JPPT | Compatibility Study

Chemical Compatibility of N-Acetylcysteine After the Simultaneous Intravenous Administration of Ondansetron

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OBJECTIVE This study evaluated the chemical compatibility of N-acetylcysteine (NAC) and ondansetron to simplify the treatment of acute nausea and vomiting during intravenous (IV) NAC administration. NAC is commonly used to treat acetaminophen overdose, but its 21-hour IV infusion is often interrupted for ondansetron administration, which can pose risks.

METHODS High-performance liquid chromatography with ultraviolet detection was used to quantify NAC. To simulate IV administration, a closed-circuit pump with multiple independent lines, was plumbed with Y-sites to circulate NAC at concentrations matching 30- and 100-kg loading doses and 4-mg ondansetron was pushed into the flow paths. Control lines without ondansetron were also maintained. Samples were collected at 10, 20, and 30 minutes postondansetron introduction. NAC concentrations in single-drug and combination lines were compared using an unpaired *t*-test with Welch's correction ($p = 0.05$).

RESULTS The mean concentrations for the 100-kg dose were 55.23 and 55.28 mg/mL for control and with ondansetron, respectively. The 30-kg cohort included 36.38 mg/mL for control and 36.49 mg/mL with ondansetron. The results of the unpaired *t*-test for either weight illustrated that no statistical significance was achieved. Furthermore, the *t*-values of 0.2013 for 100 kg and 0.8556 for 30 kg support a less likely chance of significant difference.

CONCLUSION Based on this experiment, ondansetron can be introduced into an NAC infusion via IV push *in vitro* without affecting the NAC concentration in the solution. The likelihood of IV compatibility for NAC and ondansetron could permit no infusion interruptions, reducing unnecessary risk of acetaminophen toxicity.

ABBREVIATIONS: HPLC, high-performance liquid chromatography; IV, intravenous; LC, liquid chromatography; LD, loading does; MS, mass spectrometry; NAC, N-acetyl cysteine; NS, normal saline; UV, ultraviolet

KEYWORDS acetaminophen; NAC; N-acetylcysteine; ondansetron; compatibility

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Introduction

Acetaminophen is associated with more than 50,000 annual emergency room visits and approximately 500 deaths, mostly attributed to unintentional overdose.[1] Acetaminophen overdose poses a significant risk of hepatotoxicity, especially in children and adolescents. N-acetylcysteine (NAC) is the most effective therapy for acetaminophen poisoning. However, oral and intravenous (IV) administration of NAC are often complicated by nausea and vomiting, with a reported occurrence of 23% and 9%, respectively, necessitating the use of antiemetics.[2] Ondansetron, a 5-HT3 receptor antagonist, is an antiemetic often employed to treat acute nausea and vomiting in the setting of NAC use. While NAC can be used both orally and IV, IV administration has been associated with fewer side effects, shorter treatment duration, and lower cost.[3] Protocols for NAC administration involve 20- to 21-hour-long infusions. Any interruption of the infusion for incompatible or unknown compatibility medications involves delays in the treatment due to flushing, medication administration, flushing, and restart of the NAC. Errors could be made at any point during this interruption, including a failure to restart the NAC infusion. The risks associated with unknown compatibility or infusion interruption are minimized by determining the compatibility of ondansetron with NAC. The need to mitigate nausea and vomiting applies to up to 60% of patients and is often most urgent within the initial hours of NAC therapy. It also coincides with when acetaminophen serum concentrations are also at their peak.[4,5] Administration of ondansetron is shown to increase overall tolerance of NAC treatment to a positive clinical endpoint.[6,7] As such, we investigated the IV compatibility of NAC when combined with ondansetron to reduce the steps in treating acute nausea and vomiting. Some hospitals have already demonstrated the utility of a simpler "one-bag"

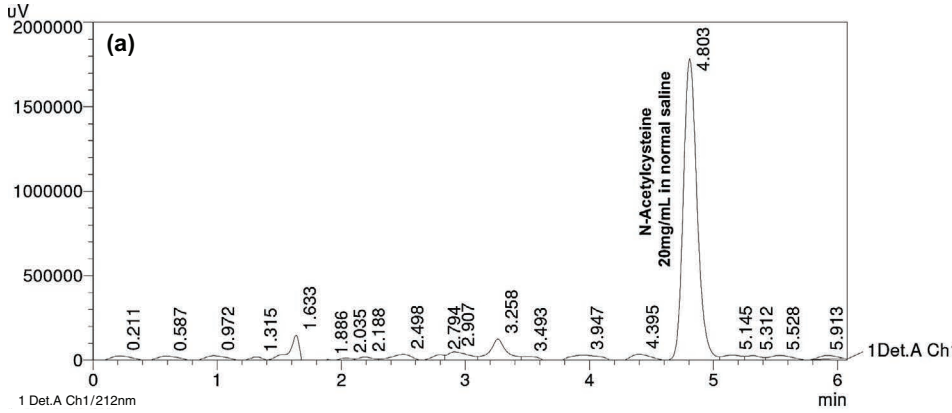
method for administering NAC, which could include ondansetron if chemical compatibility is confirmed.[8]

Methods

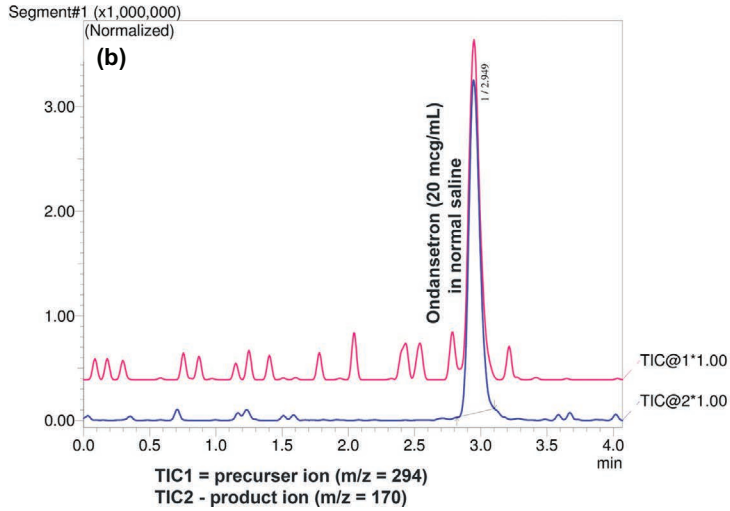
Equipment and Chromatographic Conditions. A Shimadzu high-performance liquid chromatography (HPLC) system, equipped with an autosampler, column oven, in-line degasser, and ultraviolet (UV) detection set at 212 nm was used for all chromatographic measurements (Shimadzu Scientific, Kyoto, Japan). NAC calibration solutions (1–60 mg/mL) were prepared in HPLC-grade water. The NAC standard was acquired from Alfa Aesar (Haverhill, MA, USA). The chromatographic separation used isocratic conditions with 90% water with 0.1% trifluoroacetic acid (A) and 10% acetonitrile (B) at a flow rate of 0.5000 mL/min (Honeywell Burdick & Jackson, Morris Township, NJ, USA). The analytical column was a Waters XBridge C18 column (4.6 × 150 mm; 3.5 µm), maintained at 50°C (Waters Corporation, Milford, MA, USA). All injected samples were filtered using a 0.22-µm syringe filter before injection (Signa Aldrich, St. Louis, MO, USA). All sample injection volumes were 1 µL, and the autosampler was purged with 2-propanol between injections (Fisher Scientific, Waltham, MA, USA).

The HPLC method for quantification of NAC in normal saline (NS) was fashioned after Gowda et al,[9] who used an isocratic mixture of 0.1% trifluoroacetic acid in water and acetonitrile (96/4) on a reversed-phase column. The concentration of aqueous was adjusted in our method to ensure the retention time of NAC differed from that of ondansetron. While ondansetron was not quantified using HPLC-UV, as the concentration in the experimental samples was too low, its retention time was verified by

Figure 1. (a) Example HPLC-UV chromatogram (212 nm) of N-acetylcysteine in normal saline (20 mg/mL)* (b) Example ion chromatogram (+ESI-LC-MS/MS) of ondansetron in normal saline (20 mcg/mL)



*Det, detector; HPLC-UV, high-performance liquid chromatography with ultraviolet detection; UV, Ultraviolet



ESI, electrospray ionization; LC, liquid chromatography; MS, mass spectrometry; m/z, mass-to-charge ratio; TIC, total ion chromatogram

a positive electrospray ionization liquid chromatography with tandem mass spectrometry experiment. An example of a UV chromatogram of NAC in NS and an electrospray ionization liquid chromatography with a tandem mass spectrometry ion chromatogram of ondansetron are shown in Figure 1.

Table 1. Interday Precision and Accuracy for HPLC-UV Assay Used in the for Quantification of N-Acetylcysteine in Normal Saline

Concentration Spiked, mg/mL	Concentration Measured,* mg/mL ± SD	% Error (n = 20)	% RSD (n = 20)
30	29.77 ± 0.65	0.75	2.19
20	21.37 ± 0.42	6.86	1.97
15	15.97 ± 0.38	6.46	2.40
10	10.37 ± 0.40	3.73	3.88
5	4.92 ± 0.37	1.62	7.55

HPLC-UV, high-performance liquid chromatography with ultraviolet detection; % RSD, % relative standard deviation

* n = 5 replicate measurements per concentration per day, collected over 4 separate days.

Method Validation. The HPLC-UV method for NAC was validated in the range of 1 to 30 mg/mL over 4 nonconsecutive days. The precision, as represented by the percent relative standard deviation and accuracy, as represented by the percent error, were assessed for each calibration point on 4 separate nonconsecutive days by 2 different analysts. On each day of validation, a calibration curve was prepared with the following points: 1, 5, 10, 20, and 30 mg/mL NAC, and an additional 5 quality control samples were prepared at each concentration. The results of the interday and intraday precision and accuracy are summarized in Tables 1 and 2, respectively. The designation of spiked concentration refers to the intended concentration in the preparation of the calibration standards. System suitability parameters were monitored throughout the validation, including tailing factor, resolution, and theoretical plates. System suitability indicated symmetrical peaks that were well resolved

Table 2. Intraday Precision and Accuracy for HPLC-UV Assay Used in the for Quantification of N-Acetylcysteine in Normal Saline

Validation Day	Parameter	Spiked Concentration, mg/mL				
		30	20	15	10	5
1	Concentration measured, mg/mL (n = 5)	30.00	21.84	16.27	10.67	5.19
	SD	0.22	0.06	0.05	0.03	0.44
	% Error	0.01	9.19	8.47	6.69	3.71
	% RSD	0.73	0.28	0.32	0.24	8.45
2	Concentration measured, mg/mL (n = 5)	30.03	21.31	16.12	10.75	5.00
	SD	1.16	0.61	0.04	0.17	0.02
	% Error	0.10	6.53	7.45	7.55	0.00
	% RSD	3.88	2.85	0.24	1.54	0.39
3	Concentration measured, mg/mL (n = 5)	29.81	21.29	16.10	10.25	5.00
	SD	0.23	0.21	0.16	0.22	0.27
	% Error	0.62	6.46	7.34	2.45	0.00
	% RSD	0.77	0.99	1.02	2.12	5.40
4	Concentration measured, mg/mL (n = 5)	29.25	21.05	15.39	9.82	4.49
	SD	0.28	0.10	0.29	0.05	0.23
	% Error	2.50	5.25	2.57	1.78	10.19
	% RSD	0.94	0.46	1.86	0.48	5.20

HPLC-UV, high-performance liquid chromatography with ultraviolet detection; % RSD, % relative standard deviation

from other components in the chromatogram, with theoretical plates averaging 5036, average resolution of 1.92, and tailing factor of 1.34. Method selectivity was verified by injecting blank 0.9% sodium chloride from Baxter (LOT P419764, EXP Dec 2022; Deerfield, IL, USA) and ondansetron (Baxter, Deerfield, IL, USA; Lots A0E1015A [11/23] and O61096 [6/24]) diluted in NS at the experimental concentrations.

Chemical Compatibility Experiment. For this experiment, NAC from Sagent (Schaumburg, IL. USA; LOTS 7606333 [2/23], 7606476 [4/23], A000040574 [6/23], and 7606953 [10/23]) was diluted in NS to replicate loading dose (LD) concentrations for a 30- and 100-kg patient.[10,11] For the 30-kg patient LD, this involved diluting 22.5 mL of the NAC drug product into 100-mL NS for an approximate final NAC concentration of 36.7 mg/mL. For the 100-kg patient LD, 75 mL of NAC was diluted into 200-mL NS for an approximate final NAC concentration of 54.5 mg/mL. Two iterations of each LD solution were prepared, and each was introduced to a 250-mL Erlenmeyer flask. Each flask was connected on independent channels to a multichannel pump (MCP 3000 Digital Multichannel Pump, Model #13-310-662, Fisher Scientific, Waltham, MA, USA) using medical-grade tubing (Component Supply Company, Tygon ND100-65 Med/Surgical Tubing, 1/16" ID, 1/8" OD, 1/32" wall, Sparta, TN, USA). The pump was set to continuously recirculate the preparations through 4 independent channels at the lowest pump flow rate setting. The tubing was plumbed to accommodate a y-site junction. After allowing the NAC solutions to circulate through the pump for 10 minutes, 2 mL of ondansetron drug was administered via IV push into the y-site for 1 channel of the 30-kg LD and 1 channel of the 100-kg LD. For the other 30- and 100-kg LD flasks, 2-mL NS was introduced via IV push to maintain consistent volume with the experimental samples (ondansetron flasks). Three samples (1-mL each) were removed from each flask at 10, 20, and 30 minutes of circulation and filtered into autosampler vials. These times indicate the duration of contact between the 2 drugs in the IV solutions. These samples were subject to immediate HPLC analysis using the aforementioned method, with 1 injection per sample. Peak areas from the samples were compared to freshly prepared calibration standards of NAC in water, and NAC concentrations in the samples were calculated. The average calculated concentrations from the NAC + ondansetron samples were compared with the NAC (no ondansetron) samples for each loading dose using an unpaired, 2-tailed students *t*-test with a p value of 0.05 (Graph Pad Prism, v 9.03, La Jolla, CA, USA).

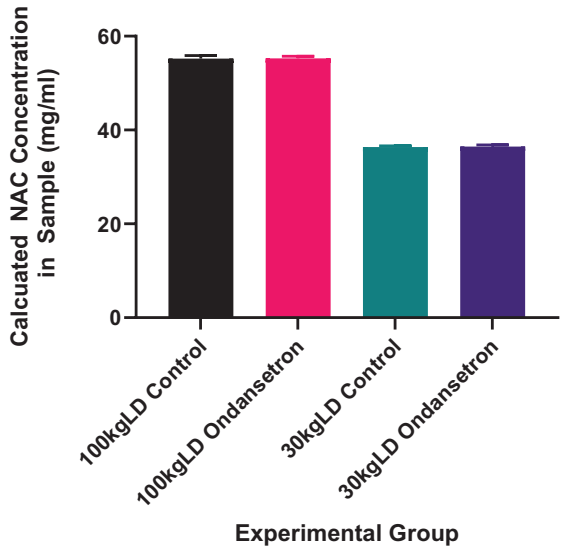


Figure 2. Graphical representation of calculated concentrations of N-acetylcysteine infusion solutions with and without the introduction of ondansetron. No statistically significant difference was detected between the groups using an unpaired student's *t*-test ($p < 0.05$)

Results

Chemical Compatibility. For the 30-kg patient LD, the NAC concentrations in the samples with ondansetron added were 36.49 ± 0.32 mg/mL, while those without ondansetron (controls) contained 36.38 ± 0.23 mg/mL NAC. For the 100-kg patient LD, the NAC concentrations in the samples with ondansetron added were 55.28 ± 0.44 mg/mL, while those without ondansetron (controls) contained 55.23 ± 0.63 mg/mL NAC. These data are graphically represented in Figure 2. Of note, these were in line with the approximate concentration of NAC expected in the preparation of NAC solutions in NS, 36.7 and 54.5 mg/mL, respectively. When the control and experimental groups were compared using an unpaired, 2-tailed student's *t*-test, no statistically significant differences were found (30-kg LD group, $p = 0.4062$; 100-kg LD group, $p = 0.8432$). Additionally, the retention time of the NAC peak remained unchanged.

Discussion

Based on this chemical compatibility experiment, the introduction of IV push or infusion of up to 30 minutes of ondansetron via a y-site into an IV infusion of NAC at loading doses appropriate for a 30- and 100-kg patient does not affect the NAC concentration in a statistically significant way. While those of the 30- and 100-kg patients represent a high and low concentration, in terms of loading dose, for NAC, additional experiments at concentrations appropriate for second and third doses of NAC could investigate if this compatibility is compromised at lower NAC solution concentrations. As such, our data indicate

that the administration of IV push or infusion of up to 30 minutes of ondansetron (4 mg) via a y-site into an NAC infusion of loading dose concentration does not compromise the concentration of NAC delivered to the patient.

Possible limitations of our study include the absence of ondansetron quantification during the compatibility experiment with NAC. Future experiments may consider an assay that can simultaneously quantify both drugs. Additionally, our experimental conditions were limited because an *in vitro* closed-circuit pump may not capture all potential variables in clinical IV administration. Despite these limitations, our data support that ondansetron and NAC can be co-administered without compromising the chemical integrity of NAC, the most critical component of mitigating acetaminophen toxicity.

Article Information

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References

1. Agrawal S, Khazaeni B. Acetaminophen toxicity. In: *StatPearls*. Treasure Island, FL: StatPearls Publishing; 2024.
2. Bebarta VS, Kao L, Froberg B, et al. A multicenter comparison of the safety of oral versus intravenous acetylcysteine for treatment of acetaminophen overdose. *Clin Toxicol* (Phila). 2010;48(5):424–430.
3. Miller MA, Navarro M, Bird SB, Donovan JL. Antiemetic use in acetaminophen poisoning: how does the route of N-acetylcysteine administration affect utilization? *J Med Toxicol*. 2007;3(4):152–156.
4. Bateman DN, Dear JW, Thanacoody HKR, et al. Reduction of adverse effects from intravenous acetylcysteine treatment for paracetamol poisoning: a randomised controlled trial. *Lancet*. 2014;383(9918):697–704.
5. Sandilands EA, Bateman DN. Adverse reactions associated with acetylcysteine. *Clin Toxicol* (Phila). 2009;47(2):81–88.
6. Clark RF, Chen R, Williams SR, et al. The use of ondansetron in the treatment of nausea and vomiting associated with acetaminophen poisoning. *J Toxicol Clin Toxicol*. 1996;34(2):163–167.
7. Tobias JD, Gregory DF, Deshpande JK. Ondansetron to prevent emesis following N-acetylcysteine for acetaminophen intoxication. *Pediatric Emergency Care*. 1992;8(6):345.
8. Mullins ME, Yu M, O'Grady L, et al. Adverse reactions in patients treated with the one-bag method of N-acetylcysteine for acetaminophen ingestion. *Toxicol Commun*. 2020;4(1):49–54.
9. Gowda P, Schaefer A, Schuck T. A simple RP-HPLC method for the stability-indicating determination of N-acetyl-L-cysteine and N,N'-diacetyl-L-cystine in cell culture media. *Cell Gene Ther Insights*. 2020; 6:303–323.
10. Dart RC, Mullins ME, Matoushek T, et al. Management of acetaminophen poisoning in the US and Canada: a consensus statement. *JAMA Netw Open*. 2023;6(8):e2327739.
11. Drugs.com. Acetylcysteine dosage guide + max dose, adjustments. Accessed July 3, 2024. https://www.drugs.com/dosage/acetylcysteine.html