Electronic Supplementary Material

A Stability Indicating RP-HPLC Method for Determination of the COVID-19 Drug Molnupiravir Applied Using Nanoformulations in Permeability Studies

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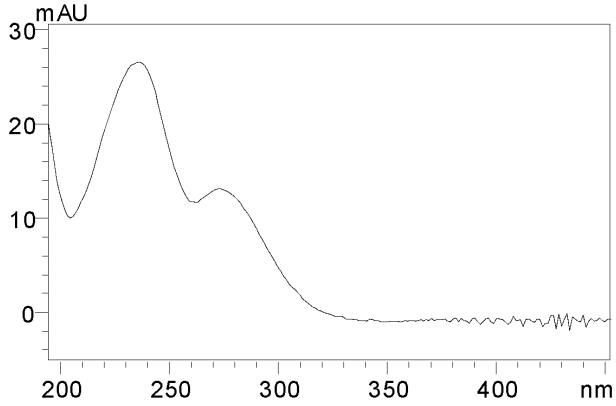
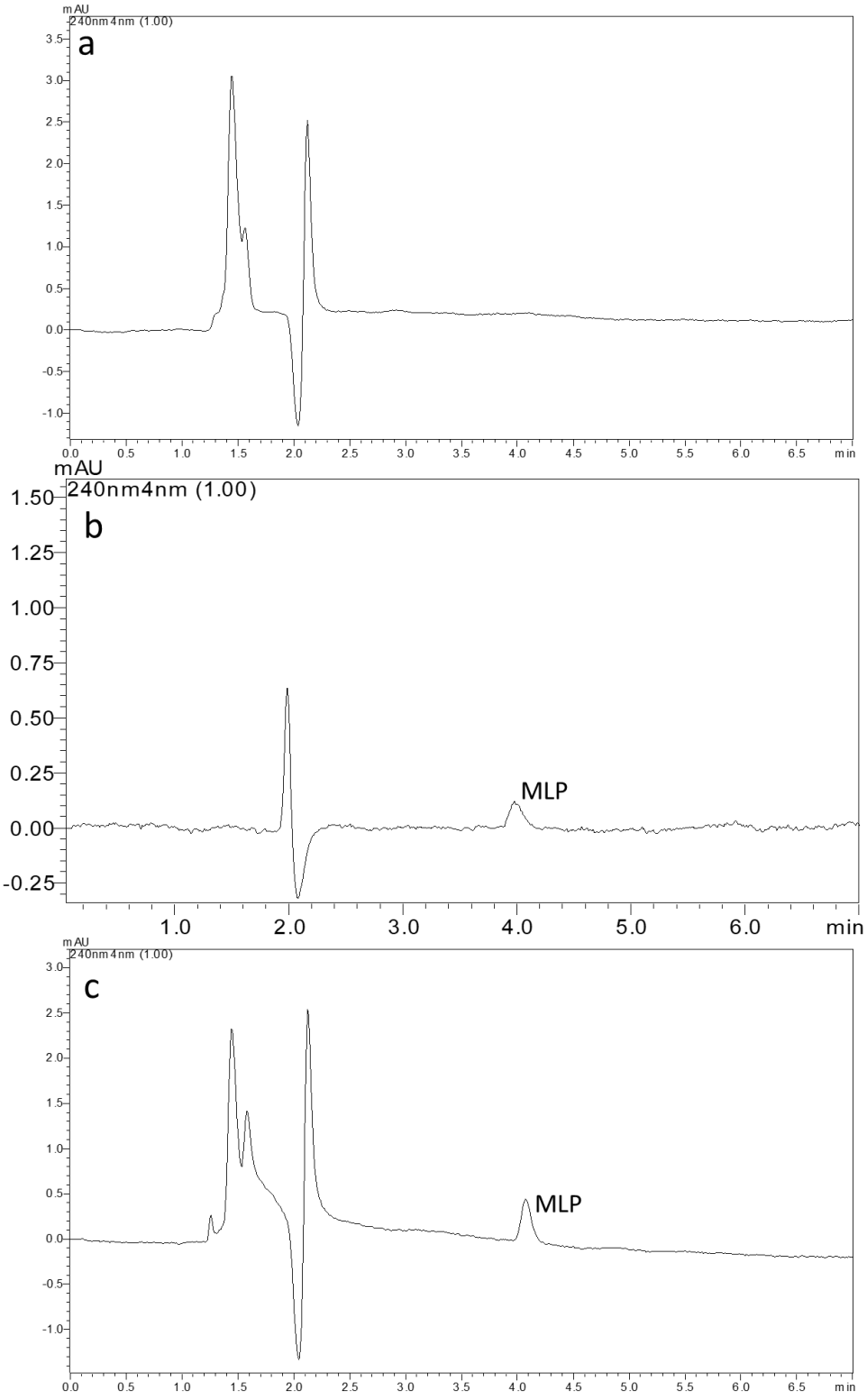


Supplementary Figure 1: UV spectrum of MLP.



Supplementary Figure 2: Chromatograms obtained under optimum chromatographic conditions; a) blank for MLP b) MLP standard spiked matrix at LOD (0.05 µg/mL) concentration c) MLP standard spiked matrix at LOQ (0.10 µg/mL) concentration.

Identification of degradation products of MLP was performed using a high-resolution mass spectrometry analysis with liquid chromatography method. Degradation conditions were applied as described in section 2.7 and solutions were analyzed on a C18 column (2.1 x 50 mm, 1.8 µm) and LC-qTOF-MS system (Agilent 6530). The mobile phase included solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile) with gradient elution (Supplementary Table 1). The flow rate was adjusted to 0.3 mL/min. The injection volume was 10 µL. MS analysis was done using negative and positive ionization modes. The LC-qTOF-MS instrument parameters were given in Supplementary Table 2.

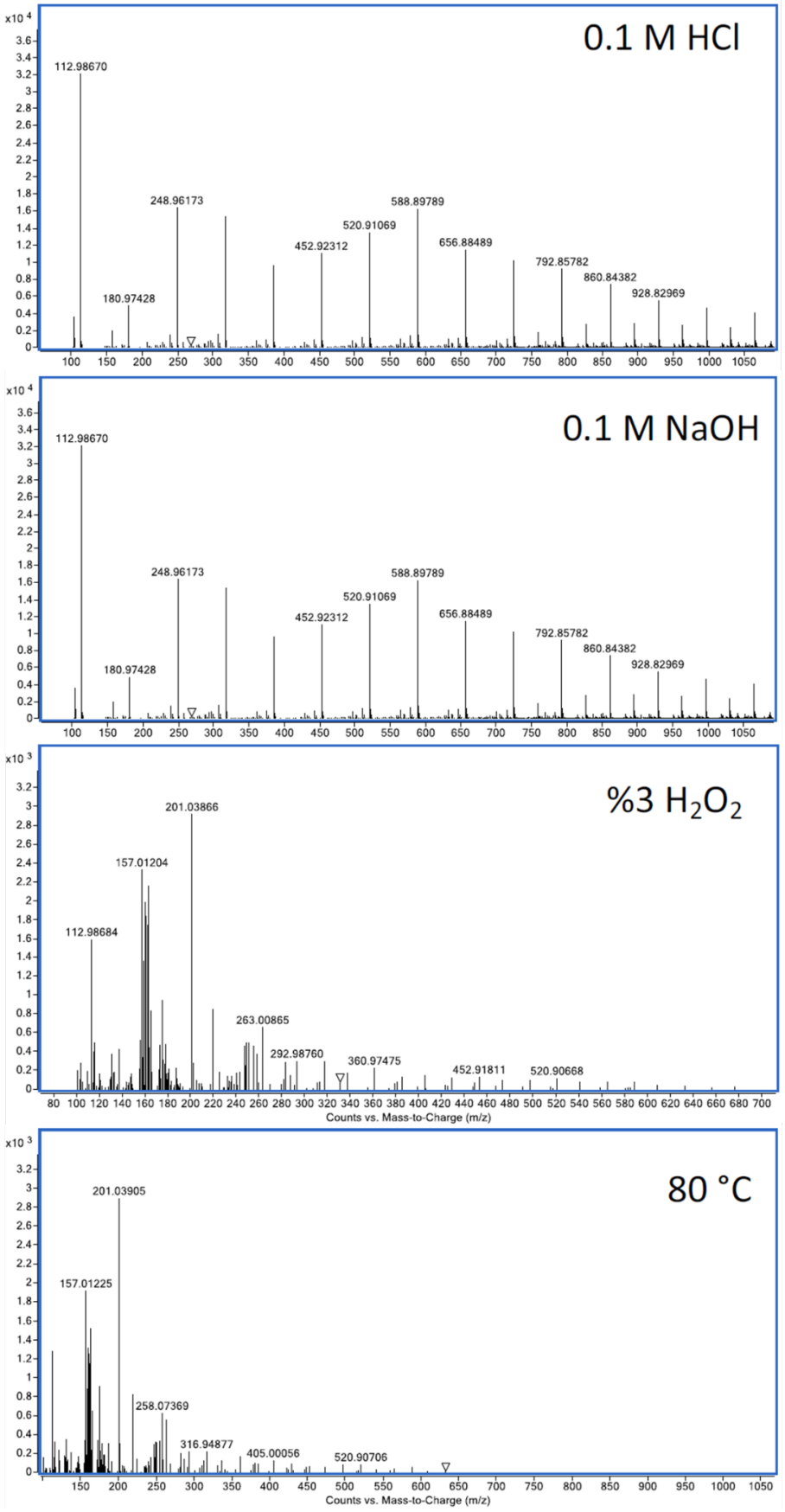
Supplementary Table 1. Gradient elution program

Time (min)	**% Mobile Phase B***
0	10
1	10
10	90
11	90
15	10
25	10

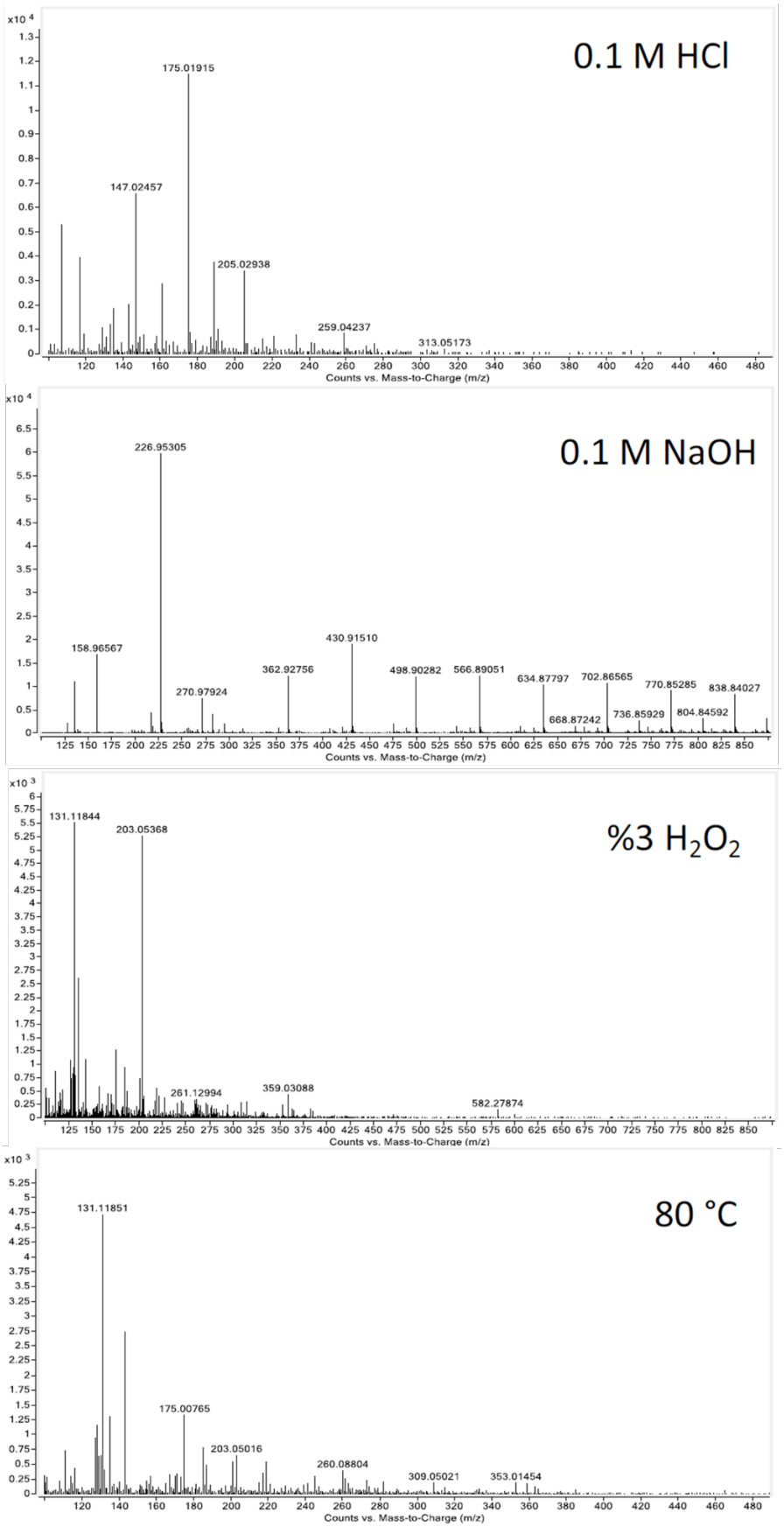
*% 0.1 FA in acetonitrile

Supplementary Table 2. LC-qTOF-MS instrument parameters

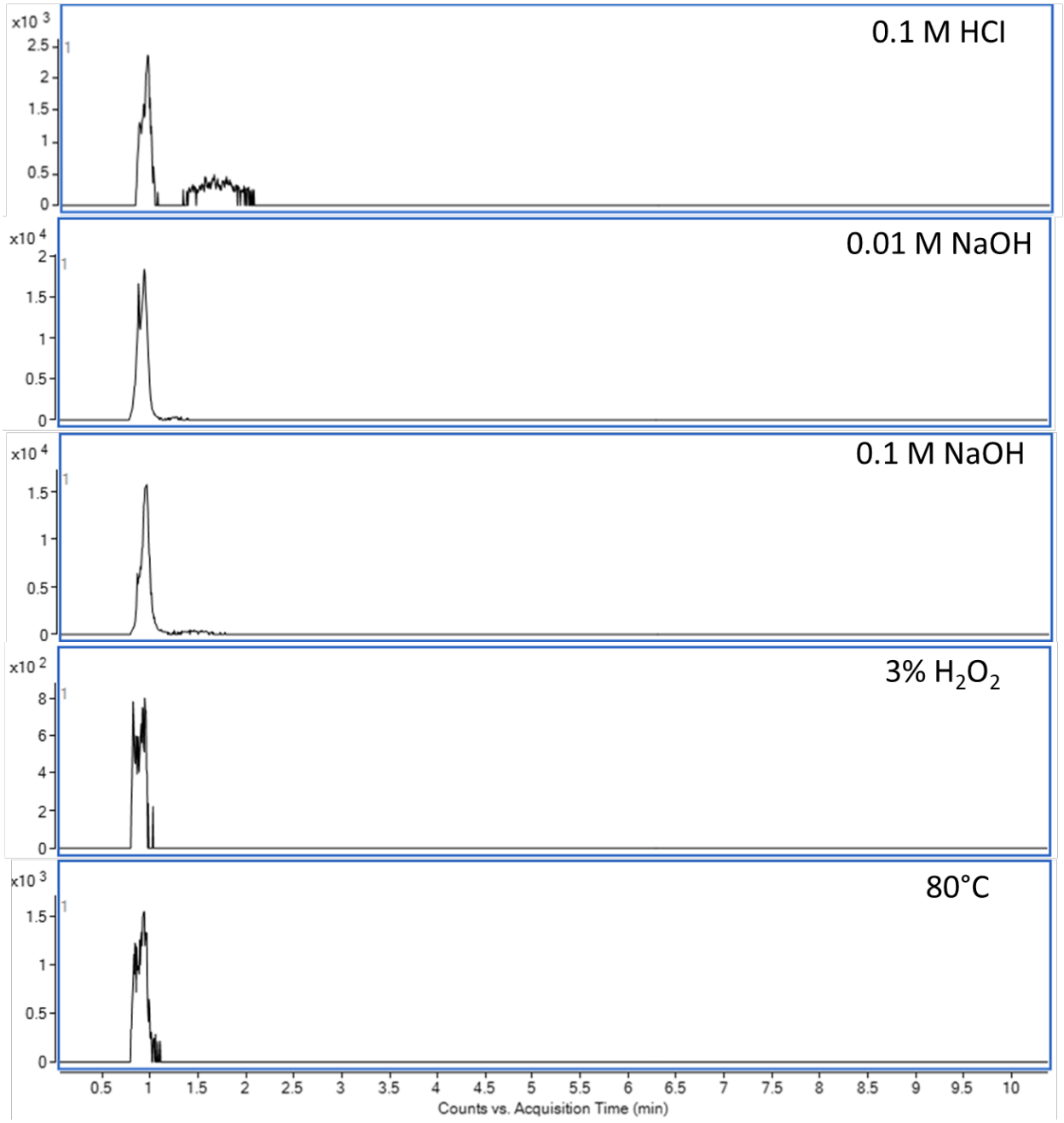
	Positive ionization	Negative ionization
Mass range	100-1700 amu	100-1700 amu
Scan Speed (spectrum/sn)	2	2
Spray voltage (kV)	3500	3500
Skimmer voltage (V)	65	65
Gas temperature (ºC)	325	325
Gas flow rate (L/dak)	10	10
Nebulizer (psi)	45	45



Supplementary Figure 3: High-resolution MS spectrum of MLP degradation products at different forced degradation conditions. Ionization mode: Negative



Supplementary Figure 5: High-resolution MS spectrum of MLP degradation products at different forced degradation conditions. Ionization mode: Positive



Supplementary Figure 5: Extracted ion chromatograms of MLP at 258 m/z for ß-d-N4-hydroxycytidine presences at different forced degradation conditions. Ionization mode: Negative