Nitrosamine Quality Risk Assessment Report of Ornithine Aspartate

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Text：

★ Purpose

Carry out nitrosamine quality risk assessment for anhydrous sodium carbonate, predict the possible future events forward-looking, through risk control, avoid hazards and ensure product quality.

★ Range

The risk assessment report is suitable for assessing the quality risk of LOLA nitrosamines.

★ Content

1. Risk assessment principles

1.1 Evaluation Basis

According to the technical guidelines for research on nitrosamine impurities in chemical drugs (for trial implementation), nitrosamine impurities belong to ICH M7 (R1) ("Assessment and control of DNA reactive (mutagenic) impurities in drugs to limit potential carcinogenic risks") Substances referred to in the guideline as "cohort of concern". According to the list of carcinogens published by the World Health Organization, both NDMA and NDEA are classified as 2A carcinogens; according to internationally recognized databases, some nitrosamine impurities have published carcinogenicity data, such as NDMA, NDEA, N-nitroso -N-Methyl-4-aminobutyric acid (NMBA), N-nitroso dibutylamine (NDBA), etc.

The drug marketing authorization holder/drug manufacturer should earnestly perform the main responsibility of drug quality management, conduct full life cycle management of drug safety and quality, and avoid the introduction of nitrosamine impurities as much as possible. If it is not completely avoided , The risk of nitrosamine impurities in drugs should be fully evaluated, and the level of nitrosamine impurities should be controlled below the safety limit.

1.2 Causes of nitrosamine impurities

According to what is currently known, there are many reasons for nitrosamine impurities, such as process generation, degradation pathways, and pollution introduction. Specifically, nitrosamine impurities may be introduced in the following ways:

(1) The risk of introducing nitrosamine impurities from the process

It is currently known that NDMA and NDEA impurities may be generated through nitrosation mechanism. That is, under certain conditions, amine compounds, especially secondary amines, react with sodium nitrite ($NaNO_2$) or other nitrosating reagents to produce nitrosamine impurities.

Materials that can introduce secondary amines and nitrosating reagents (including starting materials, solvents, reagents, catalysts, intermediates, etc.) are used in the same process step, and there is a higher risk of introducing nitrosamine impurities; even in different Materials that can introduce secondary amines and nitrosating reagents are used in the process steps, and nitrosamine impurities may also be generated.

In addition to the material itself with a secondary amine structure, possible sources of secondary amines are: primary amine, tertiary amine and quaternary ammonium may introduce secondary amine impurities; amide solvents (such as N,N-dimethylformamide, N-methyl Pyrrolidone, etc.) may produce secondary

amines under suitable conditions (such as acidity, high temperature, etc.).

The possible sources of nitrosating reagents are: nitrite, nitrite ester, nitrous acid, substances prepared from nitrite (such as sodium azide, etc.), oxidation of amine compounds, etc.

(2) Risks introduced by pollution

The use of materials (starting materials, intermediates, solvents, reagents, catalysts, etc.) contaminated by nitrosamine impurities in the production of APIs may bring risks of nitrosamine impurities.

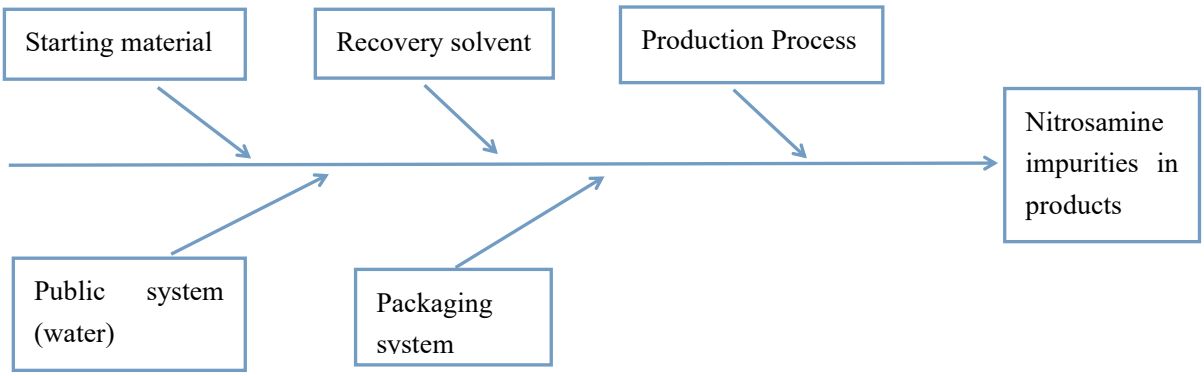
The use of recycled materials also has the risk of introducing nitrosamine impurities. Examples of recovered materials that have been found to be contaminated with nitrosamines include o-xylene, tributyltin chloride (used as a source of tributyltin azide), and N,N-dimethylformamide (DMF).

When different varieties are produced on the same production line, cross-contamination may also become a potential cause for the introduction of nitrosamine impurities.

(3) Risk of degradation

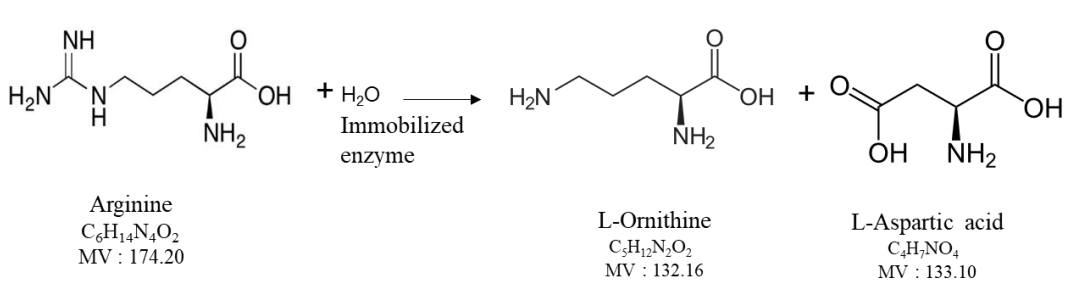
Certain drugs themselves degrade to produce nitrosamine impurities. For example, ranitidine produces nitrosamine impurities at high temperatures.

1.3 The source of nitrosamines



2. Risk assessment

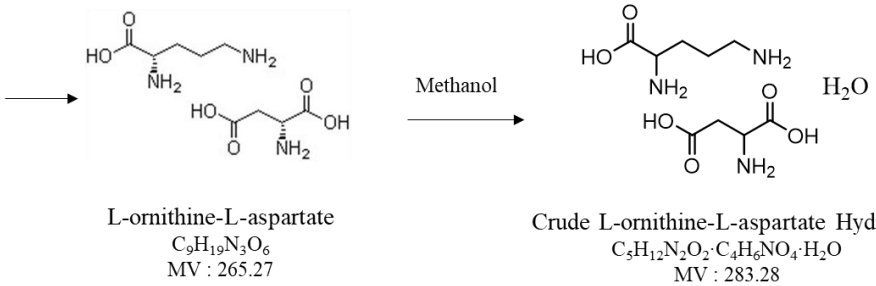
2.1. Reaction formula:



Arginine
$C_6H_{14}N_4O_2$
MV : 174.20

L-Ornithine
$C_5H_{12}N_2O_2$
MV : 132.16

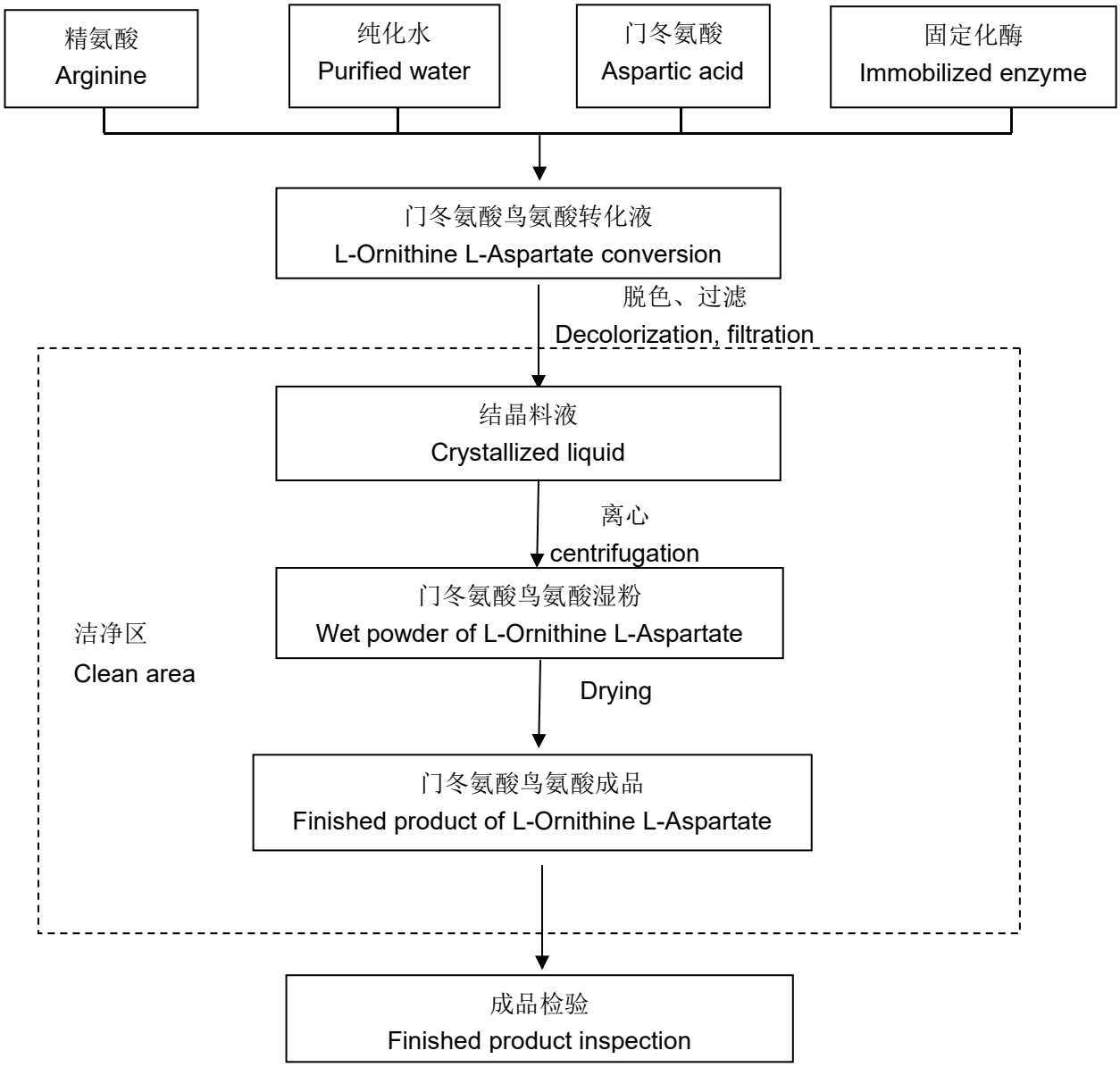
L-Aspartic acid
$C_4H_7NO_4$
MV : 133.10



L-ornithine-L-aspartate
$C_9H_{19}N_3O_6$
MV : 265.27

Crude L-ornithine-L-aspartate Hydrate
$C_5H_{12}N_2O_2 \cdot C_4H_6NO_4 \cdot H_2O$
MV : 283.28

2.2production flow chart



2.3 Summary of raw material risk analysis and assessment

NO.	Material name	Whether to introduce nitrosating reagent	Whether it can introduce secondary amine structure	Possible nitrosamines (name and structure)	Remarks
1	Arginine	No	No	None	—
2	Aspartic acid	No	No	None	—
3	Immobilized enzyme	No	No	None	—
4	methanol	No	No	None	—
5	Purified water	No	No	None	—

2.4. Summary of process risk assessment review items

No	Possible source of Nitrosamine	Yes	No	Possibility evaluation
1	Use of nitrite salts and esters (e.g. $NaNO_2$, alkyl nitrites), or other nitrosating agents (e.g. nitroso halides, nitrosodium salts, nitrogen oxides, nitro alkanes, halogenated nitro alkanes, Fremy salt, nitroso sulfonamides), in the presence of secondary or tertiary amines within the same or different steps of the manufacturing process. Sources for secondary or tertiary amines can also be starting materials, intermediates, reagents, solvents (e.g. DMF, DMAc and NMP) and catalysts, which contain amine functionality, amine impurities (e.g. quaternary ammonium salts) or which are susceptible to degradation to reveal amines.		√	No nitrite compounds, other nitrosating agents, or amine compounds are used in the production process of this product, and nitrosamine impurities will not form even under strong acidic conditions of sulfuric acid.
2	Nitrite formation by oxidation of hydroxylamine or nitrite release from nitro-aromatic precursors (e.g. by fluoro de-nitration), in the presence of secondary or tertiary amines within the same or different steps of the manufacturing process.		√	No This production process does not involve materials, reagents, intermediates, or finished products containing secondary or tertiary amines, and does not use any oxidants, resulting in no nitrite impurities.
3	Use of disinfected water (chlorination, chloro-amination, ozonisation) in the presence secondary or tertiary amines within the same or different steps of the manufacturing process.		√	No This production process does not involve materials, reagents, intermediates, or finished products containing secondary or tertiary amines, and does not use any disinfectant water or produce nitrite impurities.
4	Oxidation of hydrazines, hydrazides and hydrazones by hypochlorite, air, oxygen, ozone and peroxides in the manufacturing process or during storage. Use of contaminated raw materials in the API manufacturing process (e.g. solvents, reagents and catalysts).		√	No This production process does not involve materials, reagents, intermediates, or finished products containing hydrazine, hydrazide, and hydrazone structures, so nitrite impurities will not be produced during production and storage.。

5	Use of contaminated recovered or recycled materials (e.g. solvents, reagents and catalysts).	√		Yes The intermediate production of this product uses recycled methanol. There are clear process regulations for the use of recycled solvents, and the recycling equipment for recycled solvents is fixed. There is no situation of entrusting a third party or returning the solvent to the supplier for recycling after use. Our company's solvent recycling process, quality standards, testing methods, etc. are all approved in writing. The use of recycled solvents must meet two conditions: first, the recycled solvents must only be used in the same step, that is, at which step the recycled solvents are still used for that step; The second is that the solvent recovered from the refining step can be used for the crude product step. This product is produced using recycled solvents and does not introduce the risk of nitrosamine impurities.
6	Use of contaminated starting materials and intermediates supplied by vendors who use processes or raw materials which may contain residual nitrosamines or nitrosating agents.		√	No The suppliers of materials and reagents used in this production process have provided preparation process flowcharts, and no amine compounds or nitroso compounds are used in the preparation process. Based on the questionnaire survey of suppliers and their management measures for production, storage, and transportation, the risk of nitrosamine impurity pollution is extremely low.
7	Carry-over of nitrosamines deliberately generated (e.g. as starting materials or intermediates) during the manufacturing process.		√	No This production process does not involve materials, reagents, or intermediates containing secondary or tertiary amines, and there is no potential pathway for producing nitrosamine impurities.
8	Does the API, or one of its known impurities, have a nitrosatable nitrogen functionality?		√	No API and its known impurities are all amino acids and small

				molecule dicarboxylic acid substances, and do not involve nitroso functional group structures.
9	Degradation processes of active substances, including those induced by inherent reactivity (e.g. presence of nitro-alkyl, oxime, or other functionality) or by the presence of an exogenous nitrosating agent. This could potentially occur during both active substance and finished product manufacturing processes or during storage and could be influenced by crystal structure, crystal habit and storage conditions (temperature, humidity etc.). For more details, refer to page 6 of Referral under Article 31 of Directive 2001/83/EC for ranitidine and published literature.		√	No There are no other functional groups such as nitroalkyl or oxime in the structure of this product, and no exogenous nitrosating agents have been introduced. The degradation process does not produce nitrosamine impurities.
10	Oxidation of hydrazine or other amine-containing functional groups present in active substances or their impurities/degradants (e.g. from hydrazones and hydrazides), either in active substance manufacturing processes or during storage. This root cause has also been observed during manufacture and storage of finished products containing such functional groups. Potential oxidants include oxygen and peroxides (common impurities in some excipients).		√	No There are no hydrazine or other amine functional groups present in this product and potential related impurity structures, nor are there any potential oxidants. There will be no nitrosamine impurities generated during production and storage processes.

11	Use of certain packaging materials. Relevant nitrosamine contamination has been observed in primary packaging of finished products in blister with lidding foil containing nitrocellulose. During the blister heat-sealing process, nitrogen oxides can be generated thermally from nitrocellulose. Under these conditions, nitrosamines have been shown to form from low molecular weight amines present either in printing ink or in the finished product and to transfer to the product and/or to the cavity via evaporation and condensation.		√	No The inner packaging that comes into direct contact with this product is a low-density polyethylene bag. The supplier is a qualified supplier approved by on-site audit and has a national registration certificate for medicinal plastic bags. According to the properties of this product, theoretically it will not interact with low-density polyethylene and will not produce nitrosamine impurities when stored at room temperature. Therefore, the risk of nitrosamine impurity contamination caused by packaging systems is also extremely low.
12	Reaction of amines leaching from quaternary ammonium anion exchange resins (e.g. used for purification steps) with nitrosating agents present in the liquid phase.		√	No The production process does not use quaternary ammonium anion exchange resin.
13	Cross-contamination due to different processes being run successively on the same manufacturing line.	√		Yes Production line collinear variety of alanyl glutamine. The materials, reagents, intermediates, and finished products involved in this variety all use any nitrite compounds, other nitrosating agents, and amine substances. The compounds do not contain substances that are prone to producing nitrosamine impurities, so the risk of introducing non nitrosamine impurities through co production is extremely low.
14	Methanol is recovered using our company's dedicated distillation tower and has not been outsourced to a third party.Carry-over of impurities between process steps due to operator-related errors or insufficiently detailed batch records such as inadequate phase separations during work-up procedures.		√	No Operational instruction and detailed batch production records were prepared. And provide training to operators on cross contamination to ensure operators operate and record properly.

15	Use of contaminated recovered or recycled materials (e.g. solvents, reagents and catalysts) where the recovery is outsourced to third parties who are not aware of the content of the materials that they are processing. Recovery processes carried out in non-dedicated equipment should also be considered.		√	No Methanol is recovered using our company's dedicated distillation tower and has not been outsourced to a third party.

2.5 Risk assessment results

According to the risk assessment done, the risk of nitrosamines is assessed as:

Ignorable	May appear
√	-

2.6 Risk reduction measures

If there is a risk of nitrosamine contamination, indicate possible nitrosamine impurities, sources of contamination, and proposed risk reduction measures:

Possible nitrosamines (name and structure)	Source of pollution (specify nitrosation and nitrosatable reagents)	Risk reduction measures
—	—	—

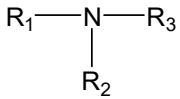
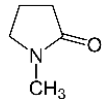
2.7 Other

Evaluation of nitrosation reagents

Nitrosation reagent（NO）	**structure**	**Is it possible to appear in the final API**
Nitrite	MNO_2	No
Nitrate	MNO_3	No
Nitrous acid	HNO_2	No
Nitrite ion	H_2O^+-NO	No
Nitric acid（N_2O_4）	HNO_3	No
Nitrite	R-ONO	No
Peroxynitrite	ONOO(-)	No

Ammonium Nitrate	NO^+	No
Nitro compound	R-NO_2	No
Nitrite Anhydride	N_2O_3	No
Dinitrogen Tetroxide	N_2O_4	No
Nitrosyl halide	Halide-NO	No
Nitrosyl thiocyanate	ONSCN	No
Nitrosophenol	Phenol-NO	No
Nitrosothiol	SH-NO	No
Aqua regia	HCl+HNO_3	No
Nitroxyl Chloride	NO_2Cl	No
other (instructions)	—	—

Evaluation of Nitrosizable Substances

Nitrosizable substances	**structure**	**by-product**	**Is it possible to appear in the API**
Secondary amine (cyclic or non-cyclic)	R_1-NH-R_2	-	No
Tertiary amine (cyclic or non-cyclic)		NHR_1R_2, NHR_1R_3,or/andNHR_2R_3	No
Hydrazine derivatives	NH_2-NR_1R_2	NHR_1R_2	No
N-methylpyrrolidone		N-methyl-4-aminobutyric acid	No

Nitrosizable substances	structure	by-product	Is it possible to appear in the API
Tertiary amide	$R_1CONR_2R_3$	NHR_2R_3	No
Chloroalkylamine	R_1R_2N-Cl	NHR_1R_2	No
N-alkyl carbamate	R_1O-CO-NR_2R_3	NHR_2R_3	No
other (instructions)	—	—	—

★ Conclusion

Through the risk assessment of raw materials and process, the ornithine aspartate produced by our company has no risk of introducing nitrosamine impurities.