A Comprehensive Review: Structure Based *N*-Nitrosamine Impurity Risk Assessment of an Anti-Coagulant Drug Rivaroxaban

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Abstract:

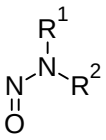
In the recent years, presence of N-Nitrosamine impurity in the drug substance as well in the drug product became the critical and serious concern. The drugs in the Sartan family have been recalled from market due to presence of N-Nitrosamine Nitrosamine impurities are carcinogenic and could cause cancer to human[1-9]. The multiple root causes of nitrosamine contamination listed in the literature can occur within the API Manufacturing process. Therefore, multiple strategies may be necessary to identify all potential source[10-18]. Regulatory agencies have recommended the through N-Nitrosamine risk assessment, nanogram level quantification analytical methods and control in the API manufacturing process[19-26].

Here we have provided the through risk assessment-based EMA guideline on Starting Material route of synthesis and API Route of Synthesis and also provided calculation for allowable limit for individual Nitrosamine impurity in an anticoagulant drug called Rivaroxaban. As nitrosamine impurities are regard as probable human carcinogen, the risk assessment of the generation of nitrosamine impurities during synthesis, production, or stability is mandated by regulatory agencies

Key words: N-Nitrosamine Impurity, Risk assessment, API Process, Carcinogenic impurities, Commercial process, Rivaroxaban,

Introduction

N-Nitrosamines are potential carcinogenic compounds having general structure:



N-Nitrosamines can be formed when an amine and nitro sating agent arc combined under favourable conditions although other generation pathways are also possible, such a e.g. oxidation and reduction processes from hydrazine-type compounds and N-nitro derivatives as per

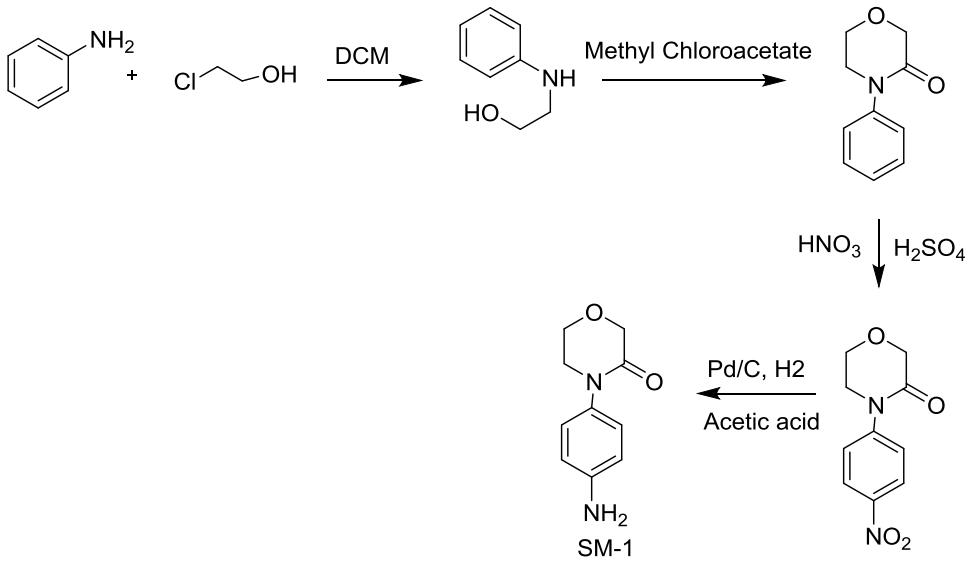
EMA/369136/2020 document "Nitrosamine impurities in human medicinal products" risk evaluation needs to be performed for the presence of N-Nitrosamines in human medicinal products containing chemically synthesized active pharmaceutical ingredients. Because the final drug substance possesses I) noxious starting materials, process intermediates, reagents at low level as impurities, and ii) the by-products of synthetic processes as toxic impurities'. But,the presences of PGIs at high enough concentrations and other toxic impurities in drug substances could cause adverse health effects in humans, for example elicit cancer to humans[27-44]

The process Scientist plays a key role in avoiding the formation or control of Nitrosamine during the development of efficient manufacturing process for the manufacture of drugs.

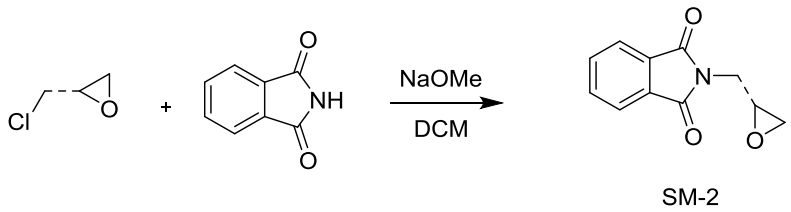
Hence, identification, characterization and control of these Nitrosamine impurity are very important. Otherwise, it could lead to holding of drug approval, clinical trials for new drug discovery programmes or delay of approval from regulatory agencies for both new and generic drugs.

Herein, **we** report the complete Evaluation of ROS and risk assessment of N-Nitrosamine impurity in the manufacturing process of Rivaroxaban.

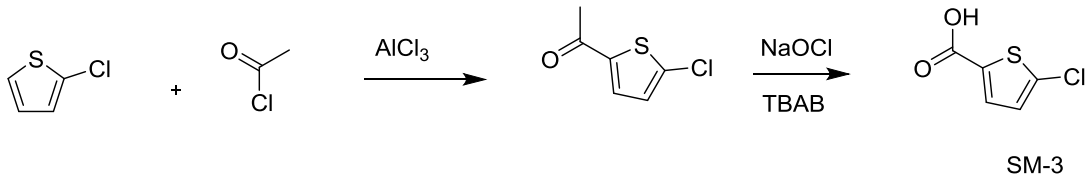
Commercially. Rivaroxaban can be manufactured by taking three starting materials and details of all three Route of synthesis provided as below[45-50']



ROS for Rivaroxaban Starting Material-2

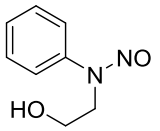
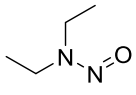
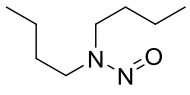


ROS for Rivaroxaban Starting Material-3

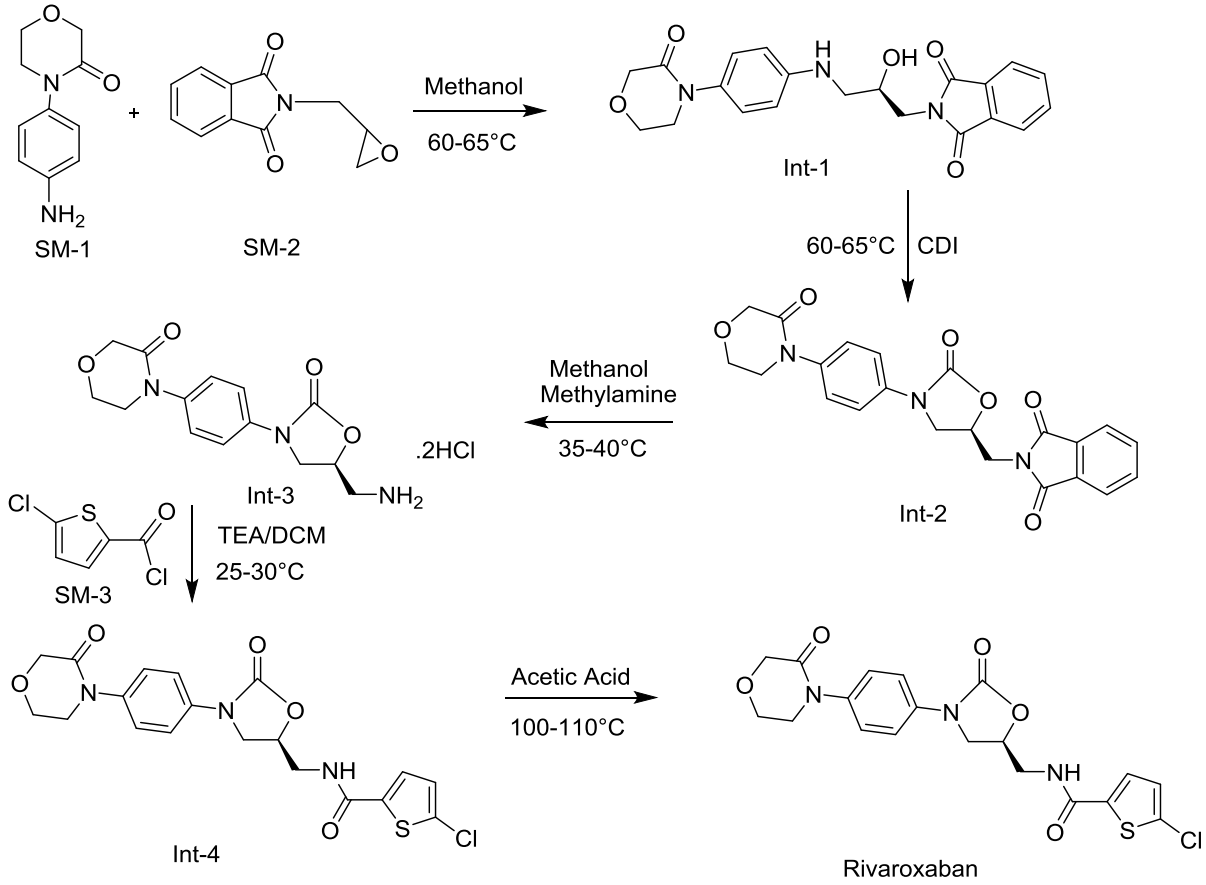


Risk Assessment for the critical N-Nitrosamine impurity formation in starting material.

Nitric acid is used in stage-II and one of the raw material of SM-1 i.e. 2-Anilinoethanol contains secondary amine it its structure. Hence, corresponding nitrosamine is possible. However, no nitrosating agent used in the synthesis of starting material-1. Therefore, possible formation of nitrosamine impurity from 2-anilinoethanol, Trimethylamine and Tetrabutylammonium Bromide have listed below[51-53].

Sr.No	**Name**	**Structure**	**Source**
1	N-Nitroso SM		2-Anilinoethanol
2	N-Nitrosodiethylamine		TEA
3	N-Dibutylnitrosamine		TBAB

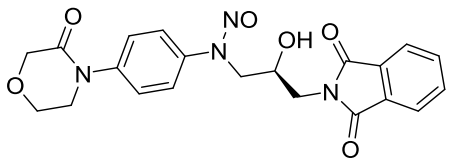
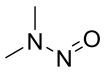
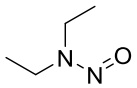
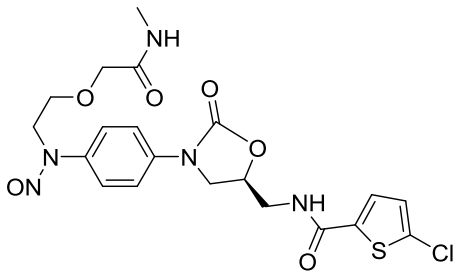
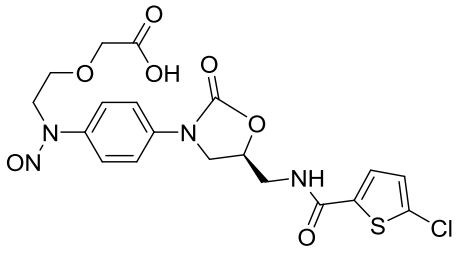
Route of Synthesis of Rivaroxaban API



There is no nitrosating agent used in the synthesis of Rivaroxaban API. However, triethylamine and *Al*, N-dimethylformatnide is used during the synthesis and therefore, corresponding nitrosamines NDEA and NDMA are possible. Further, intermediate-1 and two process

impurities i.e. Process Impurity-1 and Process Impurity-2 contains secondary amine functionality in its structure and monitored in API specification with the limit NMT 0.15%. Therefore, their corresponding nitrosamines are possible and confirmatory testing in API is required.

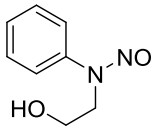
The Following are the possible nitrosamine from the ROS of Rivaroxaban.

Sr.No	**Name**	**Structure**	**Source**
1	N-Nitroso Intermediate-1		Intermediate-1
2	NDMA		DMF
3	NDEA		TEA
4	*N*-Nitroso Process Impurity-1		Process Impurity=1
5	*N*-Nitroso Process Impurity-2		Process Impurity-2

Note: For NDMA, NDEA and NDBA Allowable limit has been provided in the LAMA guideline.

Calculation of Potency category & acceptable intake using Carcinogenic Potency Categorization Approach (EMA/409815/2020 Rev. 17)

1.1 Calculation of Potency category & acceptable intake for N-Nitroso SM-S1



Count on alpha hydrogen	**Alpha hydrogen Score**	**Future Highlighted**	**Remarks**
0,2	2		Alpha methylene carbon is not part of ethyl group
Deactivating features Hydroxyl group bonded to 11-carbon* on only one side of N-nitroso group (cyclic or acyclic) **Chains of 5 consecutive non-hydrogen atoms on both side of acyclic N-Nitroso group**	+1 +1	Alcohol group is present	NA
No activating feature			NA
Potency Score = 3+1+1 = 5	Potency Category = 5	**AI = 1500 ng/day**	NA

***Maximum Daily Dose (MDD) of Rivaroxaban is 30 mg**

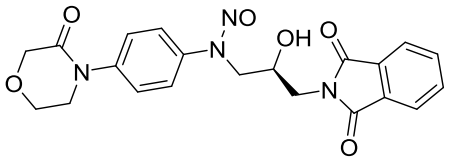
1 Calculation of acceptable limit for N-Nitroso SM-1

Acceptable limit (ppm)= Al (ng/day) /MDD (mg)

= 400/30

= 13.3

1.2 Calculation of Potency category & acceptable intake for N-Nitroso Intermediate-1



Count on alpha hydrogen	**Alpha hydrogen Score**	**Future Highlighted**	**Remarks**
0,2	2		NA
Deactivating features Hydroxyl group bonded to 11-carbon* on only one side of N-nitroso group (cyclic or acyclic)	+1	COOH group present	NA
No activating feature			NA
Potency Score = 2+1 = 3	Potency Category = 3	**Al = 400 ng/day**	NA

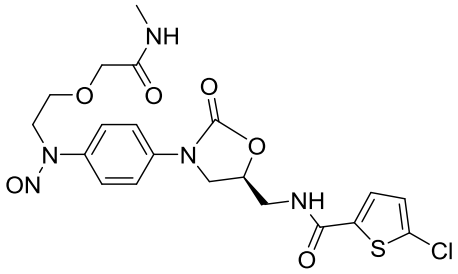
Calculation of acceptable limit for N-Nitroso Intermediate-1

Acceptable limit (ppm)= Al (ng/day) /MDD (mg)

= 400/30

= 13.3

Calculation of Potency category & acceptable intake for N-Nitroso Process Impurity-1



COUNT ON ALPHA HYDROGEN	ALPHA HYDROGEN SCORE	FUTURE HIGHLIGHTED	REMARKS
0,2	2		Alpha methylene carbon is not part of ethyl group
Chains of 5 consecutive non-hydrogen atoms on both side of acyclic N-Nitroso group	+1	NA	NA
No activating feature			NA
Potency Score = 2+1 = 3	Potency Category = 3	**Al = 400 ng/day**	NA

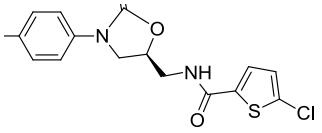
Calculation of acceptable limit for N-Nitroso Process Impurity-1

Acceptable limit (ppm)= Al (ng/day) /MDD (mg)

= 400/30

= 13.3

Calculation of Potency category & acceptable intake for N-Nitroso Process Impurity-2



COUNT ON ALPHA HYDROGEN	ALPHA HYDROGEN SCORE	FUTURE HIGHLIGHTED	REMARKS
0, 2	2		Alpha methylene carbon is part of ethyl group

Carboxylic acid group anywhere in the molecule **Chains of 5 consecutive non-hydrogen atoms on both side of acyclic N-Nitroso group**	+3 +1	NA	NA
No activating feature	No		NA
Potency Score = 2+3+1 = 6	Potency Category = 5	**Al = 1500 ng/day**	NA

Calculation of acceptable limit for N-Nitroso Process Impurity-2

Acceptable limit (ppm)= Al (ng/day) /MDD (mg)

= 1500/30 = 50

CONCLUSION:

The overall conclusions for the risks assessment, all starting material route of synthesis and API route of synthesis have been evaluated and possible formation of Nitrosamine impurity listed with potency category. Based on Potency score, calculated allowable limit for individual impurity by taking in account, with through nitrosamine risk assessment and their analysis in the drug substances and drug product can deliver safe and effective drug to the patient. The risk for the formation of nitrosamines in the final drug product by evaluating their synthesis, applying relevant controls, consulting supplier and generating relevant data and then this systematic assessment for nitrosamine impurity will be useful for the submission of drug to the regulatory authority for the pharmaceutical generic industry.

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