Original Article

A Review On Techniques For Estimation Of Nitrosamine Impurities In Antidiabetic Drugs Pioglitazones And Glifizones, Metformin

Md. Mubarakunnisa[1], R. Gandhimathi[2]

[1]Research Scholar, Department of Pharmaceutical chemistry, Vels Institute of science and technology advanced studies (VISTAS), Pallavaram, Tamilnadu, India.
[2]Professor, Department of pharmaceutical Chemistry, Vels Institute of Science and Technology Advanced Studies (VISTAS), Pallavaram, Tamilnadu, India.
*Corresponding author: Md. Mubarakunnisa Email: mubarakpharma03@gmail.com
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Abstract

Nitrosamine is family of carcinogenic impurities. Which are formed by reactions of secondary amide carbamates, amines, derivatives of urea with nitrite and other Nitrogenous agent. The contaminated starting or law materials supplies byvendor may be introduced Nitrosamine impurities in drug product. The methods of nitrosamine testing in sartan include the use of chromatographic techniques (reversed phase liquid chromatography combined with mass spectrometry (MS) etc. Impurities are undesirable chemicals present in the pharmaceuticals arising from normal manufacture. They are not chemicals accidentally or maliciously introduced. Impurities have no therapeutic value and are potentially harmful. Impurities are generally assumed to be inferior to API because they might not have the same level of pharmacological activity. Presence of impurity in the drug substance compromises the purity of the drug contains superior pharmacological or toxicological properties. Impurities in drug substance can emerge from various sources and phases of the synthetic process. During synthesis, intermediates and by-products may be carried into the drug substance as impurities or become a source of other impurities resulting from them. Impurities present in starting material may be carried into drug substance. Diabetes mellitus is a syndrome of chronically elevated glucose level in the blood either due to insulin resistance, insulin deficiencyor both. The different analytical methods available for the estimation of thedrugs which will provide the knowledge of the analysis and it can be utilized for the determination of targeted drugs (anti diabetic drugs) to enhance the therapeutic benefits of anti diabetic drugs.

Keywords: Nitrosamine, Pharmaceuticals, Diabetes mellitus, Impurities, Pharmacological activity. Analytical methods.

INTRODUCTION

Introduction to nitrosamine impurities

Nitrosamine is family of carcinogenic impurities. Which are formed by reactions of secondary amide carbamates, amines, derivatives of urea with nitrite and other Nitrogenous agent. Nitrogen has +3 Oxidation state. There is a different reason Nitrosamine present in the drug.source of Nitrosamine is During manufacturing of drug and packaging of Drug. During this process Nitrosamine impurity present in drug. We have general method for detection of nitrosamine impurities are HS-GC-MS , LC-MS/MS. (high blood pressure) and heart failure. Food and drug administration and European medical Agency investigation in year 2019 leads to detection of nitrosamine impurity present in pioglitazone are used in treatment of diabetes. The low level of nitrosamine impurity present in metformin drug investigation under FDA and EMA. Nitrosamine impurities can incorporate into drug product Basically through manufacturing process, cross contamination, degradation of product, direct introduction, process of formation

involved raw materials, intermediate, solvent and catalyst, chemical and reagent. The nitrosamine impurities formed due to carbamates, amide, N-alkyl amide, secondary or tertiary and quaternary ammonium salts with drug products.the extent of nitrosamine impurity depends on the type , structure, concentration of nitrosating Agent.Secondary amide is more reactive than other nitrosating agent (Mingjiang Sun et al., 2010, Hussain, S et al., 2018). The recovered solvent and catalyst are used in processleads to formation of Nitrosamine impurities. These solvents treated with nitrites or nitric acid in order to destroy residual azide to leads to formation ofNitrosamine impurity.

Contaminated starting or law materials supplies byvendor may be introduced Nitrosamine impurities in drug product. Cross contamination between different manufacturing process or product on same production also leads to formation of Nitrosamine impurities in drug substance or drug products. These impurities may formed due to decomposition of solvent and other material used in manufacturing process. By product formed in drug synthesis leads forward formation of Nitrosamine impurities (Parr MK et al., 2019, Alsante KM et al., 2007).

NDMA and NDEA:

Use of certain packaging materials and finished good product may be formed nitrosamine impurities. Packaging materials lidding foil containing nitrocellose, printing primer reacts with amine in printing ink to formed Nitrosamine impurities. These impurities may transfer to drug products. Another potential source of formation of nitrosamine impurities is lack of optimization of the manufacturingprocess for APIs when reaction conditions such astemperature, pH, or the sequence of adding reagents, intermediates, or solvents are inappropriate or poorly controlled. These sources of nitrosamine found European medical Agency and food and drug administration .nitrosamine impurities formed in drug substance orproduct different reasons (Singh S et al., 2012, Qiu F et al., 2007).

European medical Agency:

The European Medicines Agency (EMA) assessed the risk of formation or presence of nitrosamine during the manufacture of human medicines and provided guidance to market-authorized authorities to avoid the presence of nitrosamine contamination. Nitrosamines are chemical compounds that are classified as carcinogens that humans may be based onin animal studies. EU regulators begin noticing nitrosamine in medicines in mid-2018 when nitrosamine contamination, including N- Nitrosodimethylamine (NDMA), is detected in blood pressure drugs known as 'Sartans'. cancer in humans (Nicolas EC et al., 1998, Argentine MD et al., 2007).

Table 1: Limitations of Nitrosamine Impurity

Nitrosamine	AL limit (ng/ Day)
NDMA	96.0
NDEA	26.5
NMBA	96
NMPA	26.5
NIPEA	26.5
NDIPA	26.5

Guidance for marketing authorisation holders:

- Authorized marketing authorities should review their production processes to identify all products containing organic matter in order to identify and, if necessary, reduce the risk of the presence of nitrosamine contamination (Lee and D et al., 2014).
- The EMA has finalized a review under Article 5 (3) of Regulation (EC) No 726/2004 of June 2020 to provide guidance to market authorization authorities on how to avoid the presence of nitrosamine contaminants

in human medicines.

- CHMP has asked market permit holders to review all human chemical and biological drugs to determine the possible presence of nitrosamine andtest products at risk.
- Companies need to have appropriate control strategies in place to prevent or reduce thepresence of these pollutants and, where necessary, improve their production process.
- The EMA and competent national authorities will continue to monitor the presence of nitrosamine contaminants in medicines, in collaboration with regulators from outside the European Union (EU), and will work with marketing authorities to find quick solutions to any deficiencies (Lohr L L et al., 2001, Liu, J et al., 2020).
- The European Medicines Regulatory Network encourages marketing license holders to submit theoutcome of step 1 before the deadline once they have completed a risk assessment or risk assessment for their products.
- They should also assess the risks to patients and take appropriate action to avoid or reduce patients' exposure to nitrosamine.

Methods for detection of Nitrosamine impurities :

The FDA has released the following methods for the determination of NDMA impurities in drugs.

- GC / MS Headspace Chromatography Mass Spectrometry Approach.
- Liquid Chromatography - Tandem Mass Spectro- metry (LC - MS / MS) Method for the Determination of NDMA in Ranitidine DrugSubstance and Solid Dosage Drug Product.[7]

Analytical methods:

Methods of nitrosamine testing in sartan include the use of chromatographic techniques (reversed phase liquid chromatography - RP - LC or gas chromatography - GC), combined with mass spectrometry (MS), spectrophotometry (UV). nitrogen chemiluminescence (NCD). USP proposes four analytical methods that manufacturers can use toidentify potential nitrosamine in their products:

- The first method recommends high-performance liquid chromatography-high resolution massspectrometry (HPLC-HRMS) for measuringNDMA, NDEA, NDIPA, NEIPA, NMBA and NDBA.
- The second recommends gas chromatography- mass spectrometry (GC-MS) for NDMA, NDEA, NDIPA, and NEIPA.
- Third party recommends HPLC-Tandem Mass Spectrometry for NDMA, NDEA, NDIPA, NEIPA, NEIPA, NEIPA, and NMBA.
- Fourthly recommends GC-Tandem Mass Spectrometry for NDMA, NDEA, NDIPA, NMBA and NDMA.

Impurities

Impurities are undesirable chemicals present in the pharmaceuticals arising from normal manufacture. They are not chemicals accidentally or maliciously introduced. Impurities have no therapeutic value and are potentially harmful. Therefore, they need to be identified and controlled (Derosa, G et al., 2012, Yang, H.K. et al., 2019). Impurities are generally assumed to be inferior to API because they might not have the same level of pharmacological activity. Presence of impurity in the drug substance compromises the purity of the drug even if impurity present in it contains superior pharmacological or toxicological properties. The characterization of these related substances in pharmaceutical products is an important feature of the regulatory approval process. If the estimation indicates that the given impurity content is greater than 0.1%, it must be identified and characterized according to all regulatory requirements. Recently, not only purity profile but also the impurity profile has become essential as per various regulatory requirements. A number of articles have stated guidelines and designed approaches for isolation and identification of process-related impurities and degradation products using

Mass spectrometry (MS), Nuclear Magnetic Resonance (NMR), High Performance Liquid Chromatography (HPLC), Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS), and Tandem Mass Spectrometryfor pharmaceutical substances.

Guidelines for the control of impurities and itscharacterization

ICH guidelines on impurities in New Drug Substances have the following requirements: The studies conducted to characterize the structure of actual impurities present in the new drug substance at a level greater than 0.1% should be described. Similarly, ICH Guidelines on Impurities in New drug Products require that the degradation products observed in the stability studies conducted at recommended storage conditions be identified when present at alevel greater than the identification thresholds (1% for a maximum daily dose of <1 mg to 0.1% for a maximum daily dose of >2g). Identification of impurities below 0.1 % level, is not taken into account to be necessary, except for potential impurities are expected to be unusually potent or toxic, as per ICH guidelines. Impurities above the ICH identification threshold need to be identified and individually specified in the specifications. The limits must be qualified as safe. The limits should realistically reflect batch and stability data. The limit for any unspecified impurity should be at the ICH identification threshold. According to the ICH, the maximum daily doseidentification threshold to be considered is as follows.

Table no 1: Impurities threshold in drug

Threshold	**Maximum drug daily dose**	
	Less than 2 g/day	**More than 2 g/day**
Reporting	0.05 %	0.03 %
Identification	0.10 %	0.05 %
Qualification	0.15 %	0.05 %

Table no. 2: Regulatory guideline related to impurities

Regulatory	**Guideline details**
ICH (USA, EU, andJapan)	Stability Testing of New Drug Substances and Products Q1A(R2) Feb 2003
	Impurities in New Drug Substances Q3A(R2) Oct 2006
	Impurities in New Drug Products Q3B(R2) Jun 2006
	Impurities: Guideline for Residual Solvents Q3C(R7) Oct 2018
	Guideline for Elemental Impurities Q3D(R1) Mar 2019
	Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk. M7 (R1)
US-FDA guidelines	"NDAs -Impurities in New Drug Substances" Nov 1999
	"ANDAs – Impurities in New Drug Substances" Nov 1999
	Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches (draft) Dec 2008

Australian regulatory guideline	For prescription medicines, (TGA), Australia 1989
	Guidelines for Prescription Medicines; Appendix 18: Impurities in Active Pharmaceutical Ingredients and Finished Products Jun 2004
EP	General Chapter 5.10, Control of Impurities in substances for pharmaceutical use Jan 2017
EMA (Europe)	Assessment of Quality of Medicinal Products Containing Existing/Known Active Substances EMEA/CHMP/CVMP/QWP/450653/2006 Jul 2007
	Control of Impurities of Pharmacopoeial Substances CPMP/QWP/1529/04
	Guideline on the Limits of Genotoxic Impurities CPMP/SWP/5199/02 & EMEA/CHMP/QWP/251344/2006 Jun 2006
	Guidelines on Specification Limits for Residues of Metal Catalysts CPMP/SWP/QWP/4446/00. Jan 2007
	Guideline on Setting Specifications for Related Impurities in Antibiotics (draft) EMA/CHMP/CVMP/QWP/199250/2009 Jul 2010

Source of impurity

Impurities in drug substance can emerge from various sources and phases of the synthetic process. During synthesis, intermediates and by-products may be carried into the drug substance as impurities or become a source of other impurities resulting from them. Impurities present in starting material may be carried into drug substance. According to the ICH, impurities are classified as organic impurities, inorganic impurities, and residual solvents. Organic impurities may arise from starting materials, by-products, synthetic intermediates and degradation products. Inorganic impurities may be derived from the manufacturing process and are normally known and identified as reagents, ligands, inorganicsalts, heavy metals, catalysts, filter aids, and charcoal, etc. Residual solvents are the impurities introduced with solvents. Impurities can originate mainly from sources that are given below.

- Starting materials and intermediates
- Impurities in the starting materials
- Reagents, ligands, and catalysts
- By-products of the synthesis
- Products of over-reaction
- Products of side reactions
- Impurities originating from degradation of the drug substance.

Classification Of Impurities

Impurities are classified into various categories depending upon their origin, composition type, and biological safety. ICH has classified the impurities in drug substances in three mainmajor categories such as Organic impurities, Inorganic impurities, and residual solvent.

- Organic Impurities
- Inorganic Impurities
- Residual Solvents

Separation And Isolation Of Impurities

Usually, it is required to isolate the impurities as the use of only instrumental methods does not characterize the impurity. Generally, the chromatographic techniques are used for isolation of impurities along with classical techniques before its characterization. If instrumental methods are used, isolation of impurities is avoided, as it directly characterizes the impurities. Often the analysis of complex materials requires, as a preliminary step that is,separation of the analyte or analytes from a sample matrix. The following methods can be used for the separation of impurities from drug substances and drug products.

- Liquid-liquid extraction methods
- Solid-phase extraction methods
- Accelerated Solvent Extraction Methods
- Supercritical fluid extraction
- Column chromatography
- Flash chromatography
- Thin-layer chromatography
- Gas chromatography
- High-pressure liquid chromatography
- Capillary electrophoresis
- Supercritical fluid chromatography

Diabetes Mellitus

Diabetes Mellitus (DM) is a metabolic syndrome occurring due to relative or absolute insulin deficiency which results in a chronically elevated blood glucose level and has polyuria, polyphagia and polydipsia. There are many types of DM discussed below, all of them present at specific age and specific conditions.

Type 1 DM is due to beta cells dysfunction from attack of auto antibodies against beta cells of pancreas, and it is destroyed by antibodies, B cells and Tcells. This type of DM presents mostly in early age of life especially in females children (**Barrington, P et al., 2011, Ali AR et al., 2009, AbuRuz S et al., 2003, Khaled E et al., 2012**). Type 2 DM occurs due to insulin ineffectiveness or resistance and becauseof that Insulin doesn't work on its own receptors to lower the glucose level.

Drug classes:

- Biguanides (metformin).
- Sulphonylureas.
- Glucagon like peptide 1 analogues: Exenatide, Liraglutide.
- Dipeptidyl peptidase 4 inhibitors: Linagliptin, Sitagliptin, Saxagliptin.
- Peroxisomal proliferator activating receptor gamma inhibitors: pioglita- zone, rosiglitazone.
- Alpha Glucosidase inhibitors: Acarbose and Miglitol.
- Meglitinides: Repaglinide, Nateglinide.
- Sodium Glucose co-transporter 2 inhibitors: Canagliflozin, Empagliflozin, Dapagliflozin.
- Amylin analogues: Pramlintide.

- Monoclonal antibodies and secondary drugs: Bromocriptine, Otelixizu- mab, Recombinant human Glutamic acid Decarboxylase 65 (rhGAD65), Succi- nobucol, Teplizumab, LY2189265, AVE0010/ZP10.

Biguanides

Metformin, Buformin and Phenoformin, Proguanil, Chlorproguanil, Chlorhex-Fidine, Polyaminopropyl biguanide and Polyhexanide.

Metformin is taken orally well absorbed from gastrointestinal tract and it is excreted from body renally unchanged. Widely used in diabetes mellitus type 2,not used in type 1 DM causes hypoglycemia because Lowers gluconeogenesisin liver, increase peripheral glucose intake by increasing insulin sensitivity and increases glycolysis, however in hyperinsulinemia during fasting Biguanides de- creases Insulin level in blood because it increases insulin induced glucose uptake, so less blood glucose will cause feedback inhibition of insulin release (**Hassan SSM et al.,1999, El-Baqary RI et al., 2013, Cheng CL et al., 2001, Zarghi A et al., 2003**).

Sulfonylureas:

1st generation: Chlorpropamide (long acting), Tolbutamide (short acting), Acetohexamide, Carbutamide, Glycinamide, Metahexamide, Tolazamide.

2nd generation: Glipizide, Glimepride, Glibenclamide, Gliburnuride, Glyclazide, Gliquidone, Glisopexide and Glyclopyramide.

It closes the potassium channel present on pancreatic beta cells membranes and so cell membrane depolarizes and Ca will go into cells and binds to insulin containing vesicles causes its degradation and causing increased release of insulin.

Glucagon-Like Peptide 1 (GLP1) Receptor Agonists

GLP 1 is physiologically released in response to oral meals. It has receptors present on many tissues throughout the body. But their effect on the gastrointestinal tract is what makes them suitable for DM type 2 (**El-Gindy A et al., 2010, Sultana N et al., 2011, Sahoo PK et al., 2008**). In Beta cells of the pancreas, GLP 1 causes increased glucose-mediated release of insulin. It also de- creases glucagon release after meals. It also slows gastric emptying.

SGLT2 receptors are found on the proximal convoluted tubules of the kidney.They are responsible for reabsorbing 90% of the filtered glucose. The drugs that inhibit this transporter (dapagliflozin, empagliflozin, canagliflozin) reduce blood glucose levels by inducing osmotic diuresis. This is especially important because the diuretic effect is limited by the amount of filtered glucose, which in turn is dictated by the blood glucose level. Because of this, SGLT2 inhibitors do not cause hypoglycemia.

Alpha Glucosidase Inhibitors

The primary MOA of AGI is delaying and inhibiting the absorption of carbo- hydrates from small intestines. AGI competitively inhibit enzymes (sucrase, maltase, iso-maltase and glucoamylase) in the brush border of the enterocytes which are involved in converting non-absorbable oligo and oligosaccharides into simple absorbable monosaccharides. By delaying absorption of carbohydrates in the gut, AGI decrease the rise of blood glucose levels around 3 mmol/L post prandial.

Meglitinides

Drugs are Nateglinide, Repaglinide, Mitiglinide. The newer class of antidiabetic drug which increasing insulin secre-tion from the pancreas by blocking ATP sensitive potassium channels. This newer class of drug includes Repaglinide and Nateglinide by binding to a recep-tor different from sulfonylureas (**Kolte BL et al., 2004, S. Patil et al., 2011**).

DPP4 Inhibitors

DPP4 inhibitors is a relatively newer group of anti diabetic medications used for the treatment of type II DM in

patients, especially those who are overweight as itis associated with weight loss. Examples of drugs included in this class are Sitag- liptin, Vitagliptin, Saxagliptin etc.

DPP4 inhibitors as the name indicates inhibit the enzyme DPP4. DPP4 is re- sponsible for the breakdown of incretins including Glucagon like Peptide 1 GLP1. Incretins are released by the intestinal cells after meals which increases insulin release from the pancreas in a glucose dependent manner (**S.D. Rathod et al., 2012, P. Shakya et al., 2010, Singhvi, K et al.,2011**).

Bromcriptine

Dopamine release in hypothalamus inhibits release of many hormones such as growth hormones, prolactin, thyroid hormones and also activates dopamine re- ceptors in basal ganglia causing movement. Similarly bromcriptine is dopaminereceptor agonist and it inhibits release of excessive amount of growth hormonesso treats Hyperglycemia of excessive growth hormones in patients of acromega-ly, similarly in patients of secondary hyperthyroidism and hyper pro lactinemia there is also hyperglycemia when these high levels of prolactin (increase insulinresistance) and thyroid hormones occur in blood for years so to prevent this hyperglycemia, Bromcriptine is used.

Otelixizumab

It inhibits auto reactive T cells by attaching to the ep- silon chain of CD3 receptors, so in high doses it decreases CD3 receptor interac- tions with MHC proteins, CCR5 and CXCR4, so it limits the progression of type 1 diabetes mellitus. So simply it decreases reactivity of T cells. It is also used in other autoimmune diseases such Rheumatoid arthritis and psoriasis.

Recombinant Human Glutamic Acid Decarboxylase 65 (rhGAD65)

Glutamic acid decarboxylase enzyme is present mainly in central nervous sys- tem, is a rate limiting enzyme for catalyzing the conversion of GABA from glu- tamate, a main inhibitory neurotransmitter for brain, but some quantity of this enzyme is present in the pancreatic beta cells.

Succinobucol

Succinobucol is taken per orally currently under process of development in clin- ical trial phase 3 for anti diabetic therapy, it is derivative of lipid lowering agent called probucol it is its mono succinate ester.

Teplizumab

It is humanized monoclonal antibody targets CD3 on T lymphocytes, reduces its signaling and so if these cytotoxic T lymphocytes are not stimulated for a long period and so it undergoes apoptosis, and regulatory T cells increases consecu- tively, so it's helpful in diabetes mellitus type which is autoimmune induced T cell mediated beta cells of pancreas damage.

LY2189265

It is a long acting GLP 1 analogue works just like incretin, lowers blood glucose by three mechanisms: decrease gastric emptying so slow digestion less food intake stimulates beta cells to release more insulin inhibits alpha cells to decrease release of glucagon. It is used as adjunct therapy with Metformin to lower the glucose levels in

Lixisenatide

A GLP 1 analogue used in diabetes mellitus type 2, once daily dosing, lowers the glycemic concentration in blood, by measuring HbA1C is lowered by 0.29% to 0.69% as compared to placebo. It is a long acting GLP 1 analogue works just like incretin, lowers blood glucose by three mechanisms: decrease gastric emptying so slow digestion less food intake stimulates beta cells to release more insulin inhibits alpha cells to decrease release of glucagon.

Analytical methods for Metformin

Metformin is an oral antidiabetic belonging to the class of biguanides used for the treatment of type 2 diabetes. It acts by suppressing the glucose production by the liver. It reduces the LDL cholesterol levels and in some people it

promotes weight loss.

Spectroscopy: Pharmaceutical preparations of metformin have been analyzed by a simple and rapid near infra-red reflectance spectroscopic method. The results of the method agreed well with thoseof the UV assay method of metformin mentioned in BP 1998. The firstspectral data was observed within the wavelength range of 1000-2500nm. For the simultaneous determination of metformin and glipizide inhuman plasma, a method has been proposed where the atmospheric pressure chemical ionization source was used as a detector.

UV Spectrophotometry: Two new methods have been developedfor the analysis of metformin. These methods have been found to be simple, specific, accurate, precise and reproducible. These methods required metformin in the range of 2-12 µg/mL and 1-12 µg/mL at 237.6and 247.4 nm respectively. These methods can be satisfactorily appliedto the pharmaceutical products.

Mass spectrometry: Metformin along with pioglitazone and hydroxypioglitazone in human plasma has been determined by HPLC-elctrospray ionization-tandem mass spectrometry (ESI-MS/ MS) method. The chromatographic run time was 4.0 min. The methodhas found to be simple, selective, robust, economical and accurate. In another method moroxydine (IS-1) was used as an internal standard and the run time was 8.0 min. The recoveries were found in the range of 96.4-112.8%.

HPLC methods: HPLC is the most widely used method for the analysis of metformin in biological fluids and pharmaceutical products. Table 2 contains the analytical parameters for the assayof metformin HCl by HPLC method.

Thin layer chromatography: Metformin alone in pure form and with glimepiride in pharmaceutical products was analyzed by a simple and selective salting-out thin layer chromatographic technique.

Potentiometric methods

Method has been developed based on the use of miniaturizedpotentiometric sensors using β–cyclodextrins for the determination of metformin in biological fluids and pharmaceutical products. Coated wire electrodes have been used and the concentration range from 10^{-6} to 10^{-1} mol/L with the detection limit of 8×10^{-7} mol/L. the method has been compared with the official spectrophotometric methods and has the advantage of simplicity, accuracy and feasibility.

Analytical methods for Pioglitazones and Glifizones

The active moiety of pioglitazone hydrochloride (PIO) (5-[[4-[2-(5- ethylpyridin-2-yl)ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione) is a thiazolidinedione, a potent and highly selective agonist forthe nuclear receptor, peroxisome proliferator-activated receptor gamma (PPAR-γ). PPARs are found in tissues like adipose tissue, skeletal muscle and liver, which are critical to insulin action. Activation of PPAR-γ modulates the transcription of a number of insulin-responsive genes involved in the control of glucose and lipid metabolism (**V. Sriram et al., 2012, Karthik, G et al., 2008, D. Jain et al., 2008**).

Electrochemical methods

These membrane sensors incorporate ion association complexes of PIO cation and sodium tetraphenylborate (NaTPB) or phosphomolyb-dic acid (PMA) or phosphotungstic acid (PTA) as electroactivematerials. Direct determination showed an average recovery of98.5%, 99.0% and 98.4% correspondingly. These sensors were applied for direct determination of PIO in some pharmaceuticalpreparations and have been used as indicator electrodes forpotentiometric titration.

Capillary electrophoresis (CE)

CE methods have excellent performance for separation of phar- maceuticals, which makes it the first-choice technique for separa- tion of stereoisomers. For PIO analysis few authors have used CE as a separation and determination technique.

HPLC

This method is used for the determination of PIO in biological samples like plasma, serum and urine.

LC–MS

The identification, isolation and characterization of potential degradation products using LC–MS, ^{1}H NMR, ^{13}C NMR, MS and IR etc.. The method consists of water:trifluoroacetic acid in the ratio of 100:0.05 (v/v) as mobilephase–A and acetonitrile: trifluoroacetic acid in the ratio of 100:0.05 (v/v) as mobile phase–B using gradient/elution (T/%B: 0/10, 12/62, 16/65,17/10) at a flow rate of 1.0 mL/min and the eluents are monitored at 225 nm.

HPTLC

It is carried out for separation on aluminum plates precoated with silica gel using the mixture of toluene:ethyl acetate:formic acid (10:3:1, v/v) as the mobile phase.

Linearity: The linearity of detector response is determined by plotting a graph with series of standard concentration range of 10- 18µg/ml with concentration on X-axis and absorbance on Y-axis at 268nm. The correlation coefficient for pioglitazone hydrochloride was found to be 0.999. This data demonstrates that the method developed showed adequate sensitivity to the series of concentrations of analyte in sample.

Precision: The precision of the analytical method was determined by analysis of multiple sampling of the same homogenous sample at 268nm.

Accuracy: Accuracy for pioglitazone hydrochloride is determined by spiking triplicate concentrations of standard solution at different levels (50%, 100% & 150%) with a known concentration of a sample and absorbance was measured at 268nm.

Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Standard 10ppm solution was scanned at two different wavelengths i.e., 267nm & 269nm.

Ruggedness: The degree of reproducibility of test results obtained by the analysis of samples under various test conditions such as within laboratories variation: different days, different analyst, different equipments.10ppm solution was scanned 6 times at 268nm by different analysts and different apparatus.

LOD & LOQ: The LOD & LOQ were separately determined and calculated based on the calibration curve of standard solution.

Conclusion

The nitrosamine contamination is a carcinogenic and mutagenic contaminant leading to cancer. TheEuropean Medical Agency and the Food and Drug Administration are strictly taking strong action against these contaminants so that these contaminants can be controlled through alternatives. The sources of these catalysts catalysts, solvent, raw materials react with nitrosating Agent lead to the formation of contaminants. Nitrosamine contamination is detected using several technologies such as Gas chromatography, mass spectrometry, liquid chromatography process. These methods help in to low level of Nitrosamine impurities in drug substance or drug product in human medicine. The Isolation and characterization of impurities are mandatory for acquiring and evaluating data that establishes biological safety, which reveals the need and scope for impurity profiling of drugs in pharmaceutical research. The recognition and regulatory contemplation of organic impurities is an extremely complex problem owing to numerous sources ranging from microbial contamination to degradation products of APIs apart from traces of intermediates.

The diabetes Mellitus is a globally progressive pandemic that affects people chronically from children to aged people. There is a need to control the disease proper early diagnosis is immensely in need followed byproper treatment is needed. The treatment strategies focus initially on diet, exercises, followed by use of drugs. The different analytical methods can be utilized for the determination of metformin and other anti diabetic drugs (Charles BG et al.,1981, Aburuz S et al., 2005, M.B. Shankar et al., 2005, G. Nirupa et al., 2013). A broad range of techniques are available for the analysis of PIO in biological samples and pharmaceutical formulations. The HPLC is extensively used for the determination of PIO in various matrices like plasma, serum and urine. For determination of PIO in biological samples, we recommend the HPLC–MS/MS method, since this method combines the HPLC separation ability with MS sensitivity and selectivity, allowing the unambiguous identification of PIO and its metabolites. For analysis of

pharmaceuticals, HPLC with UV detection is applicable because this method provides accurate results and low cost compared to more advanced detection techniques.

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