires highly sensitive analytical methodologies, such as LC/MS, LC-MS/MS, and RP-LC. The present review emphasized on the various methods used for the detection and quantification of genotoxic impurities.

Keywords: Genotoxicity, Mutations, Teratogenicity, Cancer Chemotherapy

INTRODUCTION:

Genotoxic impurities induce genetic mutations, chromosomal rearrangements, chromosomal breaks and act as carcinogenic compounds (Mc. Govern and Jacobson-Kram, 2006). Genotoxicity deals with mutagensis, carcinogensis, teratogensis. Impurities present in active pharmaceutical ingredients responsible for deleterious action on a cell's genetic material affecting its integrity (Miller, 2010).Therefore, exposure to even low levels of such impurities present in final active pharmaceutical ingredient (API) may be of significant toxicological concern (Kram, 2008). These compounds cause damage to DNA by different mechanism such as alkylation or other interactions that can lead to mutation of the genetic codes. Thus, the term "Genotoxic" is applied to those agents that interact with DNA and its associated cellular components (eg. the spindle apparatus) or enzymes (eg. topoisomerases) (Dearfield *et al*., 2002, Robinson, 2010). The genetic changes are responsible for heritable effects on germ cells and impose significant risk to future generations (Jena *et al*., 2002). They cause alterations in the genetic material within living cells, which can be transmitted from one cell generation to another (somatic mutatios) or to the progeny of affected individuals through germ cells (germinal mutations) (Waykar, Sharma, 2013).

Origin of Genotoxic impurities in active pharmaceutical ingredients:

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

Although genotoxic and carcinogenic properties can be acceptable for some active pharmaceutical ingredients depend upon clinical circumstances (e.g., cancer chemothrapies). These substances add significant risk without any benefit to the active pharmaceutical ingredients. Impurities in drug substances and drug products generally do not have any beneficial effects and may impose a risk without associated benefit. Hence, there is a need of suitable guidelines and commitment from pharmaceutical industries to address this issue in the drug substance or drug products (Guidence for industry, 2008).

Genotoxic agents alter the structure, information content, segregation of DNA, including those which cause DNA damage by interfering with normal replication process. Components that include in genotoxic impurities are interact with DNA either directly or indirectly and modification in DNA will takes place eg. alkylating agents, intercalating agents (Bercu *et al*., 2009).

*Corresponding author: Usha Yadav | Email: yadav.usha19@gmail.com

1.1 Types of Genotoxic impurity:

1.1.1 Carcinogens:

These are the agents which cause cancer by affecting the genome or disrupt the cellular processes. It can cause and facilitate the propagation of cancer. The mutagenic and clastogenic activity act as carcinogenicity. The risk of causing cancer is increased by altering the cellular metabolism or directly damaging DNA by carcinogenic agents. They induce the uncontrolled, malignant division, ultimately leading the formation of tumors due to interfering the processes in cells. Apoptosis is occurs due to severe damage of DNA. Carcinogens mainly classified into two groups such as genotoxic and non genotoxic. Genotoxins are the substances which cause irreversible genetic changes or mutations with binding to DNA. They are categories into two types such as chemical agents and non chemical agents. Some chemical agents such as N-nitroso-N-methyl urea and non chemical agents such as ultraviolet light and ionizing radiation.

Non genotoxins agents affect the DNA by indirect way and they promote the growth. Some organic compounds and hormones are includes in non genotoxins. In 1965, established an international agency for research on cancer (IARC) which is an intergovernmental agency a part of world health organization of the united nations.
In 1971, it has published a classification of possible carcinogens:
Group1: The agents which includes in groups. 1st are definitely carcinogenic to humans.
Group2: The agents are suspected to carcinogenic to humans.
Group3: These substances are not classifiable as a human carcinogen.
Group4: Substances is not suspected as a human carcinogen
(http.//en.wikipedia.org/wiki/carcinogen)

1.1.2 Mutation:

The irreversible change in DNA sequence of a genome is known as mutation. Mutation in DNA sequence can alter the sequence of amino acid of the protein encoded by the gene (learn.genetics.utah.edu). DNA made by smaller units but in long sequence strung together. Mainly four basic types of units: A, T, G and C. These letters represent the base of DNA sequence such as adenine, thymaine, guanine and cytosine. Even in large mutations the number of chromosomes will changes, where sequence of the DNA with in chromosomes break and then rearrange.

1.2 Types of mutations:

There are many different types that DNA can be caused mutation such as substitution, insertion and deletion.

- Substitution: In which exchanges or replaces the one base pair for another.
- Insertion: In which the extra base pairs are inserted into a new place in the DNA sequence.
- Deletion: In which the base pair or the section of DNA sequence is lost or deleted. (evolution.berkeley.edu/evolibrary/article/0_0_0/mutations_03).

1.3 Assessment and control of genotoxic impurities:

These impurities could be limited to new applications for existing active substances where assessment of the route of synthesis, process control and impurity profile does not provide reasonable assurance that no new or higher level of genotoxic impurities are introduced. The pharmaceutical industries regulate it to recognize their respective obligation to limit genotoxic impurities. Therefore, substantial efforts are made during development to control all impurities at safe concentrations (Kulkarni *et al*., 2011).

Genotoxic impurity can be identified by different methods by already known genotoxic impurity, possessing the similar functional group with known genotoxic impurity, positive test by genotoxicity assay.
Muller classified the genotoxic impurities into 5 groups:

- ✓ **Group 1:** This impurity is more dangerous, it is known genotoxic carcinogens that need to be avoided as much as possible.
- ✓ **Group 2:** The compounds that are genotoxic but with the unknown carcinogenicity and need to be controlled with a threshold of therapeutic concern (TTC) approach.
- ✓ **Group 3:** These impurities have alerting structures that are different to the parent drug substance. The assessment of this genotoxic compound will place it into group 2 or as an ordinary impurities group 5.
- ✓ **Group 4:** These impurities are parent related alerting structure in which the genotoxicity studies on the API have already been performed and applied to the related impurities.
- ✓ **Group 5:** These impurities have no alerting structure or indication of genotoxic potential and cosidered ordinary impurities that falls within the scope of ICH guidelines (Muller *et a*l., 2006).

1.4 Importance of genotoxic impurity:

During the synthesis of active pharmaceutical ingredients various reaction steps are involved for conversion of basic starting material to the final products. Various intermediate products are formed during the synthesis and reaction involves the byproducts, catalysts, solvents and

reagents which act as impurity. Even low levels of these are present in the final product as an impurity. Some unwanted toxicities including genotoxicity and carcinogenicity are observed by some reactive chemicals and they may react with DNA bases causing mutations. Mutations can be rearrangement, breaks of chromosomes, covalent binding with DNA during replication. Genotoxic substances indirectly cause mutations by activating the cells. Various modifications in genetic material which can be caused by exposure to even very low level of genotoxins, can cause cancer. Due to these reasons, identification and control of genotoxic substances at very low levels are most important to ensure safety to the humen (Algre, 2012).

2. Regulatory Aspects:

The assessment of genotoxic impurities and determination of acceptable limits for such impurities is difficult in active substances. The EMEA guideline recognizes the limitations and proposes the use of a "Threshold of toxicological concern" (TTC) for genotoxic impurities. Genotoxic impurities arise during synthesis, purification and storage of new drug substance should be identified, based on a scientific appraisal of the chemical reactions involved in the synthesis. When a potential impurity contains structural alerts, additional genotoxicity, testing of impurity should be considered (Dobo *et al.,* 2006, Muller *et al.,* 2006).

The EMEA recommends the acceptability of genotoxic impurities for which no threshold mechanism are identified for pharmaceutical evaluations. In general, pharmaceutical measurements should be guided by a policy of controlling levels "as low as reasonably practicable" (ALARP principle). A rationale of the proposed formulation strategy should be provided based on available formulation options and technologies. The reacting substance which show "alerting substances" in terms of genotoxicity which are not shared with the active substance should be considered (Dobo et al., 2006).

In EMEA guidelines, a threshold of toxicological concern (TTC) has been developed to define the exposure level of any unstudied chemical that will not pose a risk of significant carcinogenicity or other toxic effects (Munro et al., 1999, Kroes and Kozianowski, 2002). TTC originally developed as a " Threshold of regulation" at the FDA for food materials was (Rulis 1989, FDA 1995) established based on the analysis of 343 carcinogens from a carcinogenic potency database. The TTC value was estimated to be 1.5µg/person/day (Gold et al., 1984). The concentration limit (in ppm) of genotoxic impurity in drug substance derived from the TTC can be calculated based on the expected daily dose to the patient using equation:

Concentration limit (in ppm) = TTC (µg/day)/dose (g/day) (http://www.emeu.eu.int)

2.1 Various Methods developed for detection of genotoxic impurities:

Kakadiya *et al*., (2011) developed and validated a LC/MS method for determination of methyl methane sulphonate (MMS) and ethyl methane sulphonate (EMS) in Emtricitabine in active pharmaceutical ingredients (API). Chromatographic separation was achieved on Zorbax SB C-18 column using a mixture of 0.1 % formic acid and acetonitrile in the ratio of (70:30 v/v) with electro spray ionization (ESI) technique in multiple reaction monitoring (MRM). Linearity of method was found to be in the concentration range of 0.0025 µg/ml to 0.3 µg/ml with co-rrelation coefficient was > 0.999 in both cases. In this study, the LOD and LOQ were found to be 0.3µg/ml and 0.4µg/ml respectively for both the analytes.

Li et *al*., (2012) developed and validated a LC-MS/MS method for the pharmacokinetic study of Metoprolol in beagle dogs. The plasma sample was simply precipitated by methanol and chromatographic separation was achieved on XB C-18 column with methanol, water containing 0.2 % formic acid as the mobile phase at the flow rate of 0.2 ml/min. Monitoring ions of Metoprolol and internal standard (Hydroxypioglitazone) were m/z 268.1/115.6 and m/z 373.1/150.2 respectively. The linear range was 3.03ng/ml to 416.35ng/ml with an average co-rrelation coefficient of 0.9996, and the limit of quantification was 3.03ng/ml.

Chorilli et *al*., (2011) developed and validated a LC-MS/MS method for the quantification of Mirtazapine in plasma. Diazepam was used as an internal standard, added to 200 µl of plasma sample prior to liquid–liquid extraction using hexane. Chromatographic separation was achieved on an Agilent® Eclipse XDB C-18 column in isocratic mode with 10mM ammonium acetate, acetonitrile, formic acid (60/40/0.1 v/v/v) as a mobile phase. The LOD and LOQ were 0.17ng/ml and 0.50ng/ml respectively. The extraction recoveries for Mirtazapine and Diazepam were found between 84.9 % to 93.9 %.

Kakadiya *et al*., (2011) developed and validated a LC/MS/MS method for determination of methyl methane sulphonate (MMS) and ethyl methane sulphonate (EMS) genotoxic impurities in Lopinavir and Ritonavir active pharmaceutical ingredients (API). Chromatographic separation was achieved on Atlantis T3 with electro spray ionization (ESI) technique in multiple reaction monitoring mode for the quantitation of impurities. Linearity of method was found to be in concentration range of 0.01µg/ml – 0.23µg/ml for MMS and 0.05 µg/ml–0.23

µg/ml of EMS with correlation coefficient was > 0.99 in both the cases. The LOD and LOQ was found to be ~ 0.002 µg/ml and ~ 0.01 µg/ml respectively and the developed method had an accuracy within 80 %– 120 % for both the analytes.

Kakumanu *et al.,* (2006) RP-LC method was developed and validated for detection and quantification of model drug X. Chromatographic separation was achieved by using the column Lichrocart C-18 column, mobile phase composed of ammonium acetate buffer (ph 5.0) and acetonitrile (62:38) and at a flow rate of 1 ml/min. The detection was observed at 235 nm and a column temperature was 30ºC. The retention time for both the isomers of Model drug X was 12.5min. and 14.5min., respectively. The method was found to be in the linear within the concentration range of 5µg/ml to 150µg/ml. The correlation coefficient was found to be >0.999 for both the isomers.

Narayana *et al*., (2012) developed and validated a suitable LC-MS/MS method for the quantitative determination of sodium salt of 4-chloro-1-hydroxy butane sulphonic acid (genotoxic impurity) at ppm level present in Sumatriptan drug substance. The chromatographic separation was achieved on Zorbax SB C-8 column with the mobile phase consisting a mixture of 0.05 % formic acid in water and acetonitrile using isocratic composition of 90:10 (v/v) at a flow rate of 0.8 ml/min. Ion source was electronspray ionization, source temperature was 325ºC, gas flow was 8 l/min., nebulizer pressure was 40 psi, capillary voltage was 4000V. Under these conditions impurity was quantified by selecting most stable multiple reactions monitoring (MRM) pair (187/81). Validation was carried out as per ICH guidelines.

Sarat *et al*., (2010) developed and validated GC/MS method for the analysis of (ppm level) methyl methane sulphonate (MMS) and ethyl methane sulphonate (EMS) as genotoxic impurities in pharmaceutical drug substances. Chromatographic separation was achieved on a capillary column DB-624 with 6% cyanopropyl phenyl and 94 % dimethyl polysiloxane stationary phase with the mixture of methanol and chloroform in (80:20) ratio as a diluent and sample solvent in single reaction monitoring (SRM) mode. The LOD was 0.17µg/g (0.17 ppm) for MMS, 0.18 µg/g (0.18 ppm) for EMS and LOQ was 0.52µg/g (0.52 ppm) for MMS, 0.54µg/g (0.54 ppm) for EMS were achieved for alkyl sulphonate.

Bhatta *et al*., (2011) developed and validated a new selective, sensitive LC-MS/MS method for the quantification of Natamycin in rabbit tears using Amphoterin B as an internal standard. Chromatographic separation was achieved on a Luna Cyano column with ammonium acetate buffer (pH 4): methanol (10:90 v/v) as the mobile phase with negative electro spray ionization in multiple reaction monitoring (MRM) mode. The linearity concentration range was from 25ng/ml to 800ng/ ml and LOD was 12.5ng/ml.

Sampath *et al*., (2011) developed and validated a simple, sensitive, specific and reproducible LC-MS/MS method for determination of ethambutol in human plasma. Chromatographic separation was achieved by reverse phase chromatography on a Hypurity Advance C-18 column with a mobile phase composition of methanol: buffer (90:10 v/v). The retention time of ethambutol and internal standard was found to be 0.97 min. and 1.04 min. respectively. Linearity of the method was found to be in concentration range 99.635ng/ml to 6100.12ng/ml.

Harri and Ramjit *et al.,* (2007) GC-MS method was developed for the analysis of methyl methane sulfonate (MMS) and ethyl methane sulfonate (EMS) in the Bismesylate salt. By using single-ion monitoring and repetitive scanning was help to characterize and determine methyl methane sulfonate and ethyl methane sulfonate Bismesylate salt of DPI201-106, in the treatment of heart failure a positive ionotropic agent is used. Mass spectral fragmentations, leading to product ions, are rationalized and mechanisms of potential rearrangement pathways are described. The levels of MMS and EMS was measured against the n- propyl methane sulfonate (internal standard), were found to be 0.51µg/g and 1.31µg/g, respectively.

Yadav *et al*., (2012) developed and validated a gradient RP-HPLC method with PDA detector for the purity evaluation of Imatinib mesylate in bulk drug. The genotoxic impurities were N-(2-methyl-5-aminophenyl)-4-(pyridyl)-2-pyrimidine amine (impurity A) and N-[4-methyl-3-(4-methyl-3-yl-pyrimidin-2-ylamino)-phenyl]-4-chloromethyl benzamide (impurity B). Chromatographic separation was achieved by using Inertsil ODS 3V column (150×4.6 mm, 5µ) as a stationary phase with column oven temperature 35ºC and UV detection at 268 nm. Separation was achieved by using gradient program of buffer (a buffer used was of 0.1 % triethyl amine in water and pH adjusted to 2.9 with glacial acetic acid) and mixture of methanol and acetonitrile. The method was optimized based on the peak shapes and resolution. The method was validated as per guidelines. The LOD and LOQ value were found to be 0.024µg/ml and 0.08µg/ml respectively. The method was linear within the range of 0.08µg/ml – 0.3µg/ml for both the impurities.

Devi and Ravi, (2012) developed and validated a specific, sensitive RP-HPLC method with PDA detector and UV spectrophotometric method for quantitative determination

of Iloperidone in tablet dosage form. Chromatographic separation was achieved on a Lichrospher® RP-18 HPLC column (5µ particle size, 25cm×4.6mm internal diameter) using 0.1 % trifluoroacetic acid: acetonitrile in the ratio of 50:50 v/v (pH 5.02) as mobile phase and Paracetamol as the internal standard and the effluent was monitored at 275 nm. The two sharp peaks were obtained for internal standard and Iloperidone at 2.8 min. and 7.6 min. respectively. UV spectrophotometric method was performed at 229 nm using methanol as the solvent. Linear range was 1-10 µg/ml for HPLC method and 2-20 µg/ml for UV spectrophotometric method.

Kaleemullah *et al.,* (2011) developed and validated a simple and reliable head space gas chromatographic method for the determination of residual methyl chloride, ethyl chloride and isopropyl chloride in Ziprasidone hydrochloride. The proposed method based on flame ionization technique with DB 624 as stationary phase. Linearity of detector response was established up to 13.5µg/g and the LOD was 0.8µg/g for methyl chloride and ethyl chloride, 0.9µg/g for isopropyl chloride respectively.

Susantkumar *et al*., (2011) developed and validated a simple, specific, accurate and precise LC/MS/MS method for the determination of Acyclovir in human plasma using Ganciclovir as an internal standard. Chromatographic separation was achieved on Hypersil GOLD C-18, 5µ column having 4.6mm internal diameter in binary gradient mode with flow rate 0.5 min/ml. Mobile phase used were containing ammonium acetate and acetonitrile while the eluting solution consisting of acetonitrile and water (80:20 v/v), diluent solution of methanol and water (50:50 v/v) were monitored on a triple quadrupole mass spectrometer, operating in multiple reaction monitoring (MRM) mode. The LOD and LOQ were found to be 5.0 ng and 30.0 ng respectively. The retention time for Acyclovir and internal standard were 1.24 min. and 1.65 min. respectively.

Thiyagarajan *et al.,* (2010) developed and validated RP-LC method for quantification of Idebenone and its related impurities in drug substance. The method was optimized in a zorbax SB C-18 stationary phase for separation of known impurities with excellent detection limit in drug substance. Mobile phase composed of water, acetonitrile and trifluoroacetic acid. The wavelength and flow rate was set as 215 nm and 1.0 ml/min., respectively. The LOD and LOQ were found as a signal to noise ratio 3:1 and 10:1, respectively. The developed RP-LC method was validated in accordance with ICH guidelines. The developed method can be used for the determination of synthetic and degradation impurities in regular quality control analysis for the drug substance.

Sreekanth *et al*., (2009) developed and validated a simple, accurate RP-HPLC method for the estimation of Ropinirole hydrochloride in bulk and pharmaceutical dosage forms. The separation was achieved using C-18 column 250×4.6mm internal diameter, 5µm particle size in isocratic mode, with mobile phase comprising of buffer (pH 6.0) and acetonitrile in the ratio of 50:50 v/v. The flow rate was 0.5 ml/min and detection was carried out by UV detector at 245nm. The retention time of Ropinirole hydrochloride was found to be 4.867min. The proposed method had permitted the quantification of Ropinirole hydrochloride over linearity in the range of 5µg/ml–50 µg/ml and the percentage recovery was found to be 99.3 % – 100.4 % .The intra and inter day precision was found to be 0.27 % and 0.26 % respectively.

Sivakumar *et al*., (2007) developed and validated a simple RP-HPLC method for determination of Domperidone and Pentaprazole in capsules. The separation was achieved on an ODS Analytical column with a mixture of methanol, acetonitrile and triethylamine solution (10mM, pH 7.0 ± 0.05 adjusted with 85 % phosphoric acid) in the ratio of 20:33:47(v/v/v) as mobile phase with flow rate of 1.0 ml/min. UV detection was performed at 285 nm. The retention time of pentoprazole, acetophenone (internal standard) and domperidone was found to be 4.34 min., 5.52 min. and 9.46 min. respectively. Linearity range was 0.5µg/ml–5µg/ml and 1µg/ml-10µg/ml for Domperidone and Pentoprazole respectively. The LOD were 15.3ng/ml and 3.0 ng/ml and LOQ were 51.0ng/ml and 10.0ng/ml for Domperidone and Pantoprazole respectively.

Sathyaraj *et al*., (2011) developed a rapid, sensitive, efficient and reproducible RP-HPLC method for the determination of Raloxifene hydrochloride. Separation was achieved on a RP- Kromosil C-18 (150 × 4.6 mm, 5µm) column using UV detection at 280 nm. The elution was carried out by using a mobile phase consisting of acetonitirile: water (30:70 v/v).

Patel *et al*., (2011) developed and validated a simple rapid, sensitive, specific, accurate HPLC method as per ICH guidelines for the determination of Ofloxacin in eye drop. Thermo separation products C-8 (250 cm × 4.6mm internal diameter, 5µm) column with a mobile phase consisting acetonitrile: buffer in the ratio 35:65 v/v with a flow rate of 1.5 ml/min. was used. Detection was carried out at 315 nm using UV detector. The method was linear over the concentration range of 50 µg - 300 µg/ml. The good recoveries (99.8 % - 103.73 %) and relative standard deviation (RSD) of intra and inter day assay were 0.554 % and 0.677 %, respectively. The proposed method was found to be precise, accurate, selective and rapid for the determination of Ofloxacin in quality control and assay.

Jianguo *et al.,* (2008) developed and validated LC/MS/MS method for low (ppm) level determination of alkyl esters of sulfonates or sulfates in drug substances. In this method, the some specific derivatizing reagents were used such as triethylamine for methyl esters and trimethylamine for ethyl/propyl/isopropyl esters. The resulting quaternary ammonium derivatization products are highly polar (ionic). They can be retained with hydrophilic interaction liquid chromatography column and separated from the main interfering active pharmaceutical ingredient (API) peak that is usually present at very high concentration. The recoveries observed 85 % for all the alkyl esters in the various APIs at 1-2ppm. The excellent RSD was observed in the range 0.4% – 4%. Linearity range has been established with $R^2 \geq 0.99$ for concentrations range from 0.2 ppm to 20 ppm.

Rao *et al.*, (2008) developed and validated a simple, precise, isocratic RP-HPLC method for the determination of Tamsulosin hydrochloride pellets 0.2 %. Good separation was achieved on an Inertsil ODS 3V (5 µ, 150 × 4.6 mm) column, in an isocratic mode with an acetonitrile: buffer (30:70) as a mobile phase, the pH was adjusted to 3.0 with perchloric acid. Flow rate was 0.2 % and the elution was monitored at 220 nm. The method was validated as per guidelines. The method was linear in the range of 75 ppm to 150ppm.

Gerd *et al.,* (2009) LC/MS/MS method for the analysis of hydrazines. The N-acyl derivatives showing higher retention time in RP-LC and good delectability was found by mass spectrometer by using hexachloroformate as a pre-column derivatization reagent. Acetonitrile was used for dissolving the Vitamin C at 100 mg/mL and spiked with Methyl hydrazine at 1 ppm level. The chromatographic separation was achieved on a Zorbax Eclipse Plus Column (15 cm x 3 mm i.d. 3.5 µm). By using mobile phase 0.05% formic acid in water (solvent A)/ Acetonitrile (solvent B) gradient from 10%B to 100%B at 19 min. with 0.5 mL/min. flow rate. Detection is achieved by mass spectrometer in SIM mode using electro spray ionization in positive ion mode.

Smith *et al.*, (2012) developed and validated a HPLC method for quantification of Ritonavir in pure form and in pharmaceutical formulation. Separation was achieved on Hypersil (ODS) C-8 (250×4.6mm, 5µm) column with an isocratic mobile phase containing acetonitrile: methanol: 0.01mM potassium dihydrate orthoposphate buffer (30:20:50) at the pH 3.0 with flow rate of 1ml/min. The method was linear (r^2>0.999), precise (RSD < 0.66 %) and the recovery was 100.29 %.

Mamilla et al., (2010) a sensitive GC-MS method was developed and validated for analysis of residues of allyl chloride, 1, 3-dichloro-2-propanol and 2, 3-dichloro-2-propanol genotoxic impurities in Atenolol drug substance. The separation was achieved by using 30m×0.53mm i.d. capillary column coated with 5.0µm film of DB-5. High purity Helium gas was used as a carrier gas at a constant flow rate of 1.0 ml/min. The recoveries were found to be 97.1% to 99.3%, 97.6% to 104% and 90.1% to 96.8%, respectively. The LOQ and LOD were found to be 0.003mg/g to 0.001mg/g, respectively. Linearity range was 0.003mg/g to 0.018mg/g. The co-relation coefficients were found to be 0.9910, 0.9964 and 0.9960, respectively.

Harikrishna *et al.,* (2010) HPLC-MS method was developed and validated for low-level determination of 4-hydrazino benzoic acid (4-HBA) in drug substances. The 4- HBA impurity has been highlighted as potential genotoxic. Chromatographic separation was achieved by using symmetry C-18 (150 x 4.6 mm,5µm) column in isocratic mode using 0.01 M ammonium acetate and acetonitrile (45 : 55) v/v. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.17 ppm and was 0.52 ppm, respectively. Excellent recoveries of 104.6 % were obtained at the 0.5 ppm level for one drug substance.

Ramakrishna *et al.,* (2008) GC-MS method was developed and validated for the determination of methyl methane sulfonate and ethyl methane sulphonate in Imatinib mesylate. Separation was observed by using DB-1 column (100% dimethyl polysiloxane). The optimization of the method was based on resolution and peak shapes of MMS and EMS. The LOD and LOQ were found to be 0.3µg/g and 1.0µg/ml, respectively. For both the compounds method was linear within the range of 1-15µg/ml. The correlation coefficient values of MMS (0.9998) and EMS (0.9996), respectively.

3. CONCLUSION:

GC-MS, LC-MS, HPLC, RP-LC, HPLC-MS, LC-MS-MS, RP- HPLC and RP-LC methods were specifically used for the detection and quantification of genotoxic impurities in drugs like Imatinib mesylate, Atenolol and Tamsulosin hydrochloride etc. The limit of detection (LOD) and limit of quantification (LOQ) were determined successfully by many researchers and it is suggested that the determination of genotoxic impurities is an essential step for analysis of active drug(s) so as to minimise their harmful effects.

4. REFERENCES:

1. Bercu JP (2010) Advancing toxicology based cancer risk assessment with informatics. [Dissertion].Bhatta RS, Chandasana H, Rathi C, Kumar D, Chhonker YS, Jain GK

(2011) Bio analytical method development and validation of Natamycin in rabbit tears and its application to ocular pharmacokinetic studies. J Pharm Biomed Analysis. 54(5):1096-1100.

2. Chandrul KK, Srivastava B (2010) A process of method development: A chromatographic approach. J Chem Pharm Res. 2(2):519-545.
3. Chatwal GR, Anand SK (2007) Instrumental methods of chemical analysis. Himalya Publishing House. 5:2.272-2.287.
4. Chorilli M, Bonfilio R, Louvandine CR, Goncalves FARM, Salgado HRN (2011) Development and validation of an LC/MS/MS method for quantitative analysis of Mirtazapine in human plasma. American J Analyt Chem.2:650-657.
5. Deshmukh RS, Umarkar AR, Bavaskar SR, Narkhede PS, Chaudhari VG, Dhande MG (2011) Impurity profile in pharmaceutical substances- A comprehensive: A review. Int J Pharm biological Sci. 1(4):382-392.
6. Dobo KL, Green N, Cyr MO, Caron S, Ku WW (2006) The application of structure based assessment to support safety and chemistry diligence to manage genotoxic impurities in active pharmaceutical ingredients during drug development. Reg Tox Pharm. 282-293.
7. Elder DP, Teasdale A, Lipczynski AM (2008) Control and analysis of alkyl esters of alkyl and aryl sulfonic acids in novel active pharmaceutical ingredients (APIs). J pharm biomed analysis. 46:1-8.
8. Gaskell SJ (1997) Electro spray principles and practice. J mass spect. 32:677-688.
9. Gold LS, Sawyer CB, Magaw R, Backman GM, Levinson R, Hooper NK, Havender WR, Bernstein L, Peton R, Pike MC, Ames BN (1984) A carcinogenic potency database of the standardized results of animal bioassays. Environ Health Perspect. 9-319.
10. Harikrishna S, Reddy KN, Suresh Y, Ramakrishna M, Sarat M, Kishore K, Rambabu C (2010) Low-level detection of 4-Hydrazino benzoic acid in drug substances by HPLC/MS. J chem. 7: 403-408.
11. Hoffmann ED (1996) Tandem mass spectrometry: a primer. J mass spect. 31:129-137.
12. Hoffmann ED, Stroobant V (2007) Mass spectrometry. Principle and Applications. Jon Wiley and Sons Ltd. 3:1-476.
13. Hoja H, Marquet P, Verneuil B, Lotfi H, Penicaut B, Lachatre G (1997) Application of liquid chromatography mass spectrometry in analytical toxicology: A Review. J Analytical Toxicology. 21:116-126.
14. Holcapek M, Jirasko R, Lisa M (2012) Recent developments in liquid chromatography mass spectrometry and related techniques. J chromatography A. 1259:3-15.
15. Ingale SJ, Sahu CM, Paliwal RT, Vaidya S, Singhai AK (2011) Advance approaches for the impurity profiling of pharmaceutical drugs: A review. Int J Pharm Life Sci. 2(7):955-962.
16. Ishibashi T, Horisawa T, Tokuda K, Ishiyama T, Ogasa M, Tagashira R, Matsumoto K, Nishikawa H, Uedo Y, Toma S, Oki H, Tanno N, Saji I, Ito A, Ohno Y, Nakamura M (2010) Pharmacological profile of Lurasidone, a novel antipsychotic agent with potent 5-HT_7 and 5-HT_{1A} receptor activity. J Pharmacol Exp Ther. 1-40.
17. Janson MA (2009) Genotoxic impurity analysis in pharmaceuticals. [Dissertion]. Callifornia (USA). Jena JB, Kaul CL, Rama rao P (2002) Genotoxicity testing, a regulatory requirement for drug discovery and development: Impact of ICH guidelines. Indian J Pharmacol. 34:86-99.
18. Jianguo A, Mingjiang S, Lin B, Ted C, David QL, Kord A (2008) A practical derivatization LC/MS approach for determination of trace level alkyl sulfonates and dialkyl sulphates genotoxic impurities in drug substances. J pharm biomed analysis. 48:1006-1010.
19. Jyothirmayee M, Phenikumar K (2012) Pharmaceutical impurities-significance of impurity profiling. Int J Pharm Tech. 4(1):1822-1842.
20. Kakadiya PR, Reddy P, Chandershekhar TG, Ganguly S, Singh DK, Singh V (2011) low level determination of methyl methane sulphonate and Ethyl ethane sulphonate impurities in Lopinavir and Ritonavir active pharmaceutical ingredient by LC/MS/MS using electro spray ionization. J Pharm Biomed Analysis. 55:379-384.
21. Kakadiya PR, Chandrasekhar TG, Ganguly S, Singh DK, Singh V (2011) Low level determination of Methyl methane sulphonate and Ethyl methane sulphonate impurities in Emtricitabine active pharmaceutical ingredient by LC/MS/MS using elctrospray ionization. Analytical Chemistry Insights. 6:21-28.
22. Kakumanu VK, Arora S (2006) Development and validation of isomer specific RP-HPLC method for quantification of model drug X. J chromatography B. 835:16-20.
23. Kaleemullah T, Ahmed M, Sharma HK (2012) Development and validation of RP-HPLC method for the determination of P-toluene sulphonic acid and ethyl-p-toluene sulphonate in perindopril tert-butylamine drug substance. J Chem Pharm Res. 4(1):483-490.
24. Koizumi T, Chihara Y (2009) Absolute detection efficiencies of an ion counting system with a channel electron multiplier. J Phys. 1-4.

25. Kram DJ (2008) Guidance for industry genotoxic and carcinogenic impurity in drug substance and products- Recommended approach. Pharmacol Toxicol.

26. Kram DJ, Mc Govern T (2006) Regulation of genotoxic and carcinogenic impurities in drug substance and products. TrAC Trends Analytical Chem. 25:790-795.

27. Kram DJ, McGovern T (2007) Toxicological overview of impurities in pharmaceutical products. Advanced Drug Delivery Reviews. 59:38-42.

28. Krstulovic AM, Lee CR, Firmin S, Jacquet G, Dau CNV, Tessier D (2002) Applications of LC-MS methodology in the development of pharmaceuticals. LC-GC Europe. 1-9.

29. Kulkarni A, Gupta P, Gupta VK, Songara RK (2011) Genotoxic impurities- Regulatory requirement: A review. Asian J Pharm Sci Res. 1(7):1-18.

30. Li S, Wang X, Peng K, Ma Z, Zhang X, Fu S, Li X, Li L, Hang A, Jiang J (2012) Rapid and sensitive LC/MS/MS method for the determination of Metoprolol in beagle dog plasma with a simple protein precipitation treatment and its pharmacokinetic applications. Molecules. 17:2663-2674.

31. Mamilla YR, Chetyala KR, Venna R, Gajjela R, Katragadda K, Govindasamy S, Mulukutla S, Datta D (2010) A sensitive and selective GC-MS method for analysis of process related genotoxic impurities in Atenolol. Chromatographica. 71:733-736.

32. McGovern T, Jacobson J (2006) Regulation of genotoxic and carcinogenic impurities in drug substances and products. Trends in Analytical Chemistry. *25*:1342-1349.

33. Miller SA, Parsons RL (2011) 4-Analytical consideration for genotoxic and other impurities. separation science technol. 10:171-194.

34. Muller L, Mauthe RJ, Riley CM, Andino M, Antonis D, Beels J, Ellison D, Fagerland J, Frank R, Fritschell B, Humfrey C, Mohan G, Jacks A, Ness D, Donovan O, Smith M, Vudathala G, Yotti L (2006) A rationale for determination ,testing and control of genotoxic impurities. Regul Toxicol Pharm.

35. Narayana MBV, Chandrasekhar KB, Rao BM (2012) Quantification of Genotoxic Impurity 4-chloro-1-hydroxy Butane Sulphonic Acid sodium salt by LC-MS/MS in Sumatriptan succinate. J Bioanal Biomed. 4(6):104-107.

36. Patel SKN, Smith AA, Amuthalakshmi S, Gandhi VN, Manavalan R (2011) Analytical method development and validation of Ofloxacin eye drop by HPLC. J Curr Chem Pharm Sci. 1(1):59-64.

37. Ramakrishna K, Raman NVVSS, Rao KMVN, Prasad AVSS, Reddy KS (2008) Development and validation of GC-MS method for the determination of methyl methane sulfonate and ethyl methanesulfonate in imatinib mesylate. J Pharm biomed Analysis.46:780-783.

38. Rao MVB, Reddy BCK, Rao MS, Sreedhar B (2008) Development and validation of RP-HPLC method for the determination of Tamsulosin hydrochloride. International J. Chem. Sci. 6(3):1695-1701.

39. Robert E (2010) Mass spectrometer calibration over wide concentration ranges in multi component gas mixtures. Meas Sci Technol. 21:1-11.

40. Robinson DI (2010) Control of genotoxic impurities in active pharmaceutical ingredients: A review and respective. Organic Process Res Development. 14:946:959.

41. Sandra A, Wright B, MN, RNC, Robert DS, MD (2004) Tandem mass spectrometry in new born screening. A primer for neonatal and perinatal nurses. J Perinat Neonat Nuts. 18(1):41-58.

42. Sarat M, Ramkrishna M, Suresh Y, Harikrishna S, Rambabu C, Kishore K, Reddy KN (2010) Low level determination of residual Methyl methane sulphonate and Ethyl methane sulphonate in pharmaceuticals by gas chromatography with mass spectrometry. E-J Chem. 7(2):629-635.

43. Sampath K, Terish JD, Kumar S, Ramesh N, Sasijith SL (2011) Method development and validation of Ethambutol in human plasma by using LC/MS/MS. Int J Res Pharm Biosci. 1(1):1-6.

44. Sathyaraj A, Satyanarayana V, Rao MVB (2011) Gradient RP-HPLC method for the determination of purity and assay of Raloxifene hydrochloride in bulk drug. Research Journal of Chemical Sciences. 1(2):9-16.

45. Scheubert K, Hufsky F, Bocker S (2013) Computational mass spectrometry for small molecules. Journal of Cheminformatics. 5(12):1-24.

46. Shelke PG, Tripathi AS, Dewani AP, Bakal RL, Mohale DS, Chandewar AV (2012) Liquid chromatography in conjugation with mass spectrometry (LC/MS). International Journal of Pharmaceutical and Chemical Sciences. 1(3):1183-1189.

47. Sivakumar T, Manavalan R, Vallippan K (2007) Development and validation of reversed phase HPLC method for simultaneous determination of Domperidone and Pantaprazole in pharmaceutical dosage forms. Acta Chromatographica. 18:130-142.

48. Smith AA, Aran R (2012) Development and validation of analytical method for Ritonavir by HPLC. International Journal of Pharmacy and Pharmaceutical Sciences. 4(4):173-176.

49. Susantakumar P, Gaur A, Sharma P (2011) Development and validation of LC/MS/MS method for

the estimation of Acyclovir in pharmaceutical dosage form. Pharmacophore. 2(4):210 224.

50. Vijaylakshmi R, Kumaravel S, Anbazhagan S (2012) Scientific approaches for impurity profiling in new pharmaceutical substances and its products- An overview. Int J Pharm Chem. Sci. 1(1):386-403.

51. Wear R, Sharma C (2013) Assessment of genotoxic impurities in pharmaceuticals by in silica and in situ derivatization-head space GC-MS methodology. Int J Toxicol Pharm Res. 5(1):33-41.

52. Yadav RR, Rokde MD, Solunke SA, Gangrade DM, Holkar GS, Daphal VN (2012)Determination of potential genotoxic impurities in Imatinib mesylate by RP-HPLC method. Bio Forum Int J. 4(2):15-18.

53. Zhou S, Song Q, Tang Y, Naidong W (2005) Critical review of development, validation and transfer for high throughput bioanalytical LC/MS/MS methods. Curr Pharm. Analysis. 1:3-14.

54. Zimmer D (2003) Introduction of quantitative liquid chromatography tandem mass spectrometry. Chromatographic Supplement. 57:325-332.

