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Scientific Report

Proton NMR Analyses of RD286 Treated Xylazine HCl

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1. Introduction

Aseptic Health, LLC (Aseptic) is developing products (Sanitize IT) to neutralize potent compounds such as opioids. Aseptic has previously confirmed the degradation of fentanyl and heroin via Sanitize IT (RD286 and RD500) through ^{1}H nuclear magnetic resonance (NMR) analysis (1), (2) and requested the effectiveness of RD286 on xylazine hydrochloride using ^{1}H NMR spectroscopy. Xylazine is used in veterinary medicine as a sedative, analgesic, and muscle relaxant (3). While the Food and Drug Administration (FDA) is aware of the increasing presence of xylazine in illicit drugs and warns of the potential dangers of human exposure, there are currently no approved uses of xylazine for humans (4), (5).

For the effectiveness testing, a xylazine sample was treated with RD286 for one minute and 10 minutes incubation at room temperature after vortexing per the client's request (see the experimental section for more details). The RD286-treated samples and reference materials (xylazine HCl and RD286) were analyzed by ^{1}H NMR to determine whether the treatment of RD286 was effective in degrading xylazine HCl. **Table 1** contains details of the samples used to investigate the efficacy of RD286 on xylazine HCl. Throughout this report, xylazine HCl will be referred to as xylazine.

Table 1. Summary of samples analyzed by ^{1}H NMR spectroscopy.

Sample Description	Triclinic Labs' Identifier	Lot Number	NMR Filename
RD286	TCL19534	N/A	N/A
Xylazine HCl	TCL19551	LRAC7339	N/A
Xylazine HCl reference standard	1288-26-1	N/A	NMR1-3075
RD286 treated xylazine HCl sample (one minute incubation)	1288-26-2	N/A	NMR1-3076
RD286 treated xylazine HCl sample (ten minutes incubation)	1288-26-3	N/A	NMR1-3077
Intact RD286	1129-33-1	N/A	NMR1-2103

2. Results

In this study, the ^{1}H NMR spectra of xylazine treated with RD286 (one minute and ten minutes incubation) were compared to reference spectra (RD 286 and xylazine) in **Figures 1-3**. Analysis revealed that the ^{1}H NMR spectra of RD286-treated xylazine displayed peak shifts in the xylazine signals at 6.95 and 6.86 ppm compared to the xylazine reference standard (RS) shown in **Figure 2**. Peaks observed at 3.43 and 3.32 ppm of the RS spectrum exhibited a shift of approximately 0.43 ppm in RD286-treated xylazine samples (**Figure 3**). It should be noted that the peak at 3.43 ppm of xylazine in the RS spectrum overlapped with the RD286

signal in the spectrum of RD286-treated samples. An additional peak shift of xylazine at 2.07 ppm of RD286-treated samples (**Figure 3**) was also observed. Interestingly, a new peak was detected at 2.0 ppm in the RD286-treated samples that was not observed in the xylazine RS (**Figure 3**). These observations suggest that treatment of xylazine with RD286 caused structural changes in xylazine.

3. Conclusion

The ^{1}H NMR analysis on the xylazine reference standard and RD286-treated xylazine samples showed evidence that the treatment of RD286 changed the chemical structure of xylazine after one minute and ten minutes of incubation.

4. References

1. Triclinic Labs non-GMP report for Aseptic Health, LLC, "1H NMR analyses of RD286 and RD500 Treated Samples of Fentanyl", R2022213.01, dated April 20, 2022.

2. Triclinic Labs non-GMP report for Aseptic Health, LLC, "Proton NMR Analyses of RD286 Treated Heroin", R2022583.02, dated November 04, 2022.

3. https://www.deadiversion.usdoj.gov/drug_chem_info/Xylazine.pdf.

4. https://www.fda.gov/media/162981/download.

5. https://www.dea.gov/documents/2022/2022-12/2022-12-21/growing-threat-xylazine-and-its-mixture-illicit-drugs.

5. Experimental

5.1. ^{1}H NMR Spectroscopy

The ^{1}H NMR spectra were acquired on a Bruker Avance NEO 400 MHz spectrometer using TopSpin 4.1.4 software at Triclinic Labs. The acquired spectra were processed using TopSpin 4.1.4 and referenced to the chemical shift of the residual solvent peak (e.g., D_2O at 4.79 ppm). More detailed NMR sample preparation and acquisition parameters are provided in **Tables 2 and 3**.

Table 2. NMR sample preparation.

Sample Description	Sample ID	Sample Preparation
Intact RD286	1129-33-1	1 mL of RD286 (TCL16043) solution was directly added into coaxial tube[1].
Xylazine reference standard	1288-26-1	5.6 mg of xylazine HCl was dissolved in 1 mL of D_2O.
RD286 treated xylazine sample (one minute incubation)	1288-26-2	3.0 mg of xylazine HCl was added into a microcentrifuge tube, then 1 mL of RD286 (TCL19534) was added, thoroughly vortexed, and incubated for one minute at room temperature. After incubation, the RD286 treaded sample was filtered by a 0.45 µm (17mm diameter) nylon filter to collect the supernatant. The supernatant was then added to a 5-mm NMR tube and analyzed.
RD286 treated xylazine sample (ten minutes incubation)	1288-26-3	3.2 mg of xylazine HCl was added into a microcentrifuge tube, then 1 mL of RD286 (TCL19534) was added, thoroughly vortexed, and incubated for ten minutes at room temperature. After incubation, the RD286 treaded sample was filtered by a 0.45 µm (17mm diameter) nylon filter to collect the supernatant. The supernatant was then added to a 5-mm NMR tube and analyzed.

Table 3. NMR acquisition parameters.

Parameter Name	Parameter Value
Transmitter Frequency	400.15 MHz
Acquisition Time	4 sec
Spectral Width	6250 Hz
Number of Scans	128
Sequence	ZG
P1 (pulse width)	13.7 µsec
PLW1 (pulse power)	14.3 W
D1 (relaxation delay)	4 sec
Line Broadening	0.1 Hz

[1] At the client's request, the previously acquired spectrum of RD286 was reused.

6. Figures

Figure 1. Comparative analysis of the ^{1}H NMR spectra of the intact RD286, the xylazine reference standard, and the RD286 treated xylazine samples.

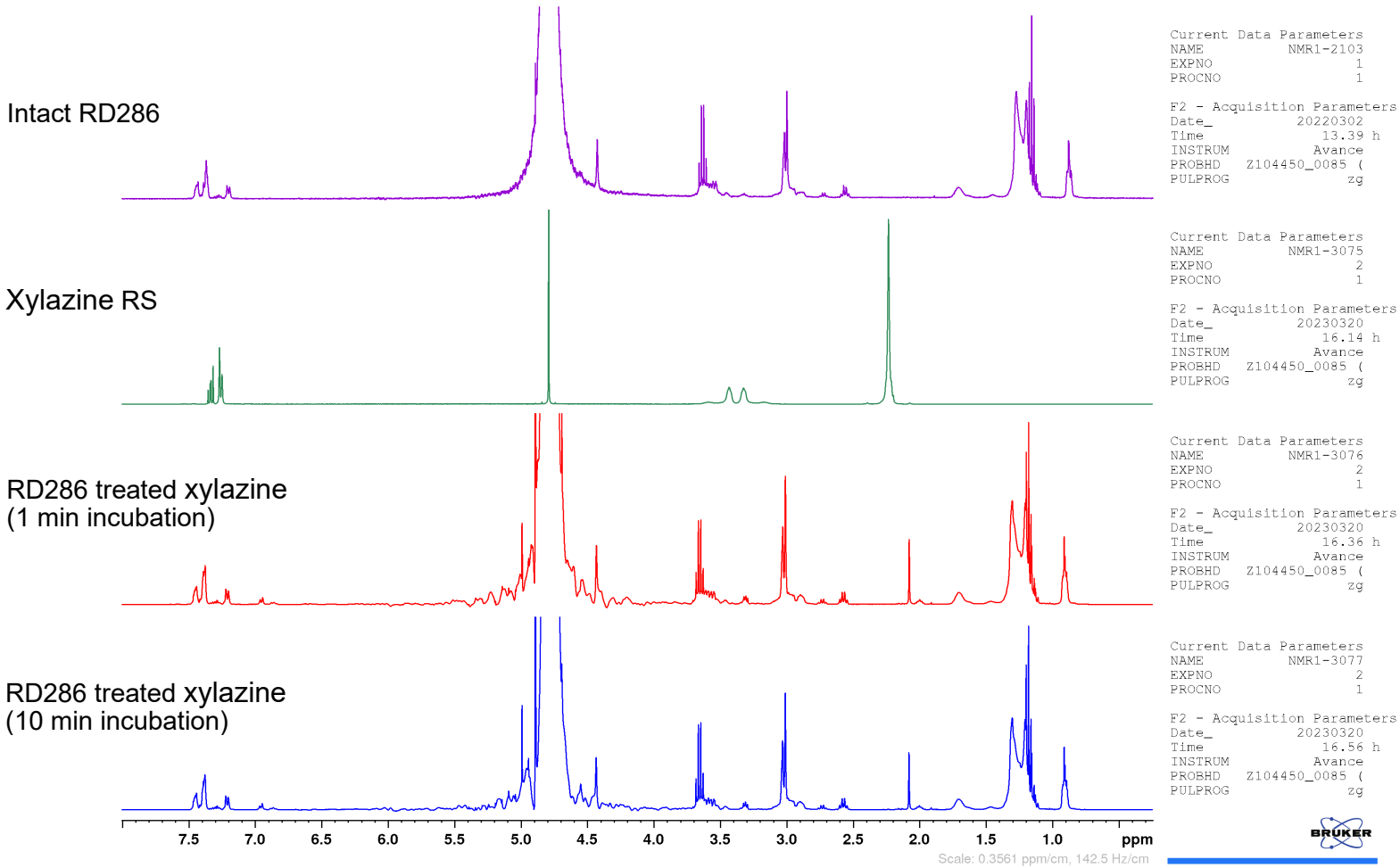

Figure 2. Comparative analysis of the ^{1}H NMR spectra (7.8 – 6.5 ppm) of the intact RD286, the xylazine reference standard, and the RD286 treated xylazine samples.

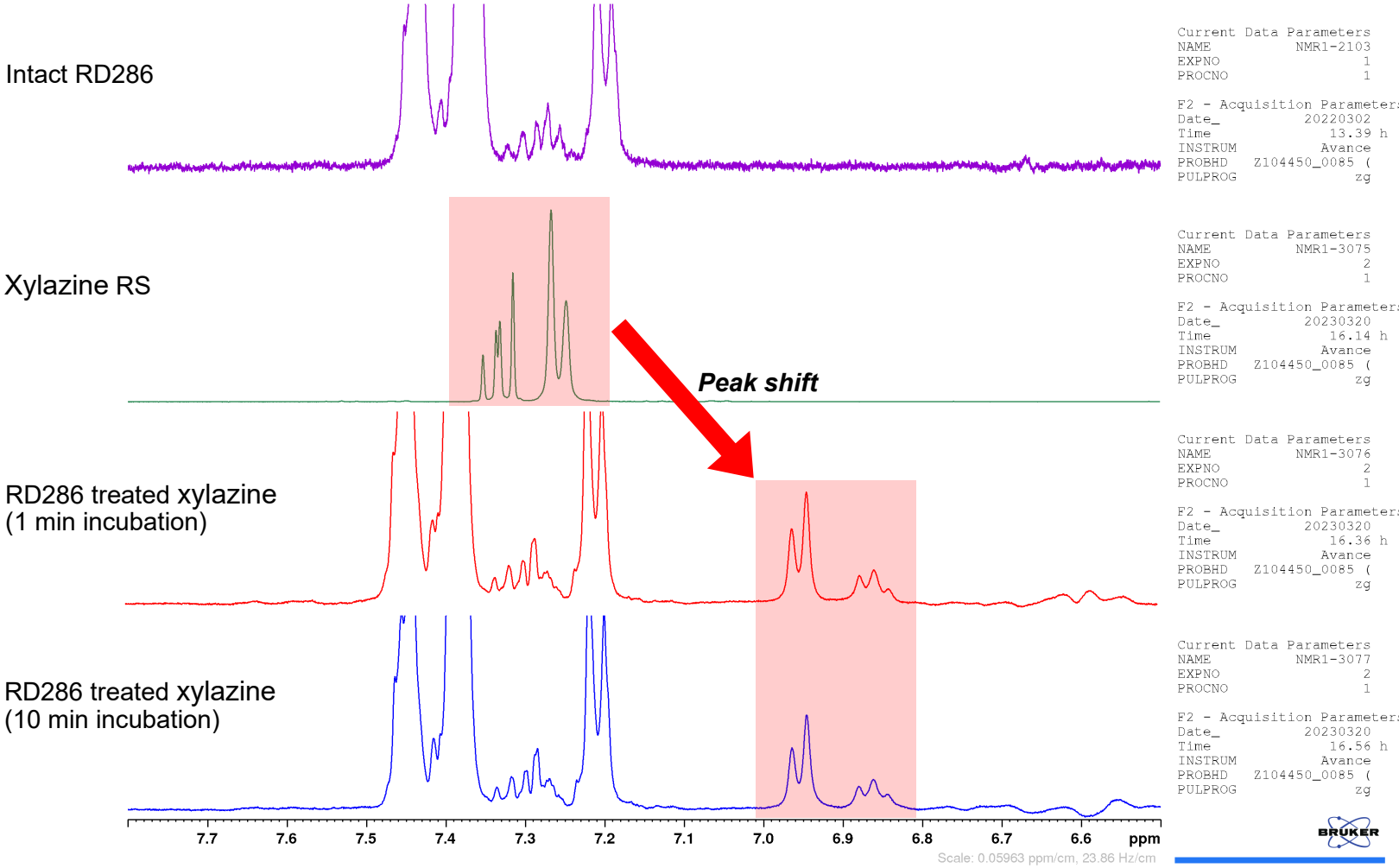

Figure 3. Comparative analysis of the ^{1}H NMR spectra (3.9 – 1.8 ppm) of the intact RD286, the xylazine reference standard, and the RD286 treated xylazine samples.