

## Determination of *N*-nitrosodimethylamine in zidovudine using high performance liquid chromatography-tandem mass spectrometry

Yujin Lim<sup>1</sup>, Aelim Kim<sup>1</sup>, Yong-Moon Lee<sup>2</sup>, and Hwangeui Cho<sup>1,★</sup>

<sup>1</sup>*Institute of New Drug Development, School of Pharmacy, Jeonbuk National University, Jeonju 54896, Korea*

<sup>2</sup>*College of Pharmacy, Chungbuk National University, Cheongju 28160, Korea*

(Received September 26, 2023; Revised November 2, 2023; Accepted November 3, 2023)

**Abstract:** Zidovudine is an antiretroviral agent prescribed for the prevention and treatment of human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS). It is typically recommended to be used in combination with other antiretroviral drugs. Zidovudine has the potential to generate *N*-nitrosodimethylamine (NDMA) in the presence of dimethylamine and nitrite salt under acidic reaction conditions during the drug manufacturing process. NDMA is a potent human carcinogen that may be detected in drug substances or drug products. An analytical method was developed to determine NDMA in pharmaceuticals including zidovudine using high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). The analysis involved reversed-phase chromatography on a Kinetex F5 column with a mobile phase comprising water-acetonitrile mixtures. The detection of positively charged ions was conducted using atmospheric pressure chemical ionization (APCI). The calibration curve demonstrated excellent linearity ( $r = 0.9997$ ) across the range of 1–50 ng/mL with a highly sensitive limit of detection (LOD) at 0.3 ng/mL. The developed method underwent thorough validation for specificity, linearity, accuracy, precision, robustness, and system suitability. This sensitive and specific analytical method was applied for detecting NDMA in zidovudine drug substance and its formulation currently available in the market, indicating its suitability for drug quality management purposes.

**Key words:** *N*-nitrosodimethylamine, zidovudine, drug substance, drug product; LC-MS/MS

### 1. Introduction

Due to the carcinogenic potential of nitrosamines, comprehensive research has been conducted for a considerable duration on the environment,<sup>1</sup> food,<sup>2</sup> rubber products,<sup>3</sup> cigarettes,<sup>4,5</sup> and cosmetics.<sup>6</sup> In contrast, the issue of nitrosamines in pharmaceuticals

emerged recently. In July 2018, European Medicines Agency (EMA) first announced that *N*-nitrosodimethylamine (NDMA) was detected in the Active Pharmaceutical Ingredient (API) of valsartan manufactured by Zhejiang Huahai.<sup>7</sup> As a result, related medicines were recalled worldwide. Following this incident, nitrosamines, including NDMA, were conti-

★ Corresponding author

Phone : +82-(0)63-219-5653 Fax : +82-(0)63-219-5638

E-mail : hecho@jbnu.ac.kr

This is an open access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

nuously detected in various API and drug products such as ranitidine, nizatidine, metformin, and rifampicin.<sup>8-10</sup> Through numerous studies by researchers, it has been revealed that the occurrence of nitrosamines in pharmaceuticals is caused by the API synthesis process, drug products manufacturing process, insufficient production process optimization or management, decomposition and oxidation of pharmaceuticals, influence of additives, and contamination from packaging materials. As a consequence, the controversy over drug safety has continued for several years.

Nitrosamine is a nitroso compound characterized by a chemical structure of  $R_1N(-R_2)-N=O$ . It is formed through the nitrosation of secondary amines and nitrates under acidic conditions. NDMA is recognized as a representative nitrosamine<sup>11</sup>. Most nitrosamines are inherently carcinogenic, and more than 90 % of them have been found to induce cancer in animal testing.<sup>12-14</sup> For this reason, NDMA and *N*-nitrosodietylamine (NDEA) have been categorized as group 2A indicating a probable carcinogen to humans and *N*-nitrosopiperidine (NPIP), *N*-nitrosobutylamine (NDBA), and *N*-nitrosopyrrolidine (NPyr) were categorized as group 2B suggesting a potential carcinogen to humans by International Agency for Research on Cancer (IARC).<sup>15</sup> The acceptable intake (AI) for nitrosamines is set by ICH M7(R1) based on

the assumption of lifetime exposure.<sup>16</sup> In the case of NDMA, the AI is set at 96 ng/day, but the maximum dose for each drug is different, so 96 ng is divided by the maximum daily dose and used as acceptable daily intake.

Zidovudine is a nucleoside reverse-transcriptase inhibitor that was first approved in 1987 for the management and treatment of Human Immunodeficiency Virus (HIV).<sup>17</sup> Typically, it is administered twice a day in combination with other antiretroviral medications, with a maximum daily dosage of 600 mg. The synthesis of the zidovudine employs dimethylformamide (DMF) as a solvent and lithium azide ( $LiN_3$ ) to replace azide (*Fig. 1*).<sup>18,19</sup> This process may lead to the generation of NDMA due to the formation of dimethylamine produced during the high-temperature processing of DMF and the generation of nitrite ions from  $LiN_3$ . Even now, concerns regarding the detection of nitrosamines in pharmaceuticals persist, and regulatory authorities are implementing follow-up measures. To accurately measure impurities at low concentration levels, it is essential to use highly sensitive and selective analytical techniques such as gas chromatography-mass spectrometry (GC-MS)<sup>5,20</sup> or high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS).<sup>21,22</sup>

Hence, in this study, a reliable method was developed to determine trace amounts of NDMA in pharmaceu-

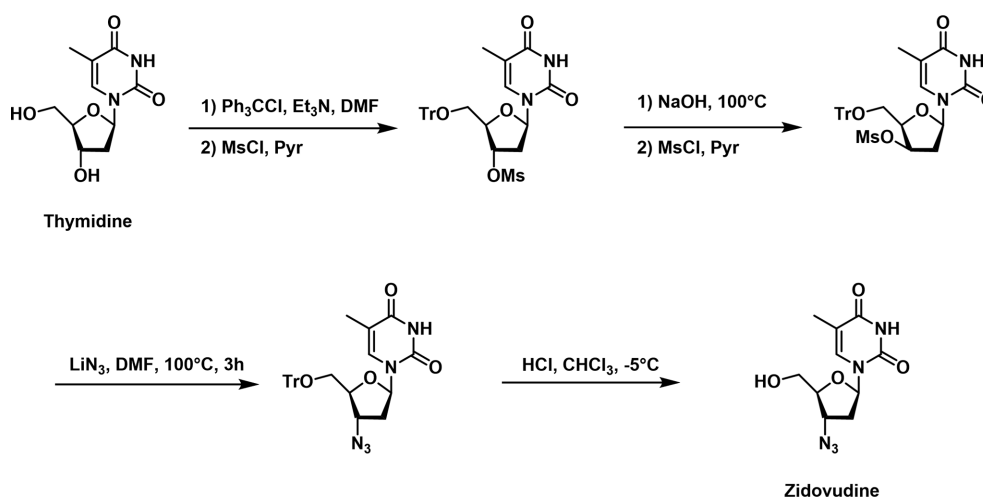


Fig. 1. Synthesis of Zidovudine.

ticals, including zidovudine, with the potential for NDMA production using HPLC-MS/MS. The content of NDMA should be controlled below 0.16 ppm in drug substance and product considering the maximum daily dose of zidovudine. The method was validated in compliance with the International Conferences of Harmonization (ICH) guideline and employed to detect the presence of NDMA in both zidovudine API and drug product currently available in the market.<sup>23</sup>

## 2. Experimental

### 2.1. Materials and reagents

*N*-nitrosodimethylamine (NDMA), the active pharmaceutical ingredients (APIs) for Zidovudine and Lamivudine were purchased from Sigma-Aldrich (St. Louis, MO, USA). *N*-nitrosodimethylamine-*d*<sub>6</sub> (NDMA-*d*<sub>6</sub>) as isotopic labeled internal standard (IS) was obtained from Sigma-Aldrich. Methanol and formic acid were obtained from Thermo Fisher Scientific (Middlesex County, MA, USA). Acetonitrile and water were purchased from J.T.Baker (Phillipsburg, NJ, USA). All of these were HPLC grade reagents. The drug product comprises 300 mg of zidovudine and 150 mg of lamivudine.

### 2.2. HPLC conditions

The chromatography system utilized in this study was a Nexera X3 series (Shimadzu, Kyoto, Japan), consisting of a system controller, solvent delivery module, autosampler, column oven, and photodiode array detector (PDA). The separation process was conducted on a Kinetex F5 chromatographic column (2.6  $\mu$ m, 100  $\times$  3 mm i.d., Phenomenex, Torrance, CA, USA) maintained at 30 °C. The mobile phases employed were water (A) and acetonitrile (B). A gradient elution flowing at a rate of 0.3 mL/min was employed as follows: 0 - 4 min: 0% (B), 4 - 10 min: 0 - 90% (B), 10 - 14 min: 90% (B), 14 - 14.5 min: 90 - 0% (B) and 14.5 - 20 min: 0% (B). The photodiode array detector was used for drug identification with full scan mode operating in the wavelength range of 200 to 400 nm.

During the initial 3 minutes and after 5.5 minutes, the eluent was redirected to a waste using a switching valve to reduce the risk of contaminating the mass ion source and the analyzer. Samples were kept at a temperature of 15 °C and a 10  $\mu$ L volume was injected for LC-MS/MS analysis.

### 2.3. Mass spectrometer conditions

The detection via multiple reaction monitoring (MRM) was conducted using a Qtrap 5500 plus mass spectrometer (AB Sciex, MA, U.S.A.). The analytes were positively ionized by an Atmospheric Pressure Chemical Ionization (APCI) and were analyzed utilizing ion transitions of *m/z* 75  $\rightarrow$  43 for NDMA, *m/z* 268  $\rightarrow$  127 for Zidovudine, *m/z* 230  $\rightarrow$  112 for Lamivudine, and *m/z* 81  $\rightarrow$  46 for the IS. The source temperature was maintained at 300 °C, and the spray voltage was set to 5500 V. APCI corona current, Curtain gas, Gas 1 (nebulizer gas), and collision activated dissociation gas (CAD) were set at 3  $\mu$ A, 25 psi, 30 psi, and 9 psi, respectively. The compound-specific mass parameters for NDMA, NDMA-*d*<sub>6</sub>, zidovudine, and lamivudine were as follows: declustering potential of 80, 56, 46, and 11 V; collision energy of 23, 23, 31, and 20 eV; collision cell exit potential of 12, 10, 14, and 12 V, respectively. All parameters were fine-tuned for liquid chromatography conditions. The data acquisition and processing were conducted using the Analyst 1.7.3 package (AB Sciex).

### 2.4. Preparation of standard solutions

The commercially available NDMA reference standard in methanol (5000  $\mu$ g/mL) was used as primary stock standard solution. A 1000  $\mu$ g/mL of intermediate stock solution was prepared by diluting with methanol using NDMA stock solution. The stock solutions were further diluted into working solutions with deionized water to achieve the required concentrations for NDMA. The NDMA-*d*<sub>6</sub> stock solution was dissolved in methanol to achieve a concentration of 1000  $\mu$ g/mL and then subsequently diluted to 1000 ng/mL with deionized water. The standards for calibration curve were prepared in 10 mL

volumetric flask by spiking an appropriate volume of the working standard solutions with 250  $\mu\text{L}$  of IS, resulting in final concentrations of 1, 2, 5, 10, 20, and 50 ng/mL for NDMA. All solutions were transferred into glass vials and then kept in a 4  $^{\circ}\text{C}$  refrigerator until the analysis.

### 2.5. Drug samples preparation

120 mg of zidovudine drug substance or powdered drug product equivalent to 120 mg zidovudine API was carefully weighed and placed into a 10 mL volumetric flask. Subsequently, 250  $\mu\text{L}$  of IS was added, followed by the addition of deionized water to reach the mark. The solution was mixed for a minute using a vortex mixer followed by ultrasonic extraction for 3 min. then centrifuged at 7,500 rpm for 10 minutes. After filtration through a 0.2  $\mu\text{m}$  PTFE syringe filter, the supernatant was carefully transferred into an autosampler vial for LC-MS analysis.

### 2.6. Method Validation

The proposed method was validated in compliance

with the ICH guideline. Validation parameters include specificity, limit of detection (LOD), limit of quantification (LOQ), linearity, precision, recovery, robustness and solution stability.

## 3. Results and Discussion

### 3.1. Optimization of the MS/MS conditions

APCI operating in the positive ion mode is known to be more efficient at ionizing NDMA.<sup>24,25</sup> During the early stages of method development, both APCI and ESI modes were examined for the ionization of NDMA. Since the use of APCI over the electrospray ionization (ESI) provided approximately 30 times greater sensitivity from our preliminary data, APCI was chosen as the ionization technique for determination of NDMA in this experiment.

The MRM transitions and MS conditions for analytes analysis were optimized using by directly infusing standard solutions. The protonated ion  $[\text{M}+\text{H}]^+$  for all analytes dominates in the Q1 full scan MS spectra. The product ion spectra of NDMA, IS,

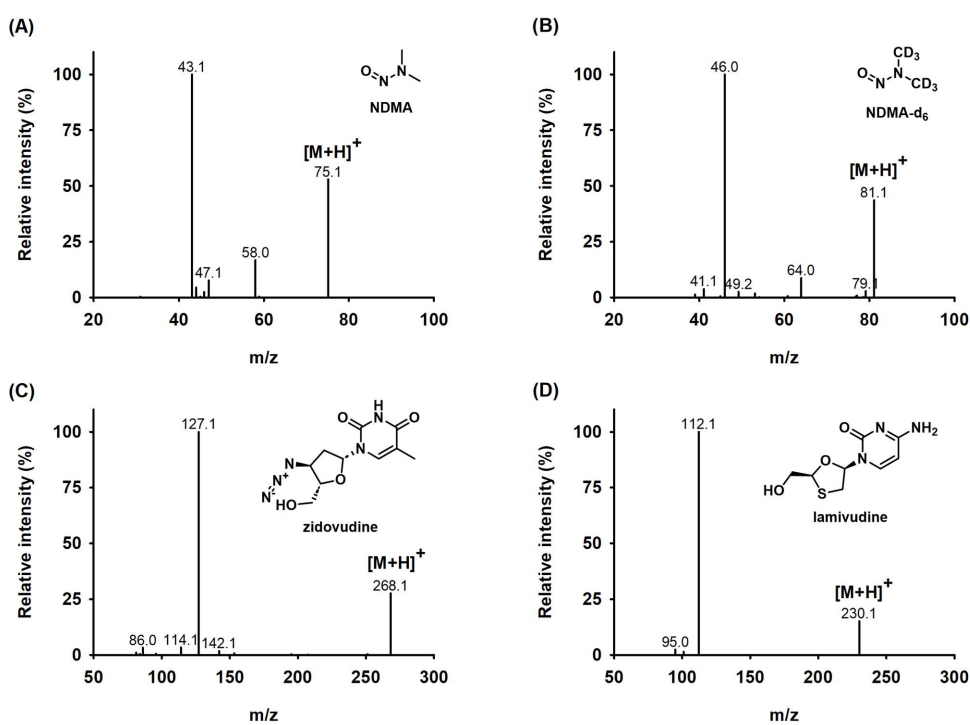


Fig. 2. Product ion spectra of NDMA (A), NDMA-d<sub>6</sub> (B), zidovudine (C) and lamivudine (D).

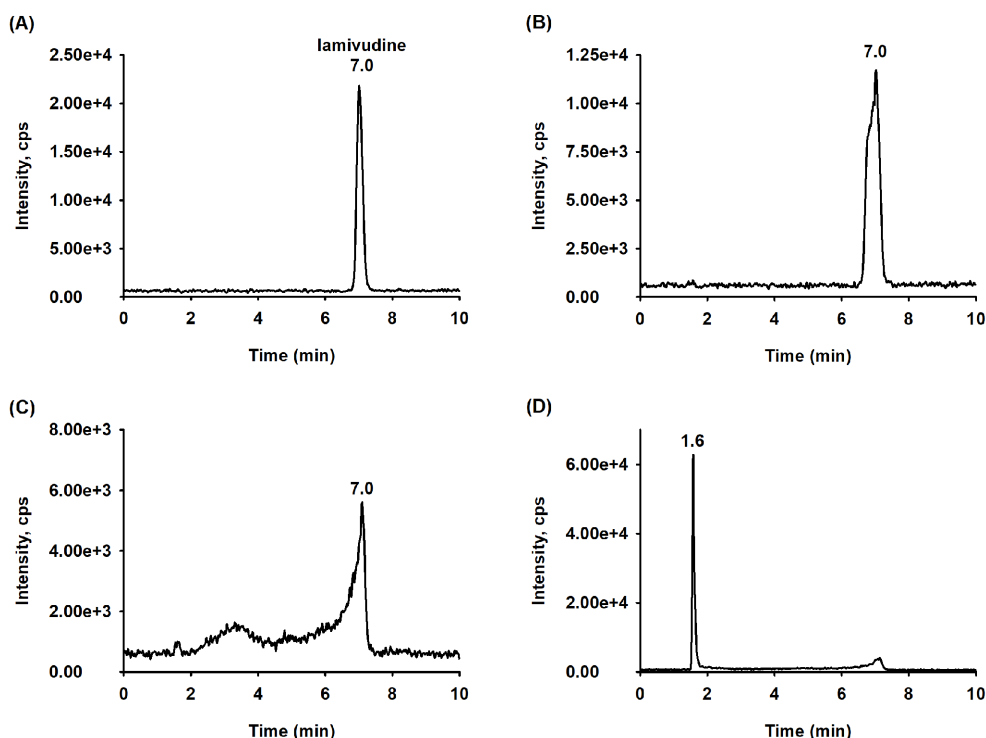


Fig. 3. Effect of injection solvents on peak shape and retention of lamivudine. (A) 100 % water, (B) 20 % MeOH, (C) 50 % MeOH and (D) 80 % MeOH as injection solvent.

zidovudine, and lamivudine are shown in Fig. 2. MRM transitions of NDMA and NDMA- $d_6$  were used for quantification of NDMA, while MRM transitions of zidovudine and lamivudine were used to optimize the chromatographic conditions during the method development.

### 3.2. Optimization of the chromatographic conditions

Conventional C18 columns often offer limited interaction with the relatively hydrophilic compound such as NDMA. In this experiment, an F5 column was employed, providing increased retention of NDMA and allowing for its adequate separation from the drug substances.

Many methods for the NDMA analysis involve direct injection into the analytical instrument after dissolution of pharmaceuticals in solvent. However, the choice of sample solvent can significantly impact peak shape and retention time, especially under

highly aqueous mobile phase conditions. When the methanol content in the sample solvent exceeds 20 %, the peak distortion was observed (Fig. 3). Moreover, using an injection solvent containing methanol  $\geq$  80 % led to the peak splitting and a loss of retention. In contrast, lamivudine was relatively well-retained with a satisfactory peak shape when injected in 100 % aqueous solvent. NDMA and zidovudine consistently retained their distinctive peak shapes and retention times regardless of the composition of the injection solvent.

Test sample is a combination tablet containing zidovudine and lamivudine. To ensure effective separation of NDMA from the components of zidovudine and lamivudine, various elution programs were investigated using water–acetonitrile with the addition of 0.1 % (v/v) formic acid for the standard solution mixture. Under initial mobile phase conditions with 5 % acetonitrile, NDMA was effectively separated from the nonpolar zidovudine API ( $k' > 3$ ), while

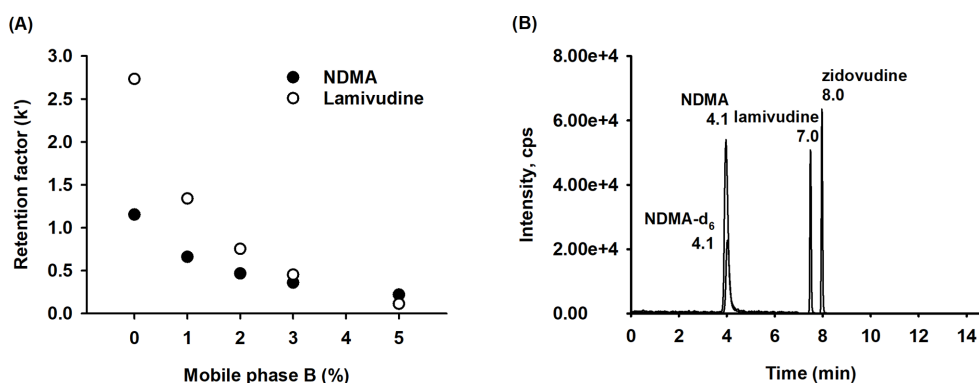


Fig. 4. Separation of NDMA, zidovudine and lamivudine according to mobile phase composition. (A) Retention factor ( $k'$ ) of NDMA and lamivudine according to proportional composition of acetonitrile in the mobile phases, (B) Separation of analytes using a 100 % aqueous initial mobile phase.

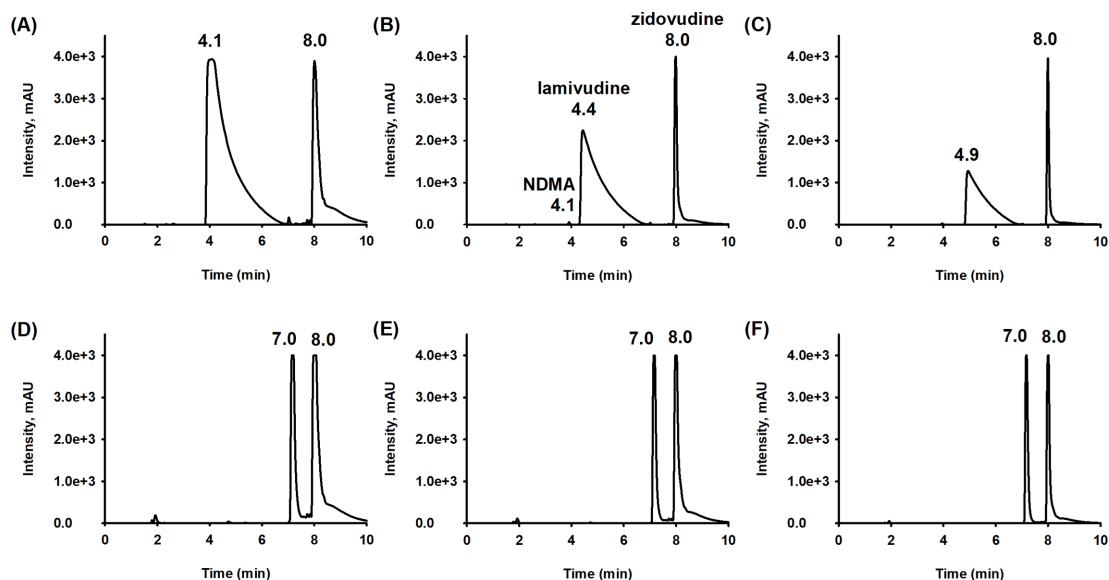


Fig. 5. LC-UV chromatogram of drug product samples. (upper) water–acetonitrile containing 0.1 % formic acid, (bottom) pure water–acetonitrile as mobile phases; (A), (D) 12 mg/mL of zidovudine and 6 mg/mL of lamivudine, (B), (E) 6 mg/mL of zidovudine and 3 mg/mL of lamivudine, (C), (F) 2 mg/mL of zidovudine and 1 mg/mL of lamivudine.

lamivudine eluted in proximity to NDMA. Satisfactory separation of the three analytes was achieved when elution began with a 100 % aqueous mobile phase (Fig. 4).

As mentioned in the sample preparation section, the prepared samples included a notably high concentration 12 mg/mL of zidovudine and 6 mg/mL of lamivudine. Further investigation was conducted to assess the impact of high-concentration sample injection on chromatography using LC-UV. When

the prepared drug product samples were injected using 100 % aqueous injection solvent into an initial mobile phase consisting of 100 % aqueous solution with the addition of 0.1 % formic acid, the retention time of lamivudine decreased and overlapped with NMDA peak, due to the solute mass surpassing the column's capacity (Fig. 5). The same phenomenon was also observed during the injection of highly concentrated standard solution. Excluding additives from the mobile phases improved early elution and

reduced peak distortion for lamivudine in the drug samples. The absence of additives in the mobile phases had no noticeable impact on the signal response of the primary target, NDMA. Consequently, pure water–acetonitrile was employed as the mobile phase.

A divert valve is employed to mitigate the potential interference caused by notable quantities of the API during trace analysis of NDMA and to prevent instrument contamination. NDMA eluted at 4.1 min and introduced to mass analyzer, while lamivudine and zidovudine were redirected to a waste at 7.0 min and 8.0 min, respectively.

### 3.3. Method validation

Selectivity was evaluated using analysis of blank, standard solution, sample solution and the sample solution spiked with NDMA. No interfering peak elutes from any samples at the retention time of NDMA. Fig. 6 shows LC–MS/MS chromatograms of NDMA analyzed for standard solution, drug substance, drug product samples.

A calibration curve of six concentration levels was generated at range of 1 – 50 ng/mL with a  $1/x$  weighing factor for NDMA. The mean equation of the regression line was  $y = 0.0357x + 0.00369$  with coefficient of

determination ( $R^2$ ) of 0.9997. Considering the sample preparation procedure, this calibration curve allowed the determination of NDMA in zidovudine across a concentration range from 0.083 to 4.17 ng/mg (50 %–2600 % of the interim limit). LOD and LOQ were established at 0.3 ng/mL and 1.0 ng/mL, respectively, defined by achieving signal-to-noise ratios (S/N) of  $\geq 3$  and  $\geq 10$ , respectively.

Precision and accuracy of NDMA was assessed at for four concentration levels (1, 3, 20 and 40 ng/mL) for both drug substance and product. The intra-day assays were conducted with five replicates on the same day, while the inter-day assays were carried out on five distinct days. Accuracy was calculated using a recovery test, which was determined by analyzing the non-spiked and spiked samples. Recoveries and precisions were 98.0 – 101.0 % and below 4.95 % as relative standard deviation (% RSD) for the intra-day assays, 97.08 – 103.65 % and below 3.92 % as for the inter-day assays, respectively (Table 1). These results demonstrate the exceptional accuracy and precision attained through the developed method.

The robustness was evaluated to examine potential impacts on peak retention and area resulting from 10 % variations in flow rate and column temperature.

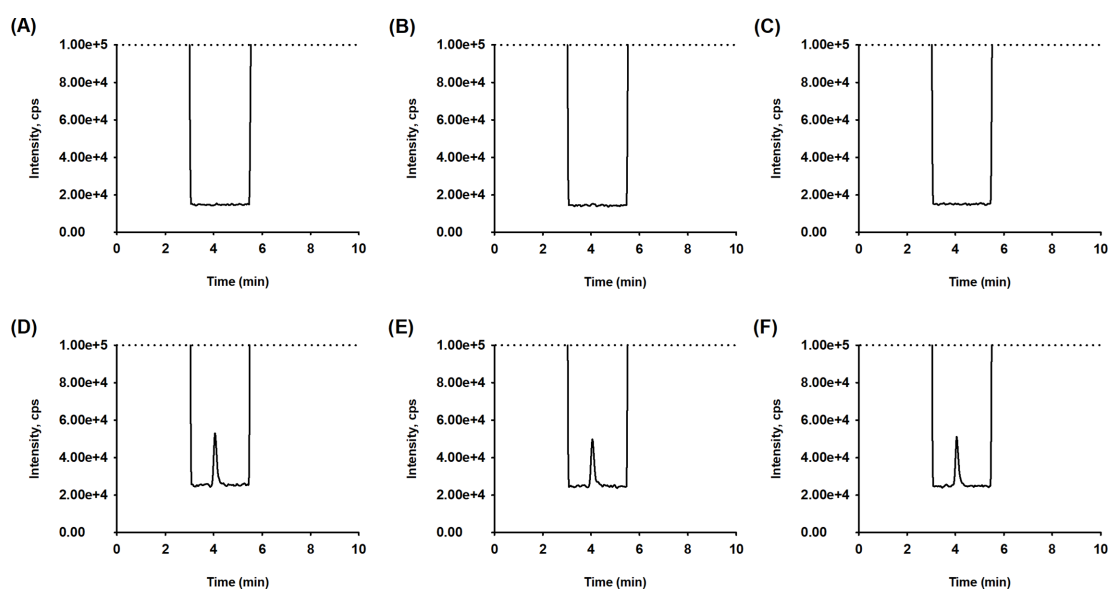


Fig. 6. LC-MS chromatograms of NDMA analysis. (A) blank solution, (B) drug substance, (C) drug product, (D) 10 ng/mL STD solution, (E) spiked Zidovudine API, and (F) spiked drug product at LOQ level (1 ng/mL).

Table 1. Accuracy and precision for NDMA in drug substance and product (n=5)

Samples	Nominal conc. (ng/mL)	Intra-day			Inter-day		
		Measured conc. (ng/mL, Mean $\pm$ SD)	Recovery (%)	Precision (RSD %)	Measured conc. (ng/mL, Mean $\pm$ SD)	Recovery (%)	Precision (RSD %)
Drug substance	1	1.01 $\pm$ 0.05	101.00	4.95	1.02 $\pm$ 0.04	102.00	3.92
	3	2.94 $\pm$ 0.07	98.00	2.42	3.0 $\pm$ 0.05	100.00	1.66
	20	20.2 $\pm$ 0.16	101.00	0.81	20.73 $\pm$ 0.33	103.65	1.59
	40	39.83 $\pm$ 0.45	99.58	1.13	38.83 $\pm$ 0.34	97.08	0.88
Drug product	1	0.99 $\pm$ 0.02	99.00	2.02	1.02 $\pm$ 0.03	102.00	2.94
	3	3.00 $\pm$ 0.08	100.00	2.58	2.99 $\pm$ 0.1	99.67	3.19
	20	20.4 $\pm$ 0.16	102.00	0.80	19.6 $\pm$ 0.16	98.00	0.83
	40	40.8 $\pm$ 0.57	102.00	1.4	39.7 $\pm$ 0.22	99.25	0.54

Table 2. Robustness and stability

Analytes	Robustness (RSD %, n = 3)				Stability (accuracy %, n = 3)			
	Column temperature 30 $\pm$ 3°C		Flow rate 0.3 $\pm$ 0.03 mL/min		12 hr		24hr	
	Area	RT	Area	RT	3 ng/mL	40 ng/mL	3 ng/mL	40 ng/mL
NDMA	3.2	10.6	2.3	3.7	94.2	103.0	99.2	97.9

The results presented that the analytical method was unaffected by small changes in the method parameters (Table 2).

The standard solutions at two concentrations (3 and 40 ng/mL) were analyzed in three replicates under different conditions to which the samples might be exposed during experiments or storage. The accuracies of NDMA were within the range of 94.2%–103.0% indicating that NDMA was stable for 12 h at 25 °C and for 24 h at 4 °C.

### 3.4. Application to API and drug sample

The suggested method was applied to determine NDMA in zidovudine API and a finished product currently distributed in the market. All samples were measured five times and NDMA was not detected in either the commercial drug substance or product (Fig. 6B-C).

## 4. Conclusions

In this study, we developed an LC-APCI-MS/MS method to quantitatively analyze trace levels of

NDMA in both zidovudine API and its formulation. The injection solvent and mobile phase composition were systematically optimized to enhance analyte separation and sensitivity. NDMA exhibited excellent chromatographic separation from the API components, and a divert valve program was implemented to effectively eliminate interference from the APIs with a very high concentration level. A comprehensive validation process was conducted to ensure the reliability and accuracy of the developed method and the results consistently met the established acceptance criteria. The developed method has been successfully applied to both active pharmaceutical ingredient and drug available in the market, and any traces of NDMA were not detected in zidovudine. The proposed method can serve as a valuable tool for the assessment of NDMA in zidovudine pharmaceuticals contributing to the quality control and safety of these essential products.

## Conflict of Interest

The authors declare no competing financial interest.



## Acknowledgments

This research was supported by a grant (20173MFDS162) from Ministry of Food and Drug Safety in 2022.

## References

1. M. J. Farré, J. Keller, N. Holling, Y. Poussade, and W. Gemjak, *Water Sci. Technol.*, **63**(4), 605-612 (2011). <https://doi.org/10.2166/wst.2011.207>
2. D. M. Pérez, G. G. Alatorre, E. B. Álvarez, E. E. Silva, and J. F. J. Alvarado, *Food Chem.*, **107**(3), 1348-1352 (2008). <https://doi.org/10.1016/j.foodchem.2007.09.064>
3. D. C. Havery and T. Fazio, *Food Chem. Toxicol.*, **20**(6), 939-944 (1982). [https://doi.org/10.1016/s0015-6264\(82\)80232-0](https://doi.org/10.1016/s0015-6264(82)80232-0)
4. S. S. Hecht and A. R. Tricker, In 'Analytical Determination of Nicotine and Related Compounds and their Metabolites', 421-488, J. W. Gorrod and P. Jacob, Eds., Elsevier Science, Amsterdam, 1999.
5. H. J. Park, J. H. Lee, S. H. Cho, S. Heo, C. Y. Yoon, and S. Y. Baek, *Anal. Sci. Technol.*, **28**(6), 385-97 (2015). <https://doi.org/10.5806/AST.2015.28.6.385>
6. L. Schettino, J. L. Benedé, and A. Chisvert, *RSC Advances*, **13**(5), 2963-2971 (2023). <https://doi.org/10.1039/D2RA06553C>
7. European Medicines Agency, 2018: "EMA reviewing medicines containing valsartan from Zhejiang Huahai following detection of an impurity" (EMA/459276/2018).
8. Korea Food and Drug Administration, Sartent's Hypertension Drug Impurity Management Standards Disclosure, Establishment of Sartan NDMA, NDEA temporary management standard and test method education, <http://www.nifds.go.kr/>, Accessed 10 April 2020.
9. US FDA Statement, 1 Nov (2019) <https://www.fda.gov/news-events/press-announcements/fda-requests-removal-all-ranitidine-products-zantac-market>, Accessed 10 April 2020.
10. US FDA, FDA updates and press announcements on angiotensin II receptor blocker (ARB) recalls (valsartan, losartan, and irbesartan), <https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-angiotensin-II-receptor-blocker-arb-recalls-valsartan-losartan>, Accessed 10 April 2020.
11. H. Robles, In 'Encyclopedia of Toxicology (Third Edition)', 584-585, P. Wexler, Ed., Academic Press, Oxford, 2014.
12. W. Lijinsky, *Cancer Metastasis Rev.*, **6**(3), 301-356 (1987). <https://doi.org/10.1007/BF00144269>
13. A. R. Tricker and R. Preussmann, *Mutat. Res.*, **259**(3-4), 277-89 (1991). [https://doi.org/10.1016/0165-1218\(91\)90123-4](https://doi.org/10.1016/0165-1218(91)90123-4)
14. L. Xu, Y.-H. Qu, X.-D. Chu, R. Wang, H. H. Nelson, Y.-T. Gao, and J.-M. Yuan, *PLOS ONE*, **10**(2), e0117326 (2015). <https://doi.org/10.1371/journal.pone.0117326>
15. International Agency for Research on Cancer. 2023. IARC monographs on the identification of carcinogenic hazard to humans. Available at : <https://monographs.iarc.who.int/agents-classified-by-the-iarc/2021/>. Accessed 6 Sep 2023.
16. European Medicines Agency. ICH M7 assessment and control of DNA (reactive) mutagenic impurities in pharmaceutical to limit potential carcinogenic risk. Amsterdam: European Medicines Agency; 2018. <https://www.ema.europa.eu/en/ich-m7-assessment-control-dna-reactive-mutagenic-impurities-pharmaceuticals-limit-potential#current-effective-version--section>.
17. S. Chaudhuri, J. A. Symons, and J. Deval, *Antiviral Res.*, **155**, 76-88 (2018). <https://doi.org/10.1016/j.antiviral.2018.05.005>
18. S. Czernecki and J.-M. Valéry, *Synthesis*, **1991**(3), 239-240 (1991) <http://doi.org/10.1055/s-1991-26434>
19. J. P. Horwitz, J. Chua, and M. Noel, *J. Org. Chem.*, **29**(7), 2076-2078 (1964). <https://doi.org/10.1021/jo01030a546>
20. H.-H. Lim, Y.-S. Oh, and H.-S. Shin, *J. Pharm. Biomed. Anal.*, **189**, 113460 (2020). <http://doi.org/10.1016/j.jpba.2020.113460>
21. Ki-Chan Han and Hekap Kim, *Anal. Sci. Technol.*, **23**(6), 551-559 (2010). <http://doi.org/10.5806/AST.2010.23.6.551>
22. R. Reddy Gopireddy, A. Maruthapillai, and M. Tamilselvi, *Mater. Today-Proc.*, **68**, A7-A20 (2022). <https://doi.org/10.1016/j.matpr.2022.11.112>
23. Validation of Analytical Procedures: Text and methodology Q2(R1), ICH Harmonised tripartite guideline. Current Step 4 version, Accessed 27 Oct 1994.
24. C.-W. Hu, Y.-M. Shih, H.-H. Liu, Y.-C. Chiang, C.-M.

- Chen, and M.-R. Chao, *J. hazard. Mater.*, **310**, 207-216, (2016). <https://doi.org/10.1016/j.jhazmat.2016.02.048>
25. F. Sörgel, M. Kinzig, M. Abdel-Tawab, C. Bidmon, A. Schreiber, S. Ermel, J. Wohlfart, A. Besa, O. Scherf-Clavel, and U. Holzgrabe, *J. Pharm. Biomed. Anal.*, **172**, 395-405, (2019). <https://doi.org/10.1016/j.jpba.2019.05.022>

---

### Authors' Positions

Yujin Lim : Graduate Student  
Aelim Kim : Graduate Student  
Yong-Moon Lee : Professor  
Hwangeui Cho : Assistant Professor