

## Nitrosamines in Pharmaceuticals

Nitrosamines are known to be carcinogenic. In 2018, N-Nitrosodimethylamine (NDMA) was found in preparations of valsartan, a sartan drug. As a result, voluntary inspections were conducted. Due to factors such as the synthesis process and storage conditions, there is a limit for NDMA and other nitrosamines in the drug substance. LC-MS/MS analysis by ESI and APCI was used to detect 7 nitrosamines.

Key words : L-column3 Ammonia eluent LC-MS/MS Nitrosamines NDMA  
 Column : USP category: L1

It has been reported in two studies that ranitidine can be transformed into NDMA by a slight increase in temperature. In one study, ranitidine was heated at 130°C for 1 hour, and the formation of NDMA was confirmed through testing.

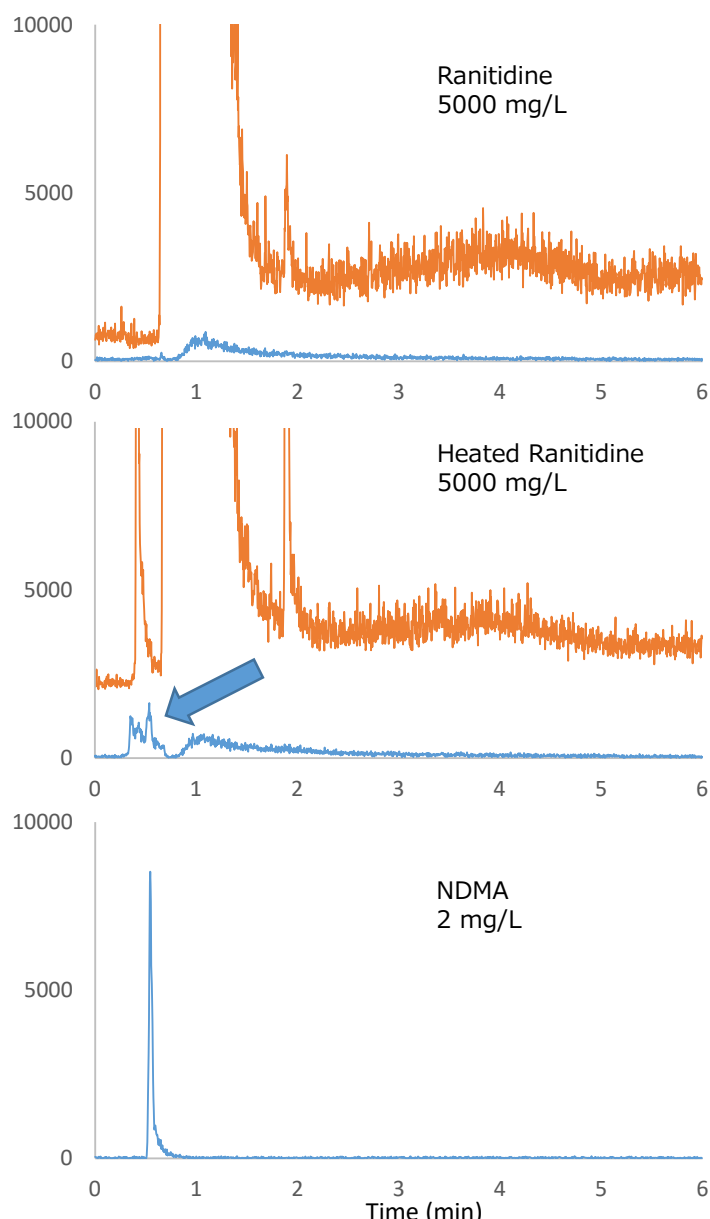


Fig.1 Chromatogram of ranitidine samples.

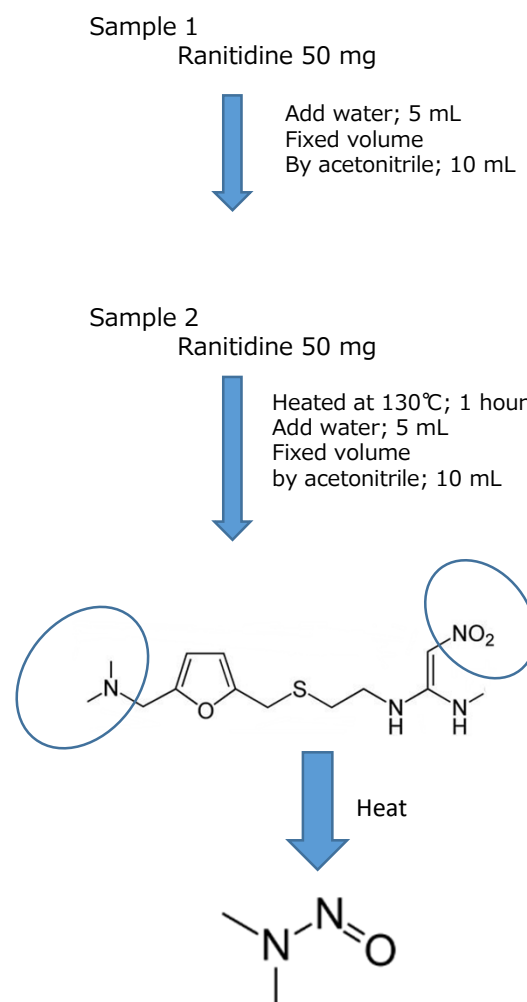
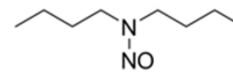
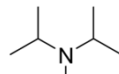
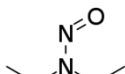
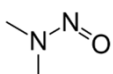


Fig.2 Formation process of NDMA. ※2

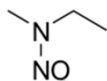
## [ Analytical conditions ]

Column : L-column3 C18 (2  $\mu$ m, 12 nm); 3.0 mm I.D.  $\times$  30 mm L.; Cat. No. 823650  
 Eluent : A: 0.1% HCOOH in H<sub>2</sub>O ; B: 0.1% HCOOH in CH<sub>3</sub>OH  
 A/B, 90/10(0-0.5 min)-5/95(5-6 min)  
 Flow rate : 0.5 mL/min  
 Temperature : 40°C  
 Detection : APCI-MS/MS(+), ESI-MS/MS(+)  
 Injection volume : 5  $\mu$ L  
 System : LC: Ultimate 3000 Bio RS (Thermo Fisher Scientific K.K.); MS/MS: 3200 QTRAP (SCIEX)  
 Sample : Nitrosamines 2 mg/L in H<sub>2</sub>O/CH<sub>3</sub>OH (9/1 v/v) each

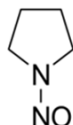
1. *N*-Nitrosodimethylamine (NDMA)  
*N*-ニトロソジメチルアミン  
 $m/z$  75.0  $\rightarrow$  58.2
2. *N*-Nitrosodiethylamine (NDEA)  
*N*-ニトロソジエチルアミン  
 $m/z$  103.0  $\rightarrow$  75.1
3. *N*-Nitrosodipropylamine (NDIPA)  
*N*-ニトロソジイソプロピルアミン  
 $m/z$  131.0  $\rightarrow$  89.0
4. *N*-Nitrosodi-*n*-butylamine (NDBA)  
*N*-ニトロソジ-*n*-ブチルアミン  
 $m/z$  159.2  $\rightarrow$  103.2



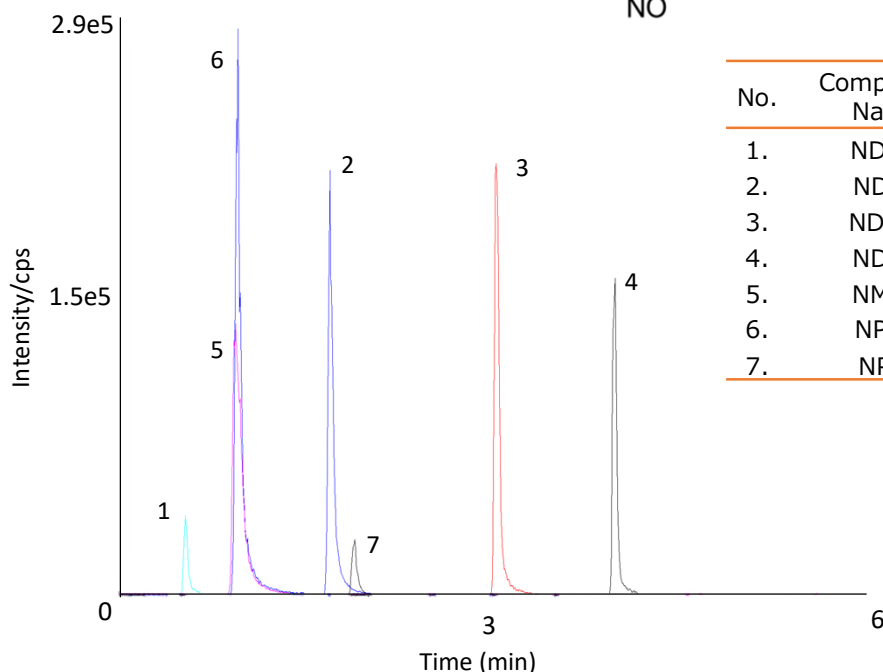
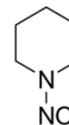
5. *N*-Nitrosomethylethylamine (NMEA)  
*N*-ニトロソメチルエチルアミン  
 $m/z$  89.1  $\rightarrow$  61.0



6. *N*-Nitrosopyrrolidine (NPYR)  
*N*-ニトロソピロリジン  
 $m/z$  101.0  $\rightarrow$  55.0



7. *N*-Nitrosopiperidine (NPIP)  
*N*-ニトロソピペリジン  
 $m/z$  116.0  $\rightarrow$  70.3



No.	Component Name	Signal / Noise	
		APCI	ESI
1.	NDMA	1333	18
2.	NDEA	8524	67
3.	NDIPA	14274	644
4.	NDBA	18602	7321
5.	NMEA	2790	51
6.	NPYR	5133	1133
7.	NPIP	1300	164

Fig. 3 Chromatogram of nitrosamines standard solution (APCI)

LC-MS/MS analysis by ESI and APCI was performed using seven nitrosamines. Here, high-speed analysis was performed using a 30 mm long column. While ESI has low sensitivity for low-molecular-weight components, APCI was able to detect even low-molecular-weight components with high sensitivity.

In September 2020, the FDA issued guidelines for nitrosamines that stipulate the use of a highly sensitive analysis method with a lower limit of quantification of 0.03 ppm or less to meet regulatory requirements. To comply with these guidelines, ionization by APCI was performed in this study, resulting in good linearity at concentrations of 20 to 200 ng/mL for all seven components. By using a SIM, the S/N ratio can be improved by 2 to 5 times. However, quantifying NDMA, which has a small molecular weight, can be challenging due to its susceptibility to the baseline and matrix, resulting in poor quantification.

