N-nitrosodimethylamine Genotoxic impurity in Valsartan products and its analytical methods for determination of NDMA.

Priyanka Dilip Mandlik[1], Sarita Pawar[2], Vrushali Khadangale[3] ,Bhushan Nalkar[4] ,Sumit Marathe[5]

1. Department of Quality Assurance(PG) , Sanjivani college of Pharmaceutical Education and Reasearch Kopargaon ,Maharashtra -423603,India.

2. Department of Quality Assurance , Sanjivani college of Pharmaceutical Education and Reasearch Kopargaon ,Maharashtra -423603,India.

3. Department of Quality Assurance(PG) , Sanjivani college of Pharmaceutical Education and Reasearch Kopargaon ,Maharashtra -423603,India.

4. Department of Quality Assurance(PG) , Sanjivani college of Pharmaceutical Education and Reasearch Kopargaon ,Maharashtra -423603,India.

5. Department of Pharmaceutics (PG) , Sanjivani college of Pharmaceutical Education and Reasearch Kopargaon ,Maharashtra -423603,India.

Abstract :

In Valsartan drug products impurity have been found such as N-nitrosodimethylamine (NDMA) which is Carcinogenic in nature and also produce toxic effect to the Human being and also to the animals ,so that action taken by the regulatory agencies .batch recalls for valsartan containing drugs products in July 2018 .It was found that NDMA was produced during synthesis of API means active pharmaceutical ingredient from the reagent nitrite and solvents dimethylformamide. This review summarizes various methods for determination of NDMA these are HPLC-MS/MS, Liquid chromatography /mass spectroscopy ,High resolution LC-MS/MS ,GC-MS/MS .also this review focus on valsartan synthesis routes ,properties , valsartan recalls ,NDMA risk assessment tools ,ICH M7 guidelines ,Valsartan impurity profiling .

Key words: NDMA N-nitrosodimethylamine, recalls, carcinogenic, risk assessment, ICH M7guidelines, valsartan synthesis route.

Introduction:

In July 2018 contamination of Valsartan containing drugs batch recalls because N-nitrosodimethylamine (NDMA) found in the drugs as impurity which is Carcinogenic in nature .It was discovered in the batch which has been produced by Chinese company Zhejiang Huahai Pharmaceutical(1) . The product recall involved nearly 2300 batches(2) that had been dispatched to Germany, Norway, Finland, Sweden, Hungary, the Netherlands, Austria, Ireland, Bulgaria, Italy, Spain, Portugal, Belgium, France, Poland, Croatia, Lithuania, Greece, Canada, Bosnia and Herzegovina, Bahrain and Malta.

. EMA indicated that the impurity of concern (N-nitrosodimethylamine i.e NDMA) had formed as “a result of a change in the manufacturing process”(2) .In September 2018, EMA(3) stated: “Medicines containing valsartan made by Zhejiang Huahai in China have been recalled by national authorities. Medicines containing valsartan from another company Zhejiang Tianyu are no longer being distributed in the EU.” In July 2018 the Danish Medicines Agency (4) recalled a variety of valsartan containing products .According to IARC (International Agency for Reasearch on Cancer) N-nitrosodimethylamine is Genotoxic ,Carcinogenic .and

also by according to ICH M7N-nitrosodimethylamine is DNA reactive mutagenic impurity which is highly potency carcinogens also called as cohort of concern.(5)

Material and Methods:

Structure of valsartan and NDMA contamination in valsartan(6)

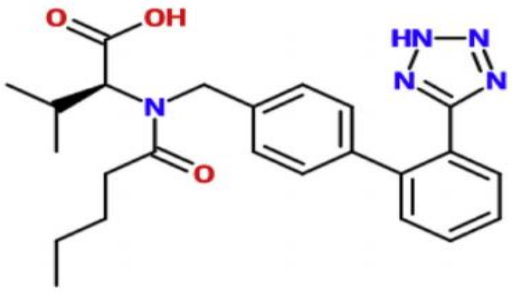
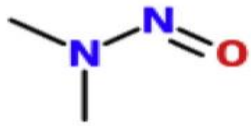
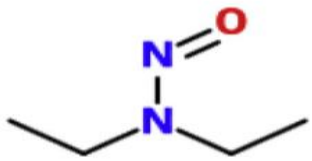


Fig. 1. Structure of valsartan.

Table 1
Typical levels of NDMA Contamination in Valsartan tablets (FDA, 2018a).

Supplier	Product	NDMA Levels (µg/ tablet)
Prinston Pharma	Valsartan 320 mg tablets	15–16
Prinston Pharma	Valsartan 320mg/HCTZ 25 mg tablets	13–20
Torrent Pharma	Valsartan 320mg/Amlodipine 10mg/ HCTZ 25 mg	10–12
Torrent Pharma	Valsartan 320mg/Amlodipine 10 mg	5–9
Teva Pharma	Valsartan 320 mg tablets	8–17
Teva Pharma	Valsartan 320mg/HCTZ 25 mg	7–10
Hetero Labs Ltd	Valsartan 320 mg tablets	0.3–0.4





N-nitrosodimethylamine (7)

Valsartan is angiotensin Ⅱ receptor antagonist which is used in the congestive heart failure , hypertonia ,myocardial infraction .

1)caicinogenecity :

N-Nitrosodimethylamine caused tumor in the rats ,mice ,pig ,hamsters ,guinea ,rabbit ,frog ,fish newts which is caused tumor of liver ,kidney ,respiratory tract ,blood vessels(8) , Malingnant and benign tumors of liver or bile(9) duct found in the :

Due to inhalation of NDMA caused lungs tumor

a) oral administration in the rat ,mice rabbits ,pigs ,fish ,guinea ,hamsters
b) Prenatal exposure in mice

c) Subcutaneous administration in the Hamsters and newborns
d) Intramuscular injection in rats
e) Exposure due to tank water in the frog and fish

2) Properties :

N-Nitrosodimethylamine is a Nitrosoamine compound is yellow liquid at room temperature .It is soluble in the water ,chloroform ,vegetable oils ,alcohols , Methylene chloride .which is less soluble in the more acidic solutions.(10)

Properties	Information
Molecular weight	74.1a
Specific gravity	1.0048 at 20°c/4°ca
Melting point	<25 °c b
Water solubility	1.000g/L at24°c b
Boiling point	151°c to 153°ca
Log K o w	-0.57a
Vapour density relative to air	2.56a
Vapour pressure	2.7 mm Hg at 20°c b

Table no : 1 Properties of NDMA .

3) Uses:

0.1%N –nitrosodimethylamine is an impurity(9) .N-nitrosodimethylamine use in the control of nematodes, in active metal anode –electrolyte system ,as plasticizer for rubber and acetonitrile polymers ,to inhibit nitrification in soil ,as a solvents in the fiber and plastics industry,in the preparation of Thiocarbonyl fluoride polymers ,as an antioxidant ,a softner copolymers and as lubricants.

4) N-Nitrosodimethylamine impurity limits:

According to the International council for the harmonisation of technical requirement for the pharmaceutical human use (ICH)for Valsartan 320 mg/d a reporting threshold of 0.05 %, an identification threshold of 0.10 %, and a qualification threshold of 0.15 % is reported (11). However, there are lower limits for (potentially) carcinogenic impurities lying far below the identification threshold (NDMA: 96 ng/d i.e. 0.30 ppm , NDEA: 26.5 ng/d i.e. 0.082 ppm; when no carcinogenicity data is available: 1.5 µg/d i.e. 4.7 ppm)(12,13) daily intake of 96 ng/d for NDMA and 26.5 ng/d for NDEA associated with this risk level. This would correspond to 0.3 ppm for NDMA and 0.08 ppm for NDEA in valsartan 320 mg tablets. How these impurities came to present in manufacture of sartans yet to be fully established such as valsartan.

NDMA detected in the food(14) and drinks having cut off value 5g/L(15) .in the ground drinking water with maximum concentration level in the lower ng/L(16) rang .Additional contamination of water NDMA can occurs and so that WHO i. e World health organization has set the drinking water guideline values to the100ng/L(17) .according to the U.S. Environmental Protection Agency (EPA) set limit in the guideline EPA screening level of 0.4ng/L in the tab water .(18)

Valsartan in 2018 regulatory agencies including US FDA and the European medicine agency (EMA)have issued allowable limits of Genotoxic impurity in the pharmaceutical product to ensure their safety is 1.5µg per day .for valsartan standard daily dose of 80mg /day .(19)

Required LLOQ < 1.5µg allowed nitrosoamine/0.080g daily dose
Required LLOQ < 18.75 µg/g

5) Formation of N –Nitrosodimethylamine impurity as follows:

In the synthesis of valsartan (S)-valin methyl ester and 4′-(bromomethyl){1,1′-biphenyl }-2-carbonitrile or 2 –cyano -4-formylbiphenyl .which is final step formation of the tetrazole moiety is formed by the reaction with azidotributyltin (20) formation of the tetrazole ring from the cyano intermediate and biphenyl coupling by using an activated tetrazole as aduct .and also by the use of sodium nitrite (NaNO2) in product generation and azide removal (21,22,23) .In the Zheijing Huahai Pharmaceutical(24) shows the formation of tetrazole by using anhydrous zing chloride and sodium azide (NaN3 in the polar solvents where(DMF) dimethylformamide followed by the quenching with NaNo2 DMF have a limited stability which may be have result in traces of dimethylamine and also shows formation of NDMA(25) which is carcinogenic in the nature and toxic agent .

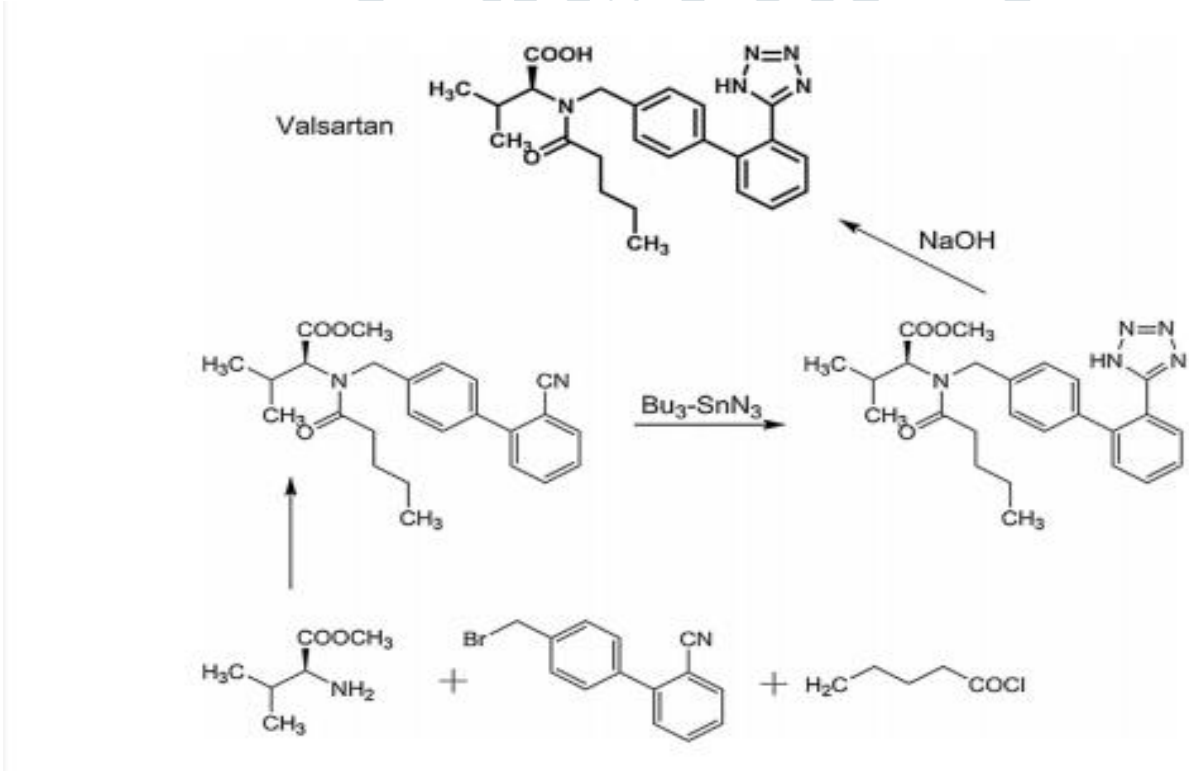


Figure 2 : Synthesis pathway of valsartan and its structure.(20)

6) Analytical methods for the determination of NDMA:

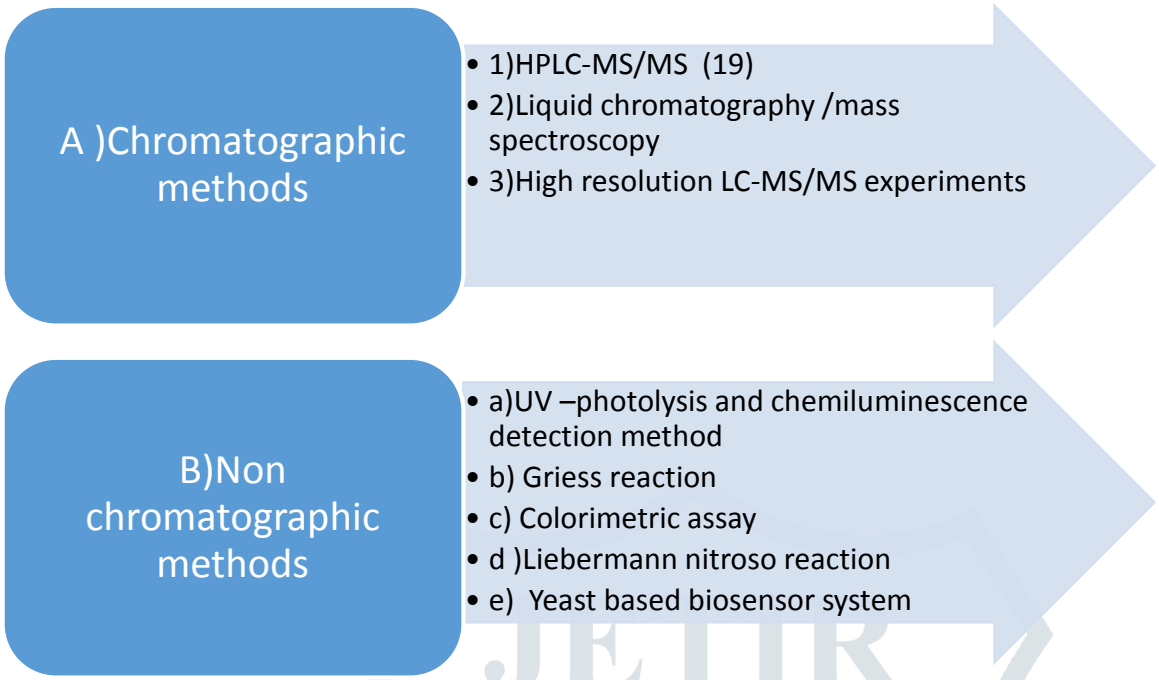


Figure 3: Analytical methods for the determination of NDMA

A) Chromatographic method :

1) By HPLC-MS /MS (19)

a)System:

The SCIEX Triple Quad 4500 system is sensitive and robust for determination and quantitation EXionLC system pair seamlessly with SCIEX mass spectrometers providing a complete LC-MS/MS solutions .

b) Methods :

sample preparation :

Samples were prepared by the 80 mg capsule dissolve in the 40 ml of 1:1 Me OH : water to get concentration 2mg/ml .This mixture was Sonicated for 20min .the solution allowed to settle for one hours at a room temperature and then centrifuge for 5min at 14k RPM then this solution was transferred in the to the HPLC vials for LC-MS /MS .then find the rang and then make final concentration 0.1,02,1.0,2.0,5.0,10,20ng/ml .the 0.1ng /mL in the extract equivalent to the 0.05µg/g in the tablet .

c) Analytical conditions:

ASCIEX EX ionLC and SCIEX triple Quards 4500 system were used for analysis if NDMA with a Phenomenex kinetex F5,2.5 µm,50×2.1mm HPLC column for the separation .

Mobile phase	**Mobile phase A:** water with 0.1% formic acid **Mobile phase B** :Methanol
Column oven temperature	40°c
Total flow rate	0.5ml/min
In the mass spectrometer Nebulizer	
Current	3µA
Curtain gas	30psi
GS1	35psi
CAD	8
Temperature	350°c

Table No 2: Analytical conditions for HPLC-MS /MS

HPLC gradients :

Time	**Buffer**	**Buffer**
0.0	85	15
0.5	85	15
5.0	5	95
5.1	85	15
6.5	85	15

Table No 3 : HPLC gradients

d) Results:

Linearity :

Working standard solution were prepared by the preparation of stock in the sample dilution buffer to 0.1,0.2,5.0,10 and 20ng/mL.The liner dynyamic rang from 0.1ng to 20ng / mL. Were correlation coefficient > 0.998

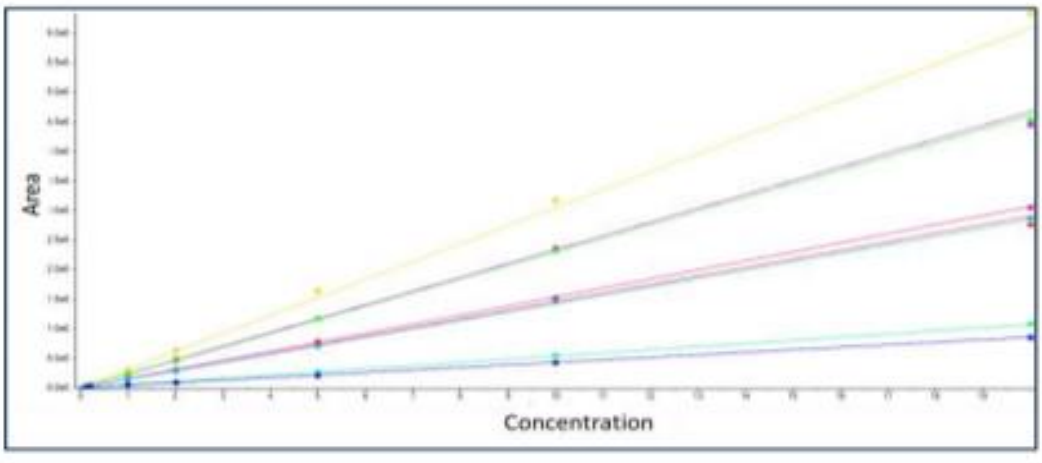


Figure 4: Calibration curves for eight Nitrosoamine compounds.

Specificity :

By dissolving standard 2mg/mL and then spiked with the 0.5ng/mL ,5.0ng/mL and 15ng /mL to make 0.25,2.5,and7.5µg/g API samples and the additional samples are prepared by the by dissolving a sultan capsule to a 2.0ng /mL concentration the prepared sample where analyzed by the LC-MS/MS .The NDMA where quantitated to 0.2ng/mL, the equivalent of 0.1µg/g the product which is below the threshold of

toxicological value.

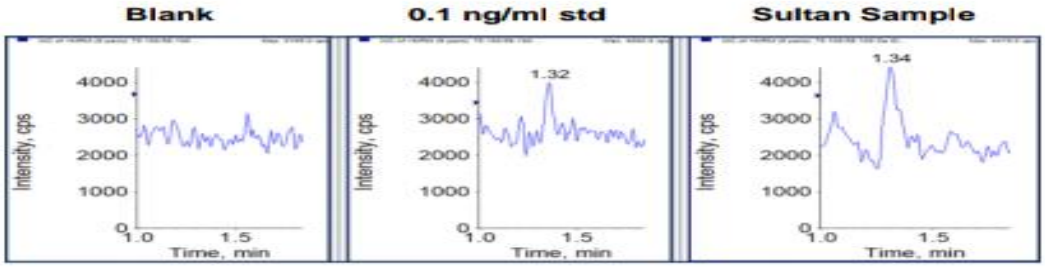


Figure 5: Sultan capsule analysis .

Recovery and reproducibility :

Compound NDMA	%Recovery	%RSD
0.5ng/mL	92.9	2.34
5.0ng/mL	90.4	2.68
15.0ng/mL	89.5	1.44

Table no 4 :Recovery and reproducibilityLLOQ reproducibility:

LLOQ Reproducibilty of NDMA was found to be 2.11% RSD.

2) Liquid chromatography /mass spectroscopy(26)

A SCIEX X500R Qq TOF system with a turbo V™ source was used for information dependent analysis (IDA) and DIA the separation was carried out by the EXionLC HPLC system containing binary gradient ,pump, autosampler ,column oven ,stationary phases ,a synergy polar 100×2mm column with 2.5µm and a luna omega polar 100×2mm,1.6µm were used in the gradient separation for IDA and DIA respectively.

Mobile phase	Flow rate	Run time	Injection volume
0.1% of formic acid (mobile phase A): Methanol (mobile phase B)	900µ/ min	-	10µL
60%A which was kept for 0.5 in .in 4.5 min was decreased to 0% and kept for 1.5	600µL	8.5	3µL
90%A was kept for 0.1 min .in 4.9 min , a was decreased to 0% and kept for 2 min	-	7.5	-

Table no 5 : Chromatographic conditions for Liquid chromatography /mass spectroscopy

Mass calibration and tuning was achieved using the integrated calibrant delivery system (CDS) with the Twin Sprayer probe (dual APCI needle). The QqTOF system was tuned to a resolution of over 27,000 at m/z 266 and over 40,000 at m/z 922 with a mass error of less than 2 ppm. For best MS/MS coverage, Dynamic background subtraction (DBS) was activated with an intensity threshold of 10 counts per second. High purity

nitrogen gas was used as nebulizer, curtain, auxiliary, and collision gas. Variable SWATH window sizes were used . The window size was optimized by using SWATH acquisition variable window calculator tool.

3) High resolution LC-MS/MS experiments:

a) Sample preparation:(27,28,29)

Take 5 tablets weighed accurately and then crush tablets in the tablet 300-600 mg sartan (irbesartan ,valsartan ,eprosartan in case of one tablet),two tablets in case of 16-100mg sartans (azilsartan,condensaratn ,losartan,olmesartan,telmisaratan ,valsaratan)one of the ground portion was accurately weighed in a 1.5mL centrifuge tube and dissolved in the 500µL of Methano l,then mix shake and then sinicate for 5 min and then suspension was diluted to final volume of 1000µL MilliQ®- water then shake for 5 min and then sonicate for 5 min the sample were centrifuged at 9727 g for 35 min at 4°c.then sample were transferred to amber colour glass vials.

4) GC-MS/MS:(30)

a) Instrument :

Gas chromatography wirh liquid auto sampler and a triple Qudrupole mass selective detector class A glass ware centrifuge ,VF-W A X ms GC column 30m×0.25mm ,1.00µm vortex mixer ,15mLdisposable glass centrifuge tubes 0.45µm Nylon filters 5mL Syringes.

b)Standard preparation:

NDMA 1µg/mL standard stock solution :

Utilizing a 100 µL gas-tight syringe, transfer 200µL of NDMA stock standard to a 20 mL volumetric flask containing approximately 18mL of IS. Add 20µL of NDEA std via a 100µL gas-tight syringe. Dilute to volume with IS and mix well.

NDMA Standard	Dilutions
NDMA 100ng/mL Standard (Std 1)	1:10 dilution of Standard Stock with IS utilizing class A glassware.
NDMA 10ng/mL Standard (Std 2)	1:10 dilution of Std 1 with IS utilizing class A glassware.
NDMA 5ng/mL Standard (Std 3)	5:10 dilution of Std 2 with IS utilizing class A glassware.
NDMA 2.5ng/mL Standard (Std 4)	5:10 dilution of Std 3 with IS utilizing class A glassware
NDMA 50ng/mL Standard (Std 5)	5:10 dilution of Std 1 with IS utilizing class A glassware
NDMA 25ng/mL Standard (Std 6)	5:10 dilution of Std 5 with IS utilizing class A glassware.
NDMA 80ng/mL Standard (Std 7)	2:25 dilution of Standard Stock with IS utilizing class A

Table no 6 :NDMA standard preparation

c) Sample preparation for drug:

By using pill cutter ,quarter one tablets place the pieces into 15mL glass centrifuge tube .add 5mL of IS by the volumetric pipette .cap tube vortex sample for 1 min and then tablet placed in the centrifuge rotate at 4000 rpm for 2.5 minutes using disposable pipette take 2mL of MeCI2 layer to a 5mL syringe then filtered with nylon filter 0.45µm .filter sample approximately 0.5mL sample into 2 ml vial .

d) Chromatographic conditions:

Gas chromatography (GC) Conditions	
Inlet temperature Transfer line temperature	250°c 250°c
Injection volume	2µL
Injection type	Pulsed Splitless : 12.285psi until 0.5min
Oven temperature	40oC for 0.5min□200oC at 20oC/min□250oC at 60oC/min and hold for 3min
Flow rate	1mL/min
Run time	12.33 min
Mass spectrometer (QQQ) conditions	
EI sources temperature	250°c
Quard 1 temperature	150°c
Quard 2 temperature	150°c
QQQ stop time	8.5min
NDMA MRM start time	4.00min
Helium quench gas	4mL/min
Nitrogen collision gas	1.5m/min
Solvent delay	6.5 min
Electron energy	-30Ev
NDMA MRM: MS1 and MS2 Resolution	MS1: Unit MS2: Wide

Table no 7: Chromatographic conditions for GC

Calculation:

Plot the response factor of the NDMA and NDEA peak areas to the IS peak area against the standard concentration (ng/mL). Determine the intercepts, slopes and coefficients of determination for each linear curve. Calculate the NDMA and NDEA impurities (ppm) using the formula below:

$$(ppm) = [(y - b) / m] \times EV \times 1\mu g/1000ng \div wt.$$

where: y = NDMA or NDEA to IS response factor

b = intercept of the linear curve

m = slope of the linear curve

EV = Extraction Volume = 5 mL

wt. = Valsartan API weight (g)

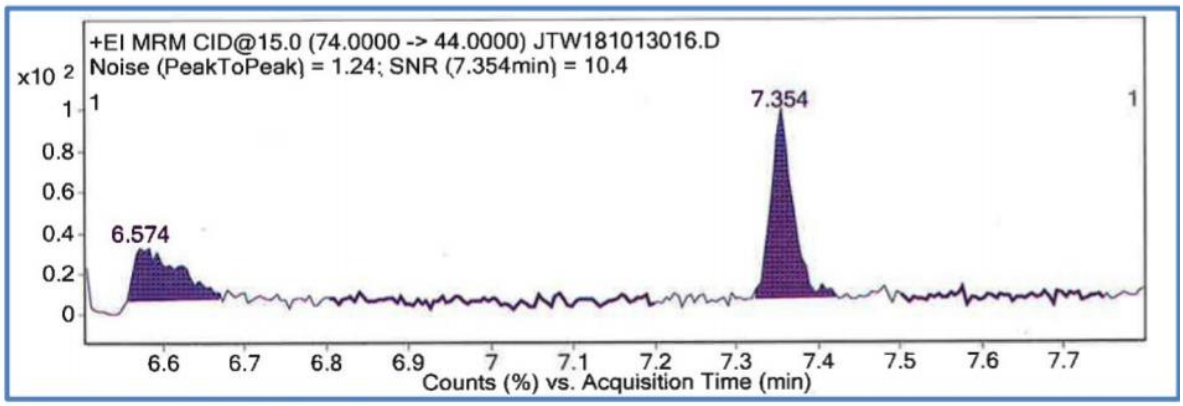
Report any NDMA peak ≥ 0.3 ppm and any NDEA peak ≥ 0.08 ppm

System suitability:

The coefficient of determination (R2) of the linear curves should be be ≥ 0.998. The S/N ratio of the 5 ng/mL linearity standard should be ≥ 10.

Chromatograms:

NDMA LOQ (0.05ppm)



*The peak at 7.354min is NDMA.

Figure 6:Chromatogram of NDMA .

e) Conclusion:

Impurity	**Drud substance limit of Quantitation (LOQ),ppm**	**Drud product limit of Quantitation (LOQ),ppm**
N-nitrosodimethylamine(NDMA)	0.05	0.08
Impurity	**Drud substance limit of Detection (LOD),ppm**	**Drud product limit of Detection (LOD),ppm**
N-nitrosodimethylamine(NDMA)	0.010	0.015

Table no 8 :LOD and LOQ

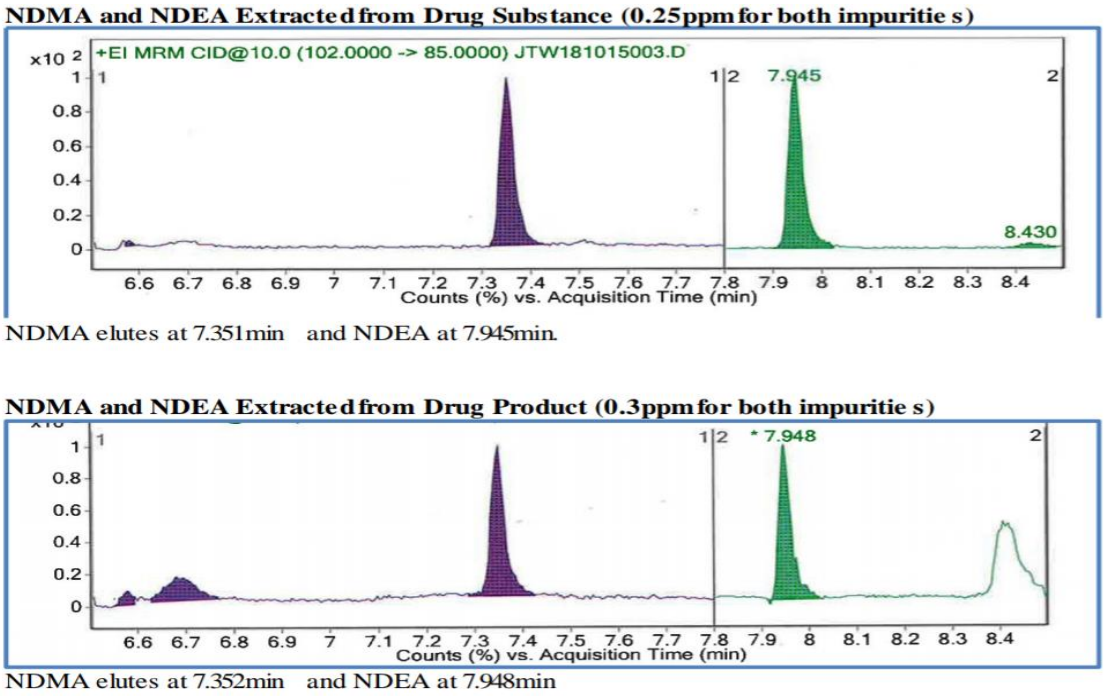


Figure 7: Chromatogram of NDMA.

B) Non chromatographic method :

a)UV –photolysis and chemiluminescence detection method :

In the water(32) NDMA were determine by the UV –photolysis and Chemiluminescence (31) detection and then spectrophotometry after photolysis .

b) Griess reaction:

This reaction shows formation of NO2- with sulphonamide and also N-(1-naphthyl) ethylenediamine(39) in concenterated hydrochloric acid(33) was used in the food industries .so that generation of NO2-,NO3- ions vary with photolysis conditions in the NDMA analysis .which is based on the amount of NO2- were photo produced (34) .this method was only used when there is high concentration of NDMA µg/mL.

c) Colorimetric assay :

Cleavage of the nitrososamine using HBr in acetic acid it gives nitrite which can be detected by the colorimetry .In the colorimetric assay(37) detection of the final product was determine by the formation of coloured compound from nitrite .which is a Viole- purple color complex when reaction with PdCI2(38) and diphenylamine (Preussmann reagent).This method based on the Eisenbrand preussman reaction (35,36) .In the general colorimetric assay were reported to relatively high limit of detection with 0.1µg of NDMA

d)Liebermann nitroso reaction :

when reaction with Phenol to p-nitrosophenol reaction with a second phenol to a blue quinon –imine (40) or reaction with NEDSA (41) reagent .The latter was reported by Fan and Tanenbaum (41)

e) Yeast based biosensor system :

In the 2005 ,Walsh et.al (42) by using yeast based biosensor system for the determination of carcinogen and procarcinogens.This is generally based on the genetically modified yeast strain that can cause DNA damage (RAD54-GFP) .for the activation of procarcinogens modidfied strains with additional cytochrome P450(CYP) enzyme were also introduced .and then this is tested for the detection of NDMA gives positive signal at a concentration of1.6mg/L (43) used for similar system and also found positive signal at a concentration of 3mg/L.

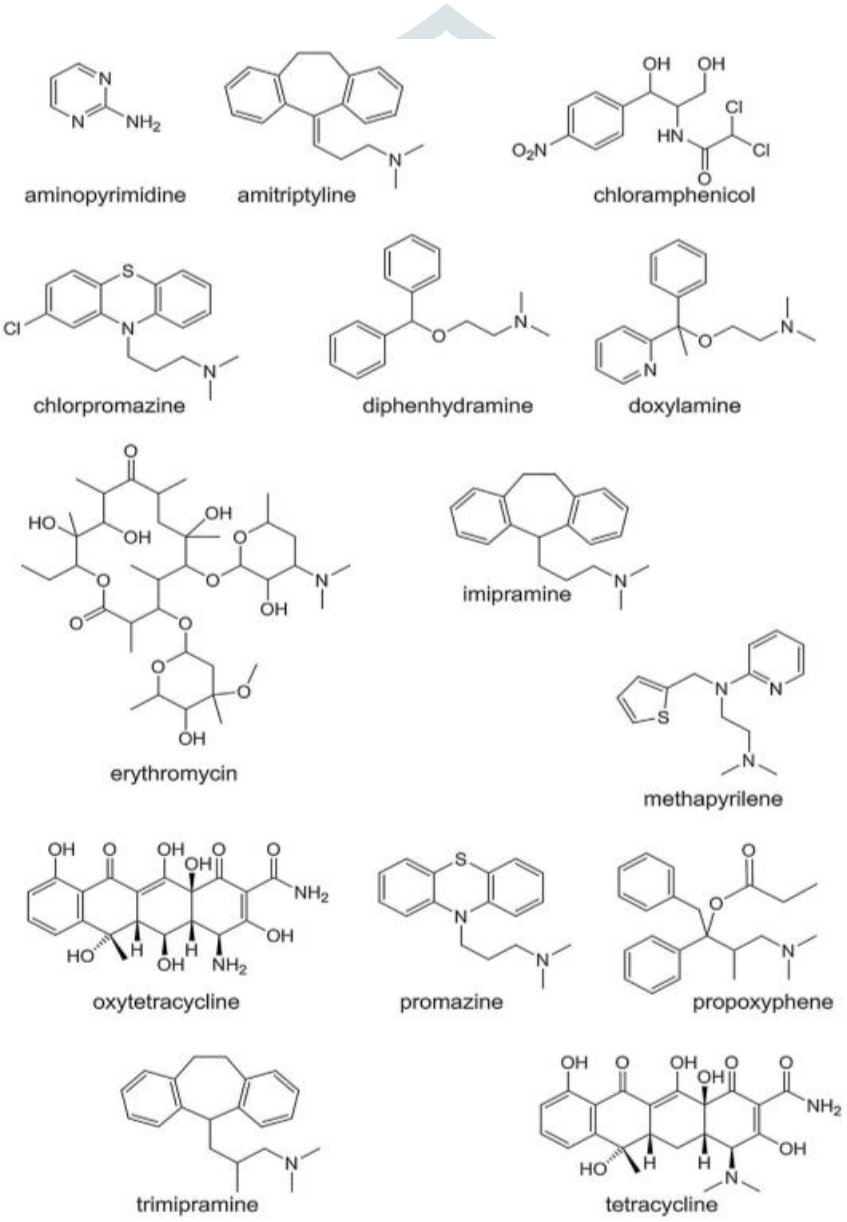


Figure 8 :Chemical structure of API containing NDMA(44)

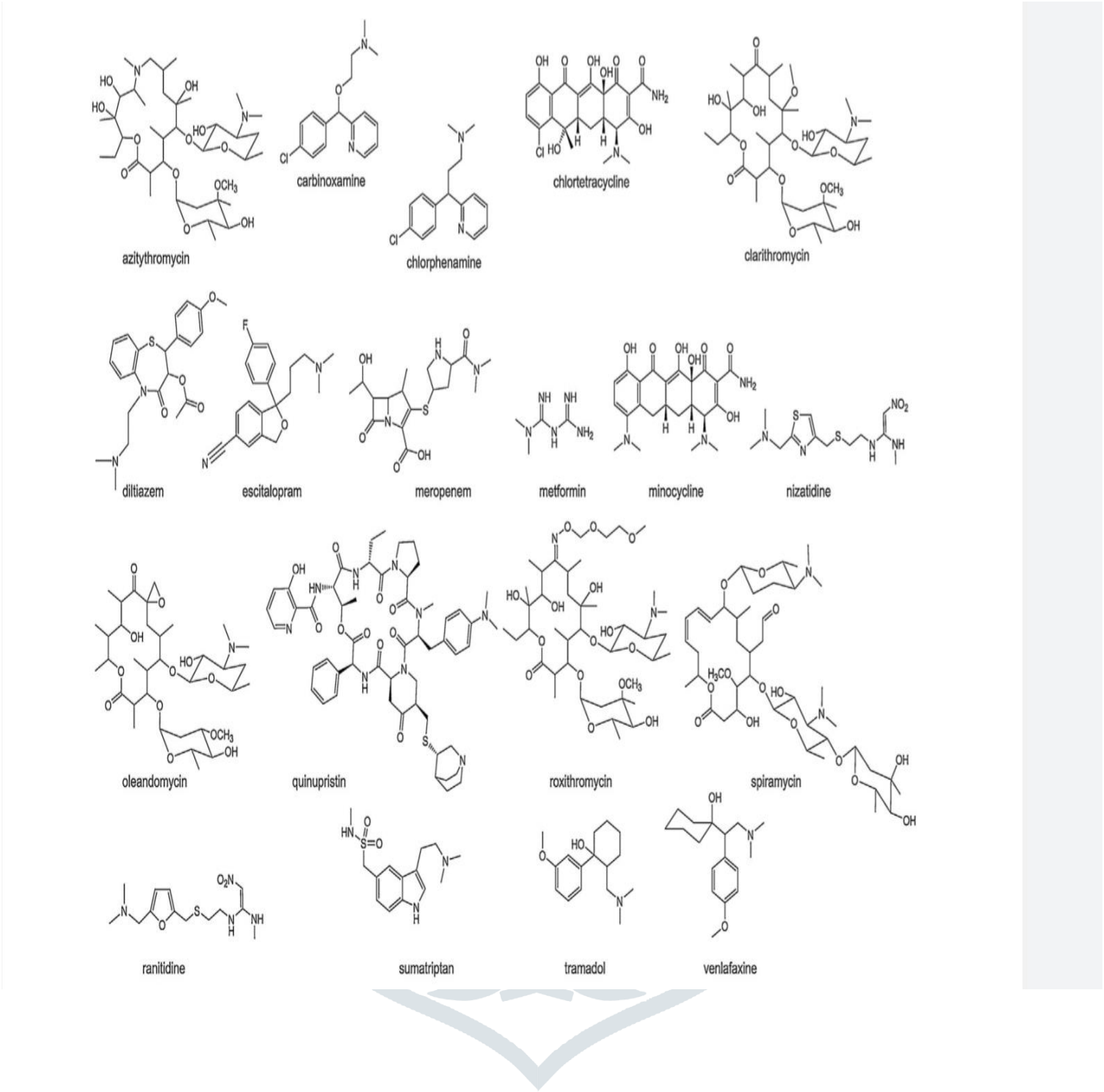


Figure 9: Chemical structure of additional precursors .(44)

8) Valsartan impurity profiling :

a) General unknown comparative screening (GUCS):

Valsartan tablet samples and different generic brands and originators were analysed using the GUCS approach. GUCS allows the comparison of a reference, e.g. 'gold' standard versus one or multiple samples by selected features. The workflow comprises the collection of LCHRMS/MS data using IDA or DIA and extracting MS chromatograms from that data. From each of these extracted ion chromatograms (XIC), peak intensity, mass spectrometric data, and retention time information was compiled to compose a peak list for every sample. In the next step, the relevant features distinguishing the samples from each other were calculated by comparing the peak data of each sample with the peak list from the 'gold' standard. The peaks

and mass spectrometric data contained in those features most correspond to contaminants or excipients that distinguish the drug products from each other. At the same time, the collected HRMS data was useful for the identification of the underlying chemical compound. Sum formulae were calculated from isotope distribution and exact mass of the pre-cursor, whereas fragment spectra were fed into a database we used Chem Spider database (45) to calculate putative structures for the unknown compounds**.**

b) Valsartan product differentiation (46)

Valsartan tablets sample of different generic brands are analysed by performing five replicate injection using the DIA .the peak aeration between the originator and generic for peak detected

7) ICH M7 guidelines on mutagenic impurity:

ICH M7 was firstly introduced by the Safety working party (SWP).in the EU guideline on genotoxic impurities .which is Threshold toxicological concern (TTC) .TTC were define as an appropriate intake for any chemical with limited or no supporting safety data that would be show a minimum risk of carcinogenicity .A TTC value of 1.5µg/day .where carcinogens referred to as the cohort of concern(COC) .Example of cohorts of concern are as follows : aflatoxin –like, N –nitroso-, and alkyl-azoxy compounds ,benzidine derivatives. Indusry had always argued that cohorts of concern structural classes were very unlikely to be encounter during the routine synthesis of the pharmaceutical drug substances(47) highlighted that the TTC which are potent carcinogenic concern was, "(a) unduly influenced by many classes of potent carcinogens of historic concern which would be impossible to generate unknowingly as pharmaceutical impurities, and (b) that the majority of reactive chemicals that would be useful to synthetic chemists are among the least potent carcinogens in the underpinning supportive analyses".

Indeed, the N-nitroso structural class is significantly over-represented in the compound libraries that underpin the TTC, with over 100N-nitroso compounds, ca. 14% being present in the Kroes (47), and Cheeseman databases (48). If carcinogenic potency data are available, as is the case for NDMA and many other N-nitrosamines in the CPDB (Carcinogenic Potency Database(49) (The Carcinogenic Potency, 2018), it is possible to determine compound specific acceptable intake (AI) limits using established methodology (ICH M7)(50)in the SWATH were calculate and then subjected to PCA after scaling and then centering was conducted to clusters sample based on their MS/MS ALL profile and then to find distinguish differences between sample by inspecting the loading plots .

9) Recalls of sartans :

Saratans are contaminated with N-nitrsoamine as such a product recalls based on the cohorts of concern such as impurity i.e NDMA that appeared be unknowingly generated .The initiall EMA(51) recalls was rapidly followed by FDA product recalls (52) and a similar action in the India(53) at the end of August 2018 , valsartan drugs from sixteen different supplier to the US market has been implicated (54) .after this EMA extended the review contains sartans such as olmesartan ,candensartan , irbesartan ,losartan was prompted by the detection of of very low level of N-nitrosodimethylamine .overall NDMA has been detected in the valsartan produced by several API manufacturers .

Impact of valsaran recalls from India after this news contamination of valsartan with NDMA on July 20,2018 the Drug controller General of India has initiated a prob into all companies .the important importing raw material of valsartan was produced by the Zhejiang Huahai .the central drug standard control organization has taken action and then all imported products where tested before they are taken up for the manufacturing the heathcare profession has been put on high alert. Novartis, India one of the companies that manufactures the product has initiated recall of specific batches of valsartan that contains NDMA. Despite the initiation of investigation into the issue, no stringent action has been taken to recall drugs containing valsartan in the Indian market. It to be mentioned Valsartan manufactured by two Indian Pharmaceuticals Hetero Pharma and Torrent pharmaceuticals in banned in USA due to impurity.

10) Risk assessment for NDMA :

It seems reasonable to ask what constitutes a virtually safe dose (VSD) for NDMA, particularly as the substance is a known environmental contaminant that is routinely found in foodstuff s dairy products and vegetables and also drinking water. Intake levels range from 0.0004 to 0.23µg in cured meat, 0.0004–1.02µg in smoked meat and 0.0006–0.13µg in grilled meat and 0.07–0.07µg in bacon (55). Based on a daily consumption of ca. 20g of processed meat per day, Danish researchers found that this resulted in a daily of exposure for adults of 33 ngkg/bw/day of nonvolatile nitrosamines and 0.34ngkg/bw/day of volatile nitrosamines these are latter class would include NDMA and NDEA (56).

NDMA can also be formed endogenously following consumption of nitrate-containing food stuff s (57). NDMA is an unintended by-product of the chlorination of waste waters and drinking waters in chemical processes that use chloramines as the disinfecting agent (58) . Inhalation and dermal exposure can occur in several industries . FDA has indicated that levels of NDMA up to 0.096µg/day, i.e. 0.1µg/day are safe, which is equivalent to 0.3ppm (0.096×1000/320) in Valsartan tablets (59) . A compound-specific assessment using ICH M7(R1)2,3 methodology also produces a value of 0.096µg/day for whole lifetime exposure based on the CPDB harmonic-mean TD50 of 0.096mg/kg/day (60).

Another risk assessment by Fitzgerald and Robinson based on a comprehensive lifetime liver-cancer dose-response study in rats (listed in the CPDB) produced TDI(tolerable daily intake) in the range 0.2–0.6µg/day, based on a bodyweight of 60kg(61) Since exposure via pharmaceuticals is unlikely to last more than a few years the ICH M72,3 less-than-lifetime (LTL) approach can be applied to the most conservative value of 0.096µg/day resulting in limits of 10/1.5×0.096=0.64µg/day if exposure is≤10 years and 20/1.5×0.096=1.28µg/day if exposure is≤1 year(62) .

Assuming that use of existing supplies of potentially contaminated valsartan is sanctioned by regulatory authorities for up to one year then the above risk assessment would lead to a limit of 4.0ppm NDMA in valsartan. Alternative risk-assessment metrics are permitted under ICH M7 (R1)2,3 which states: ".other established risk assessment practices such as those used by international regulatory bodies may be applied either to calculate acceptable intakes or to use already existing values published by regulatory authorities." One such alternative metric, sometimes called "reference point" or "point of departure – POD" is the BMDL10 – benchmark dose lower bound corresponding to a 10% increase in tumour incidence(63) . Specialist software is available that enables modelling of dose-response producing a fitted dose-response model and estimation of BMDs (small butmeasurabletreatment-relatedchanges,usuallysetat5or10%)with confidence intervals.

When dealing with genotoxic and carcinogenic impurities, EFSA (European Food Safety Authority) has concluded that: "a margin of exposure of 10,000 or higher, if it is based on the BMDL10 from an animal carcinogenicity study, and taking into account overall uncertainties in the interpretation, would be of low concern from a public health point of view", where margin of exposure (MOE) is the ratio of BMDL10 to estimated consumer intake. In relation to NDMA, SCCS (Scientific Committee on Consumer Safety) has determined a BMDL10 of 27µg/kg/day (64), equivalent to 1620µg/day in a 60kg consumer.

10) Change to synthetic route:

Zhejianjg Huahai modified the chemistry during 2011or 2013(65) in this by replacing tributyltin azide with the more reactive sodium azide as reagent used in the formation of a tetrazole ring structural moiety in which introduction of NaNO2 to remove excess azide reagent .under acidic condition nitrite can also forms nitrous acid .Impurity in the solvents DMF is dimethylamine but also dimethylamine reacted with nitrous acid it gives NDMA .

Also there is risk producing procedures from various companies in that diazonium generating reactions such as Sandmyer ,Bachmann, Gomberg ,Balz-Schiemann or in this particular case the use NaNO2 to remove

excess of azide in this aliphatic secondary amine becomes as impurity .thus formation of N-nitrosamine by product .During risk assessment process EU regulators have long identified potential presence of class 1 solvents for example toluene ,acetone,alkyl alchohols , methanol ,ethanol ,it is disappointing that risk assessment process failed to identify aliphatic amines as potential impurity in the DMF .

11) Risk assessment for NDMA :

In the routine NDMA was found in the foodstuff ,dairy milk products ,cured meats and drinking water .intake level rangs are as follows:

Food products	Level of intake
Cured meat	0.0004-0.23 µg
Smoked meat	0.0004-1.02µg
Grilled milk	0.0006-0.13 µg
Bacon	0.07-0.07 µg

Table no :9 Level of intake of NDMA by food (65)

Conclusion:

This review contains total information about N-nitrosodimethylamine impurity present in the Valsrtan drug Which gives carcinogenic and toxic effect .The objective of this paper to provide various analytical methods for determination of NDMA such as HPLC-MS/MS, Liquid chromatography /mass spectroscopy ,High resolution LC-MS/MS ,GC-MS/MS which gives .accurate ,sensitive of chromatographic methods for determine NDMA impurity in the Valsartan. .

References:

1) U.S Food and drug administration ,FDA updates on Valsartan recalls ,US food and drug administration ,2018

2)Christensen ,J 2018 access on the 7th Ocober 2018 CHMP ,2018 overall rapporteur's preliminary assessment report .referral under article 31of directive 2001/83/EC .Angiotensin-Ⅱ receptor antagonist .

3) EMA 2018 b update on medicine containing Valsartan from Zhejiang Tianyu : company no longer authoriesd to manufacture valsartan active substances for EU medicines due to presence of NDMA press release 20/08/2018.

4) Danish medicines agency , 2018 recall of the valsartan blood pressure medicines press release 5 July 2018 , accessed date 10 August 2018.

5) ICH M7 ,2017 .Assessment and control of Dna reactive mutagenic impurities in the pharmaceuticals to limit potential carcinogenic .

6) FDA 2018 a Laboratory analysis of valsartan products 5 October 2018, accessed date 20 October 2018.

7) NIOSH .1990 National occupational exposure survey (1981-83) national institute for occupational safety and health .

8) IARC 1972 N-Nitrosodimethylamine .In some inorganic substances ,chlorinated hydrocarbons ,aromatic amines, N-Nitroso compound and natural products .IARC monographs on evaluation of carcinogenic risk to human .,Vol .1 Lyon ,france :International agency for research on cancer.pp.95-106.

9) IARC 1978 N-Nitrosodimethylamine .In some N-nitroso compound .IARC monographs on the evaluation of the carcinogenic risk of chemicals to the human ,vol 17.Lyon ,france :international agency for research on cancer .pp.125-175

10) HSDB .2009 Hazardous substances data bank .national laboratory of medicines . last accessed 10/7/09.

11) Impurity in drug products , ICH Q3B(R2),2006

12) Assessment and control of DNA reactive mutagenic impurity in pharmaceutical to limit potential carcinogenic risk M 7(R1),ICH ,2017.

13)FDA updates on angiotensin Ⅱ receptor blocker (ARB) recalls including valsartan ,losartan,irbesartan .U.S .Food and drug administration .accessed2019-01-14.

14) W.A .Mitch J.O .Sharp ,R.R Trussell ,R.L .Valentine ,L.Alvarez –Cohen ,D.L Sedlak ,N-nitrosodimethylanine as drinking water contaminant a review ,Environ .Eng .Sci .20(2003) 389-404.

15) U.S Food and drug administration , Dimethylnitrosoamine in Malt Beverages , 2005.

16) S.D Richardson ,C.Postigo ,Drinking water disinfection by product , in Emerging organic contaminants and human health , springer ,2011 ,pp.93-137.

17) World health organization , guidelines for drinking –water quality ,2017

18)U.S Environmental protection agency ,technical fact sheet N-Nitrososdimethylamine NDMA ,2014.

19) (Guo et al., 2019)Guo, L., Long, Z., & Leng, X. (2019). *Rapid Analysis of Genotoxic Nitrosamines by HPLC-MS/MS Sensitive, Robust Assay Using a SCIEX Triple Quad™ 4500 System and the ExionLC™ System*. 1.

20) F. Bracher, P. Heisig, P. Langguth, E. Mutschler, G. Rücker, T. Schirmeister, G.K.E. Scriba, E. Stahl-Biskup, R. Troschütz, Arzneibuch-Kommentar, 1. Aufl. inkl. 58. Akt.lfg ed., Wissenschaftliche Verlagsgesellschaft Stuttgart, 2018

21) J. Zou, Y. Yang, W. Wang, Synthesis of Valsartan, Beijing Second Pharmaceutical Co., Ltd., Peop. Rep. China, 2009, pp. 8pp.

22) S. Jain, R.S. Shekhawat, A.K. Tyagi, A. Agarwal, Process for the Production of Sartans With High Purity, Jubilant Life Sciences Limited, India, 2012, pp. 51pp.

23) Y. Wang, G. Zheng, G. Cai, B. Chen, H. Li, Process for Preparation of Valsartan, Zhejiang Hisun Pharmaceutical Co., Ltd., Peop. Rep. China, 2009, pp. 11pp.

24) Z. Xiaoren, S. Nianping, Z. Wenling, W. Peng, Improved Method for Preparing Tetrazole for Valsartan, Zhejiang Huahai Pharmaceutical Co., Ltd., 2014, pp. 11pp

25) H. Buschmann, U. Holzgrabe, NDMA in valsartan, Apoth. 158 (2018) 22–26.

26) B. L. Williamson, L. M. Benson, A. J. Tomlinson, A. N. Mayeno, G. J. Gleich, S. Naylor, On-line HPLC-tandem mass spectrometry analysis of contaminants of Ltryptophan associated with the onset of the eosinophilia-myalgia syndrome. Toxicol. Lett. 92 (1997) 139-148.

27) T. D. Hopfgartner G, Varesio E. High Resolution Mass Spectrometry for Structural Elucidation and Quantitation of Drugs and their Metabolites based on Multiple MS and MS/MS Workflows. in Proceedings of the 59th ASMS Conference on Mass Spectrometry and Allied Topics. 2011. Denver (CO), USA.

28) G. Hopfgartner, D. Tonoli, E. Varesio, High-resolution mass spectrometry for integrated qualitative and quantitative analysis of pharmaceuticals in biological matrices. Anal. Bioanal. Chem. 402 (2012) 2587-2596.

29) S. Munster-Muller, R. Zimmermann, M. Putz, A Novel Impurity-Profiling Workflow with the Combination of Flash-Chromatography, UHPLC-MS, and Multivariate Data Analysis for Highly Pure Drugs: A Study on the Synthetic Cannabinoid MDMBCHMICA. Anal. Chem. 90 (2018) 10559-10567.

30) Paper, A. (2014). Page 1 of 6 Page 1 of 6. *Matalab*, *November 2006*, 1–6.

31) F . Breider, U. von Gunten, Quantification of total N-nitrosamine concentrations in aqueous samples via UV-photolysis and chemiluminescence detection of nitric oxide, Anal. Chem. 89 (2017) 1574–1582.

32) X. Ceto, C.P. Saint, C.W.K. Chow, N.H. Voelcker, B. Prieto-Simon, Electrochemical detection of N-nitrosodimethylamine using a molecular imprinted polymer, Sensor Actuat B-Chem 237 (2016) 613–620

33) B. Jurado-Sanchez, E. Ballesteros, M. Gallego, Comparison of the sensitivities of seven N-nitrosamines in pre-screened waters using an automated preconcentration system and gas chromatography with different detectors, J. Chromatogr. A 1154 (2007) 66–73.

34) X. Li, X. He, Y. Dong, L. Jia, Q. He, Analysis of N-nitrosodiethylamine by ion chromatography coupled with UV photolysis pretreatment, J. Food Drug Anal. 24 (2016) 311–315.

35) G. Telling, The determination of N-nitrosamines in foods and cosmetics, Trac Trends Anal. Chem. 1 (1982) 277–280

36) S. Yoon, N. Nakada, H. Tanaka, A new method for quantifying N-nitrosamines in wastewater samples by gas chromatography-triple quadrupole mass spectrometry, Talanta 97 (2012) 256–261.

37) T.-Y. Fan, S.R. Tannenbaum, Automatic colorimetric determination of-nitroso compounds, J. Agric. Food Chem. 19 (1971) 1267–1269.

38) Fiddler, The occurrence and determination of N-nitroso compounds, Toxicol. Appl. Pharmacol. 31 (1975) 352–360.

39) K. Takatsuki, T. Kikuchi, Determination of N-nitrosodimethylamine in fish products using gas chromatography with nitrogen—phosphorus detection, J. Chromatogr. A 508 (1990) 357–362.

40) D.L.H. Williams, Reagents effecting nitrosation, in: D.L.H. Williams (Ed.), Nitrosation Reactions and the Chemistry of Nitric Oxide, Elsevier Science, Amsterdam, 2004, pp. 1–34.

41) T.-Y. Fan, S.R. Tannenbaum, Automatic colorimetric determination of-nitroso compounds, J. Agric. Food Chem. 19 (1971) 1267–1269.

42) L. Walsh, P.W. Hastwell, P.O. Keenan, A.W. Knight, N. Billinton, R.M. Walmsley, Genetic modification and variations in solvent increase the sensitivity of the yeast RAD54-GFP genotoxicity assay, Mutagenesis 20 (2005) 317–327.

43) V.N. Bui, T.T. Nguyen, C.T. Mai, Y. Bettarel, T.Y. Hoang, T.T. Trinh, N.H. Truong, H.H. Chu, V.T. Nguyen, H.D. Nguyen, S. Wolfl, Procarcinogens - determination and evaluation by yeast-based biosensor transformed with plasmids incorporating RAD54 reporter construct and cytochrome P450 genes, PLoS One 11 (2016), e0168721

44) M.K. Parr, J.F. Joseph / Journal of Pharmaceutical and Biomedical Analysis 164 (2019) 536–549

45) H. E. Pence, A. Williams, ChemSpider: An Online Chemical Information Resource. J. Chem. Educ. 87 (2010) 1123-1124

46) Scherf-Clavel, O., Kinzig, M., Besa, A., Schreiber, A., Bidmon, C., Abdel-Tawab, M., Wohlfart, J., Sörgel, F., & Holzgrabe, U. (2019). The contamination of valsartan and other sartans, Part 2: Untargeted screening reveals contamination with amides additionally to known nitrosamine impurities. *Journal of*

Pharmaceutical and Biomedical Analysis, *172*, 278–284. https://doi.org/10.1016/j.jpba.2019.04.035

47) Delaney, E., 2007. J. Regul. Toxicol. Pharmacol. 49 (2), 107–124.

48) Cheeseman, M.A., Machuga, E.J., Bailey, A.B., 1999. Food Chem. Toxicol. 37 87-412.

49) CVUA (Chemisches Und Veterinäruntersuchungsamt Karlsruhe), 2018. Test Method for the determination of NDMA by LC/MS/MS in Valsartan finished products. 21 September 2018.

50) ICH M7(R1), 2017. Assessment and Control of Dna Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk. Current Step 4 Version Dated 31 March 2017

51) EMA, 2018a. EMA Reviewing Medicines Containing Valsartan from Zhejianjg Huahai Following the Detection of an Impurity. EMA/459276/2018, 5 July 2018.

52) FDA, 2018c. FDA Announces Voluntary Recall of Several Medicines Containing Valsartan Following Detection of an Impurity. July 13, 2018.

53) Mukherjee, R. India launches probe as China co recalls BP drug. 20 July 2018

54) FDA, 2018d. List of Valsartan Products Under Recall. 27 August 2018

55) FDA, 2018c. FDA Announces Voluntary Recall of Several Medicines Containing Valsartan Following Detection of an Impurity. July 13, 2018

56) Herrmann, S.S., Duedahl-Olesen, L., Christensen, T., Olesen, P.T., Granby, K., 2015. Food Chem. Toxicol. 80, 137–143.

57) Zeilmaker, M.J., Bakker, M.I., Schothorst, R., Slob, W., 2010. Toxicol. Sci. 116, 223–235.

58)Technical FactSheet:N-Nitrosodimethylamine (NDMA). UnitedStates ProtectionAgencyAccessed date: 21 November 2018.

59) FDA, 2018d. List of Valsartan Products Under Recall. 27 August 2018. Accessed date: 20 October 2018.

60) N-NITROSODIMETHYLAMINE fact sheet No. 38. IPCS Inchem Home. . Accessed on 21 November 2018.

61) Fitzgerald, D.J., Robinson, N.I., 2007. J. Toxicol. Environ. Health 70 (19), 1670–1678

62) Snodin, D.J., 24 August 2018b. Personal Communication to D Elder. Technical FactSheet:N-Nitrosodimethylamine (NDMA). UnitedStates ProtectionAgency

63) EFSA Scientific Committee, 2016. 17 November 2016.Update: use of benchmark dose approach in risk assessment. Efsa J. 1–41. 2016

64) Scientific Committee on Consumer Safety, 2012. Opinion on NDELAin Cosmetic Products and Nitrosamines in Balloons. SCCS/1486/12 18 September 2012

65) FDA, 2018c. FDA Announces Voluntary Recall of Several Medicines Containing Valsartan Following Detection of an Impurity. July 13, 2018. Accessed date: 7 October 2018.