APPLICATION NOTE

Determination of Nitrite, Nitrate and Amine in medicines using Ion Chromatography.

The U.S. Food and Drug Administration (FDA) issued a public health alert regarding the presence of N-nitrosodimethylamine (NDMA) in medicines. Recently, NDMA has been found in drug products containing ranitidine-, nizatidine-, and metformin-. N-nitrosodimethylamine is an N-nitrosamine, a compound with the generic chemical structure R2N–N¼O, which is a deprotonated amine bonded to a nitroso group. It is a well-known environmental contaminant present in drinking water & some foods.

A positive association between NDMA exposure and cancer was reported. In 2020, the FDA announced industry guidance to control N-nitrosamine impurities in human drugs. The guidance describes conditions that may introduce nitrosamine impurities into pharmaceutical products. The FDA, in collaboration with regulatory counterparts around the world, has set an acceptable daily intake limit for NDMA of 96 ng/day. If more than one nitrosamine is present in the sample, then the daily intake limit for total nitrosamine is 26.5 ng/day.

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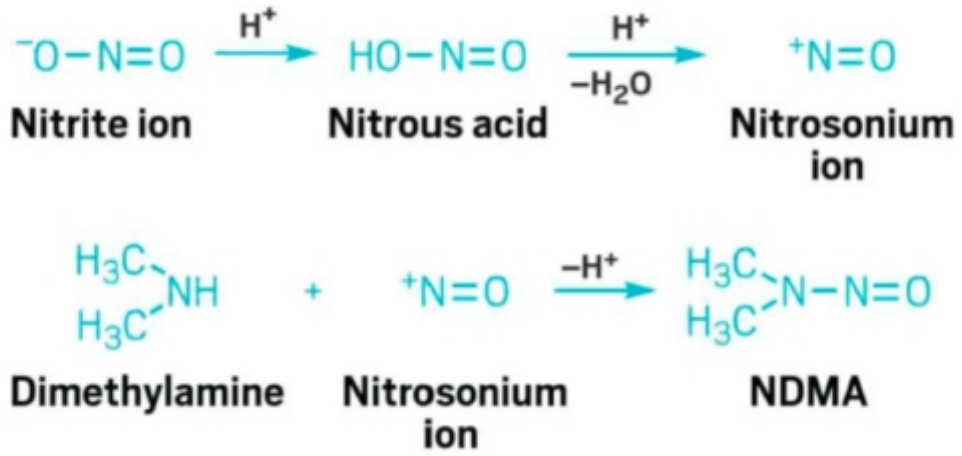
The FDA recommends that drugs be recalled by the manufacturer if the drug contains a level of nitrosamine above the acceptable daily intake limit. Drug manufacturers are trying to find out how NDMA is present in such a wide range of medicines and trying to find out how to prevent this contamination. Possible sources include side reactions from drug synthesis, the breakdown of unstable drug compounds, contamination from the manufacturing process, and the conditions under which the compounds are stored and packaged. One possible route to NDMA impurities is the side reaction of one step in the compound's synthesis. For example, in acidic conditions, DMA reacts with nitrites to produce nitrosamines. Dimethylamine is used in the synthesis of many drug substances such as metformin hydrochloride. The nitrosylation of DMA to generate NDMA was used as a model reaction that could occur during API processing..



Simulations based on the published kinetics of secondary amine nitrosylation have shown that higher nitrite levels may result in significant levels of N-nitrosamines at low pH or elevated temperatures. Therefore, limiting the precursor compounds in drug substances or products can prevent the potential formation of NDMA and other nitrosamines. In order to devise processes to reduce or eliminate nitrosamine formation, sensitive methods for the determination of amines that are nitrosamine precursors, nitrite and nitrate in pharmaceutical products are essential.

Few secondary, tertiary amines in drug products can be determined by Ion chromatography with non-suppressed (no cation suppressor is used and leads to low running cost) conductivity detection technique. Alkylamines and alkanolamines are as simple as doing cation determination in IC.

Also, we can use ion chromatography (IC) as a typical method for nitrite and nitrate determination, especially when good sensitivity is required.

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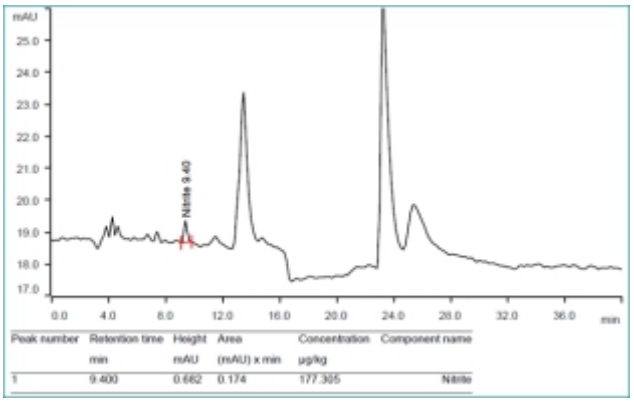
Ion chromatography is a well-accepted technique for the determination of ions in an aqueous solution. For most pharmaceutical samples it requires little or no sample preparation or analyte derivatization. Ion chromatography uses an ion-exchange separation followed typically by conductivity, electrochemical, UV absorption, or mass spectrometry detection. Ion chromatography-based procedures are included in several USP monographs and IC has been applied to all aspects of the manufacturing of pharmaceutical products, including the determination of active pharmaceutical ingredients, counter ions, ionic drug degradation products, and ionic process-related impurities.

We have developed an IC method for nitrite and nitrate in these drug samples. This method was based on an anion exchange separation coupled with UV absorbance detection at 210nm. The methods were validated with respect to calibration, detection limit and recovery. These methods were successfully applied to

Determination of nitrite in API

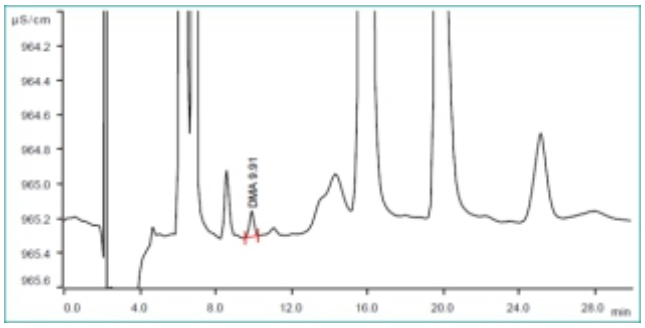


Peak number	Retention time	Height	Area	Concentration	Component name
	min	mAU	(mAU) x min	µg/kg	
1	9.400	0.682	0.174	177.305	Nitrite

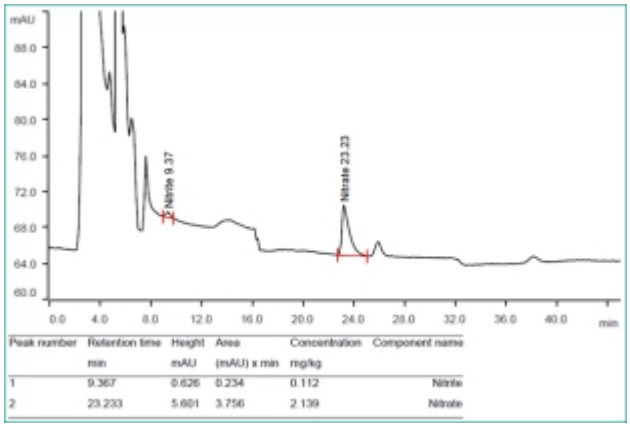
Chromatographic condition

Column	: Metrosep anion exchange column
Mobile Phase	: Sodium carbonate & sodium hydroxide
Flow rate	: 1.0 mL/minute
Column temperature	: 45°C
Detection method	: UV-visible detection at 210nm

Determination of dimethylamine (DMA) in API



Determination of nitrite and nitrate in Excipients



Peak number	Retention time	Height	Area	Concentration	Component name
	min	mAU	(mAU) x min	mg/kg	
1	9.367	0.626	0.234	0.112	Nitrite
2	23.233	5.601	3.756	2.139	Nitrate

Chromatographic condition

Column	: Metrosep anion exchange column
Mobile Phase	: Sodium carbonate & sodium hydroxide
Flow rate	: 1.0 mL/minute
Column temperature	: 45°C
Detection method	: UV-visible detection at 210nm

10 year anion suppressor warranty

Chromatographic condition

Column	: Metrosep cation exchange column
Mobile Phase	: Nitric acid & acetone
Flow rate	: 0.9 mL/minute
Column temperature	: Ambient
Detection method	: Non-Suppressed conductivity detection

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