Supplementary Online Content

Braunstein LZ, Kantor ED, O'Connell K, et al. Analysis of ranitidine-associated *N*-nitrosodimethylamine production under simulated physiologic conditions. *JAMA Netw Open*. 2021;4(1):e2034766. doi:10.1001/jamanetworkopen.2020.34766

eMethods. Supplementary Methods

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods. Supplementary Methods

Equipment, Supplies, and Chemicals

SCIEX EXIONLC AD (LC) coupled with X500R time of flight high resolution mass spectrometry (HRMS) was purchased from SCIEX (Framingham, MA). Kinetex F5 HPLC column (2.6 µm, 4.6 × 100 mm) was purchased from Phenomenex (Torrance, CA). Certified reference materials (CRM) of N-Nitrosodimethylamine (NDMA) and ranitidine (≥ 99.2 %) were purchased from Sigma-Aldrich (St. Louis, MO). Isotopic labeled NDMA standard $^{13}C_2$-D_6-NDMA was purchased from Cambridge Isotope Laboratories (Tewksbury, MA). All other chemicals and reagents are ACS grade from Sigma-Aldrich.

Experiments

Simulated gastric fluid (SGF, 2 g/L sodium chloride in water) was prepared at various pH and nitrite concentrations. To initially evaluate NDMA formation dynamic at different human stomach relevant pH conditions, 100 mL SGF containing 50 mM sodium nitrite was prepared at pH = 1.2, 2.5, 3.5, 4.5, and 5.5 using hydrochloric acid. Ranitidine prescription tablet medications of 150 and 300 mg were added to the SGF. To demonstrate that NDMA formation could occur at variable nitrite concentrations, 100 mL SGF containing 50, 25, 10, 5, 2.5,and 1 mM nitrite were prepared at pH = 2.5. A “cool mint” flavored brand Zantac 150 mg tablet was added to the SGF. All experiments were conducted at 37 °C incubation, and samples were taken at the 2-hour time point, respectively. One milliliter of incubated SGF was transferred, centrifuged, and filtered by a 0.2 µm nylon filter into HPLC vial. A known amount of $^{13}C_2$-D_6-NDMA was spiked into the sample targeting the final internal standard concentration of 40 ng/mL. The ranitidine and Zantac tablets were also directly diluted and run by LC-HRMS as control samples.

Instrumental Analysis

NDMA data was generated by a method developed from the FDA recommended liquid chromatography – high resolution mass spectrometry (LC-HRMS) method for determination of nitrosamines in ranitidine drug substance and drug products [ref #3]. Briefly, chromatographic separation started at gradient of 90% of mobile phase A (0.1% formic acid in water) and 10% of mobile phase B (0.1% formic acid in methanol) and was held for 1 minute. Mobile phase B ramped up to 100% at 5 minutes and was held for 2 minutes, followed by B ramped down to 10% and held till 10 minutes. Total HPLC flow rate was 1 mL/min. NDMA elutes at 1.71 minute. Atmospheric pressure chemical ionization mode (APCI+) was selected to ionize NDMA and its isotopic labeled internal standard. Mass identification for NDMA was done by detecting the accurate mass of $[M+1]^+$ in MRMHR acquisition mode for NDMA 75.0553>75.0553, and $^{13}C_2$-D_6-NDMA 83.0997>83.0997, respectively. The mass accuracy was set at 15 parts per million (ppm) and mass resolution was greater than 25,000.

Quality Assurance and Quality Control

Linear calibration curve was established by an eight-point calibration ranged from 0.5 to 200 ng/mL containing the same internal standard concentration as samples. Calibration is accepted if the r^2 is equal or greater than 0.99. The limit of quantification (LOQ) is defined as the lowest acceptable calibration point. The lowest calibration point must have a minimum signal to noise ratio of 10. Concentrations of NDMA in samples were quantified by internal standard method. The LOQ was 1 ng/mL for NDMA and was equivalent to 100 ng in each sample incubation. When NDMA concentration in sample extracts exceeded the calibration range, sample extracts were diluted with methanol containing internal standard for re-analysis.