Supporting Information

Design and radiosynthesis of a class-IIa HDAC inhibitor with high molar activity via repositioning the ^{18}F-radiolabel.

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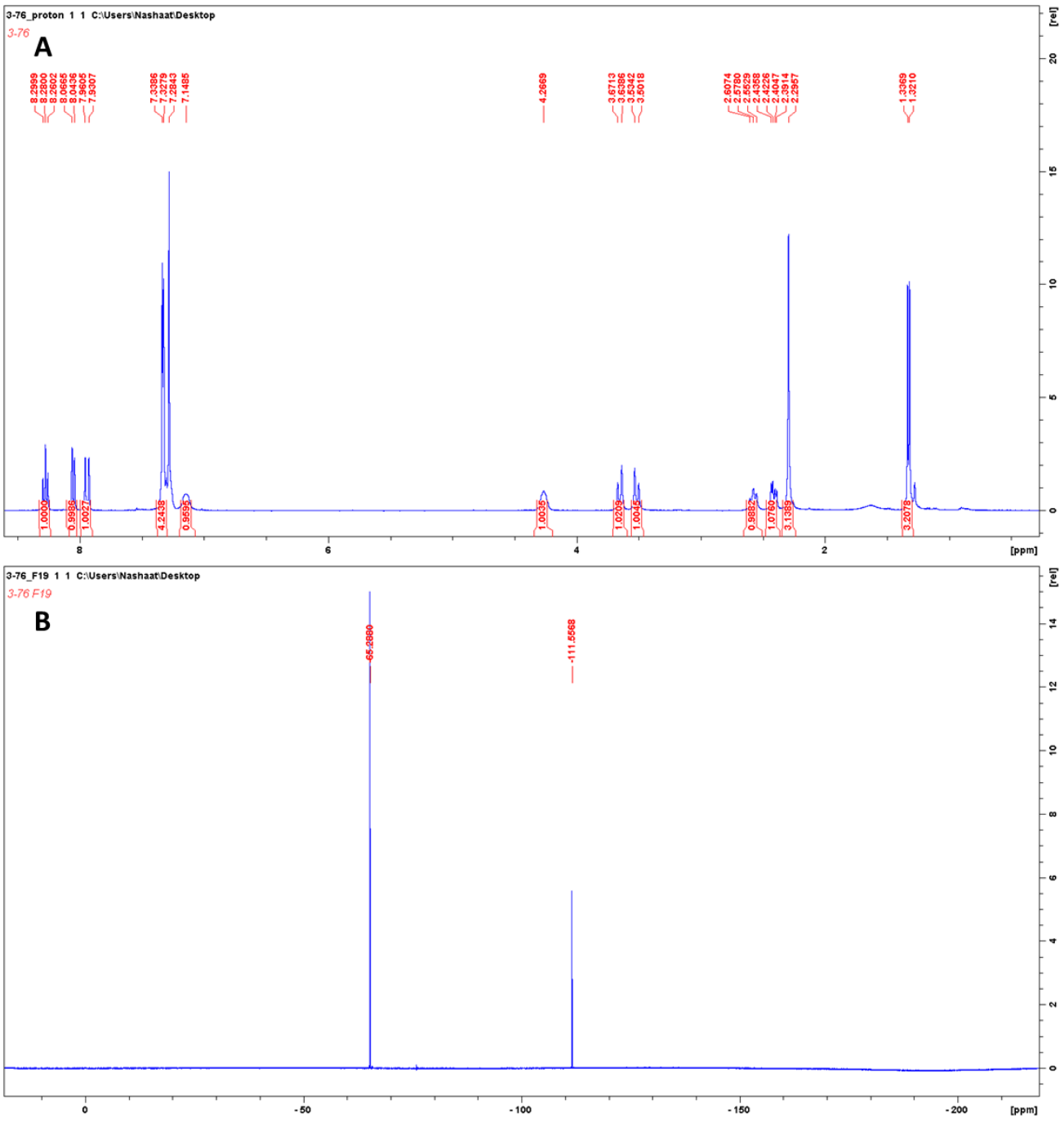

Figure 1. A) ^{1}H NMR ($CDCl_3$, 400 MHz) and B) ^{19}F NMR ($CDCl_3$, 376.5 MHz) spectroscopic characterization for NT376 was performed using 400 MHz Bruker instrument.

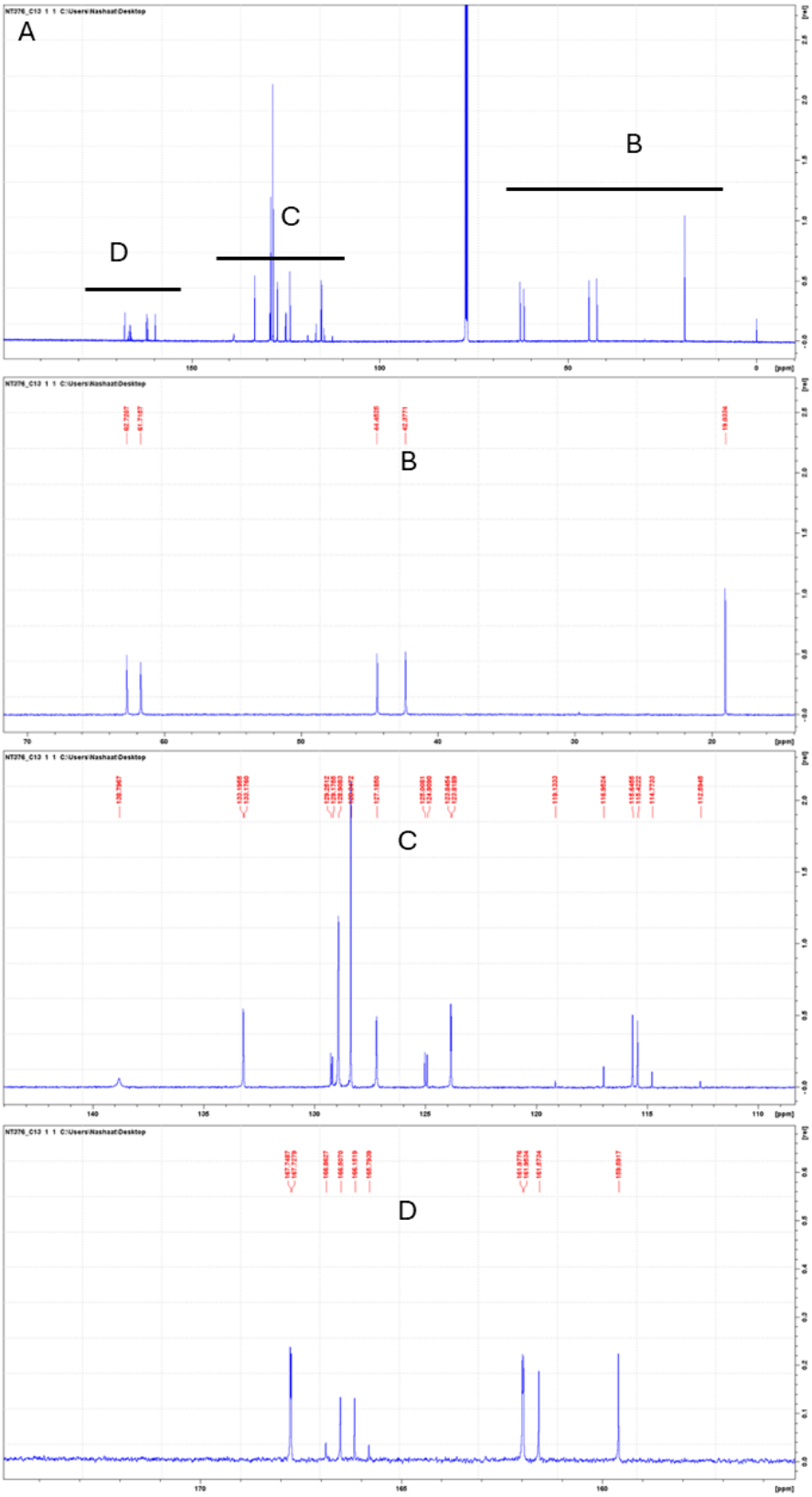

Figure 2. A) ^{13}C NMR, full spectrum for NT376 ($CDCl_3$, 400 MHz) and B-D: expanded spectrum of chemical shifts from up to down field (provided for clarity).

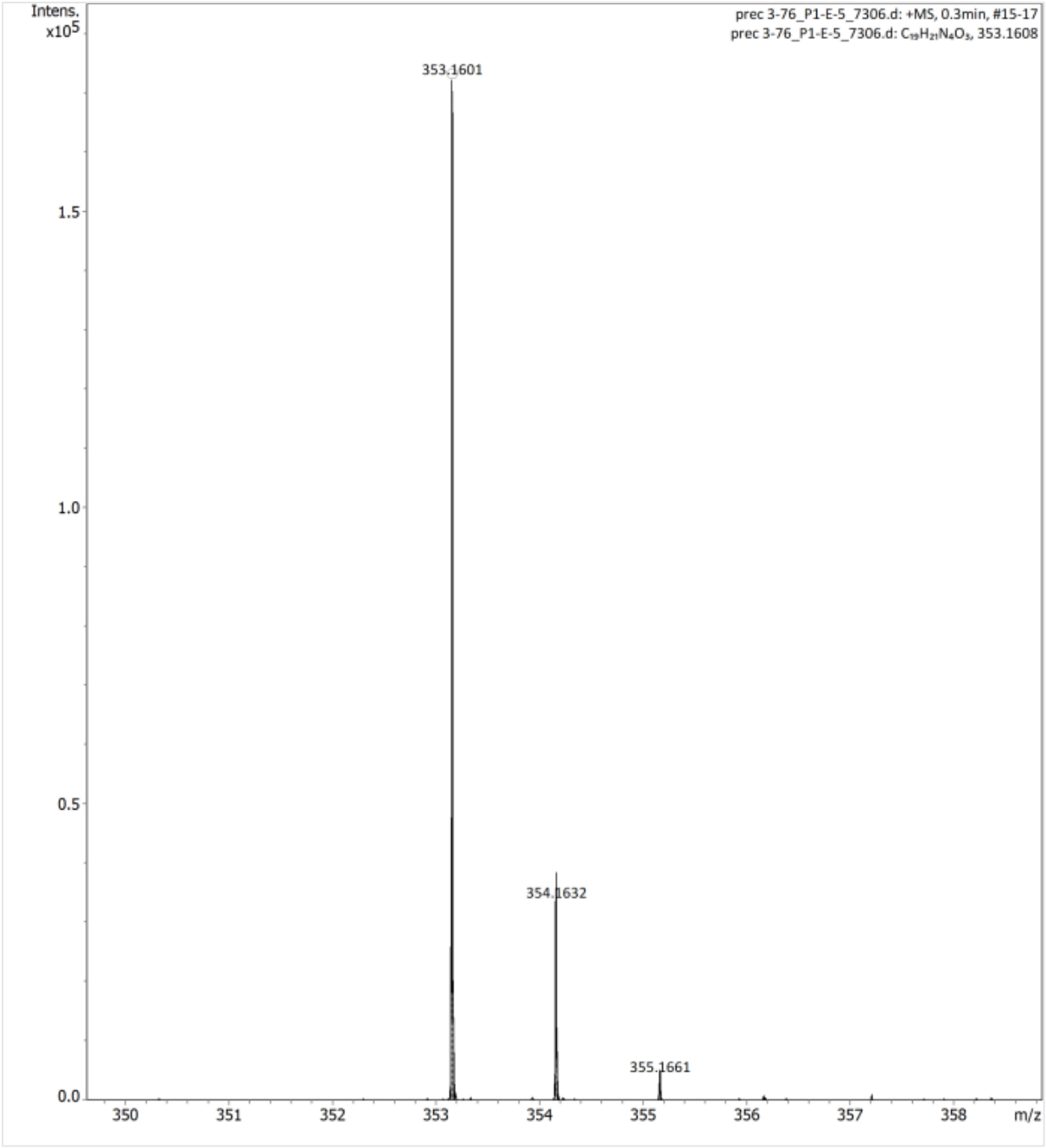

Figure 3. High resolution mass spectroscopy (HRMS) for NT376 was performed using Agilent 1260HPLC/G6224A-TOF MS.

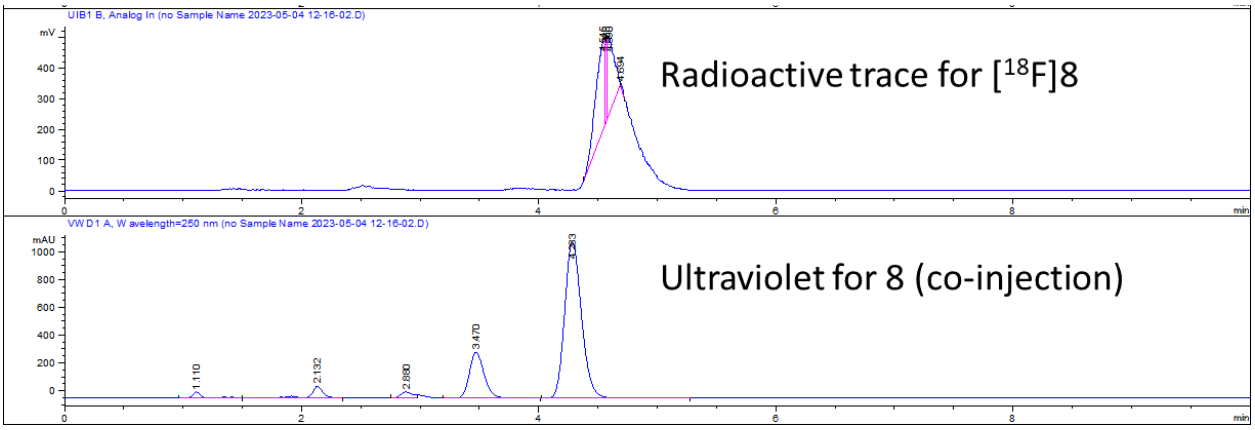

Figure 4. Analytical radio-HPLC chromatograms of [^{18}F]NT8 co-injected with authentic 8. Samples were eluted with 70% acetonitrile/30% ammonium acetate buffer (20.0 mM) at a flow rate of 1.0 mL/min.

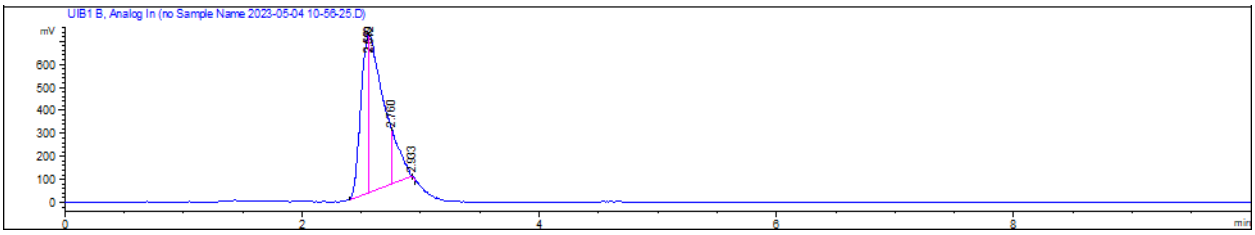

Figure 5. Analytical radio-HPLC chromatograms of [^{18}F]9 which demonstrate complete conversion of [^{18}F]8 to [^{18}F]9. Samples were eluted with 70% acetonitrile/30% ammonium acetate buffer (20.0 mM) at a flow rate of 1.0 mL/min.

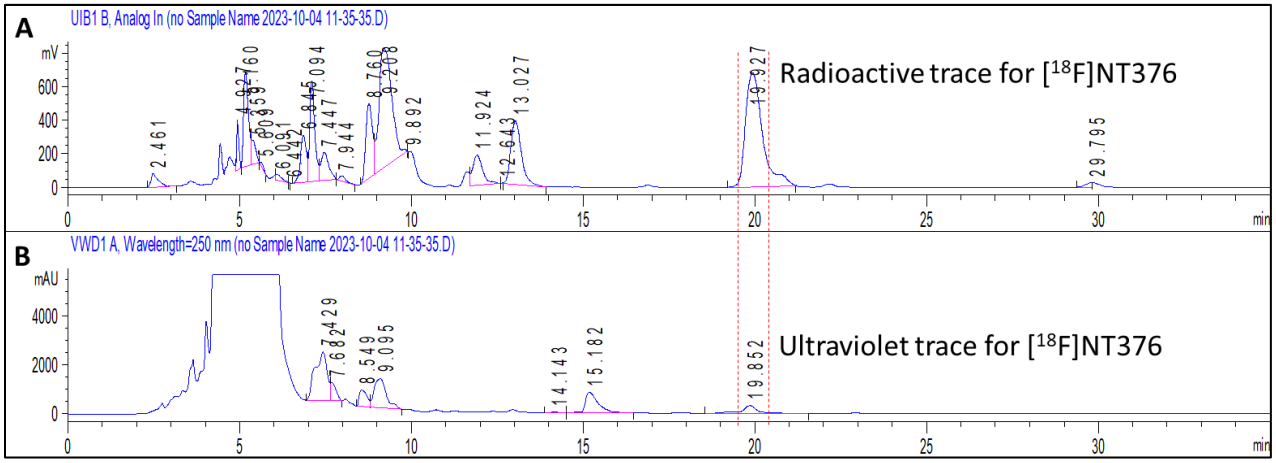

Figure 6. Semi-preparative high performance liquid chromatography (HPLC) purification of [18F]NT376 (a) radioactive peak of [^{18}F]NT376 in crude mixture detected with radioactivity detector, (b) [^{18}F]NT376 associated non-radioactive mass in the crude reaction mixture were detected with ultraviolet detector. Dashed lines shows start and stop of [^{18}F]NT376 collection which demonstrate significant improvement in molar activity compared to our previous reports.[1-3]

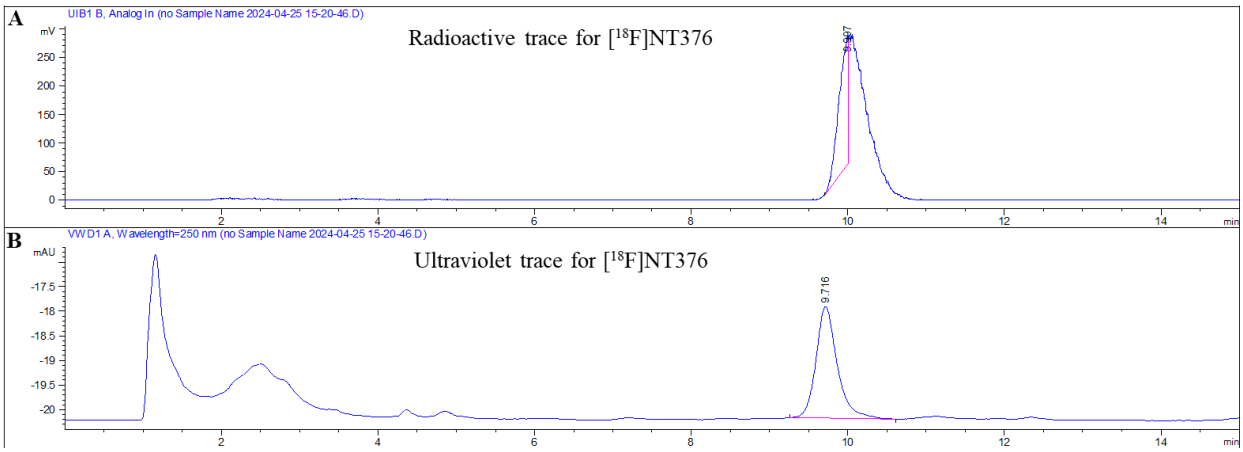

Figure 7. Analytical radio-HPLC chromatograms of purified [^{18}F]NT376: A) radioactive trace and B) ultraviolet mass trace for NT376 (note the Y-scale). Samples were eluted with 70% acetonitrile/30% ammonium acetate buffer (20.0 mM) at a flow rate of 1.0 mL/min.

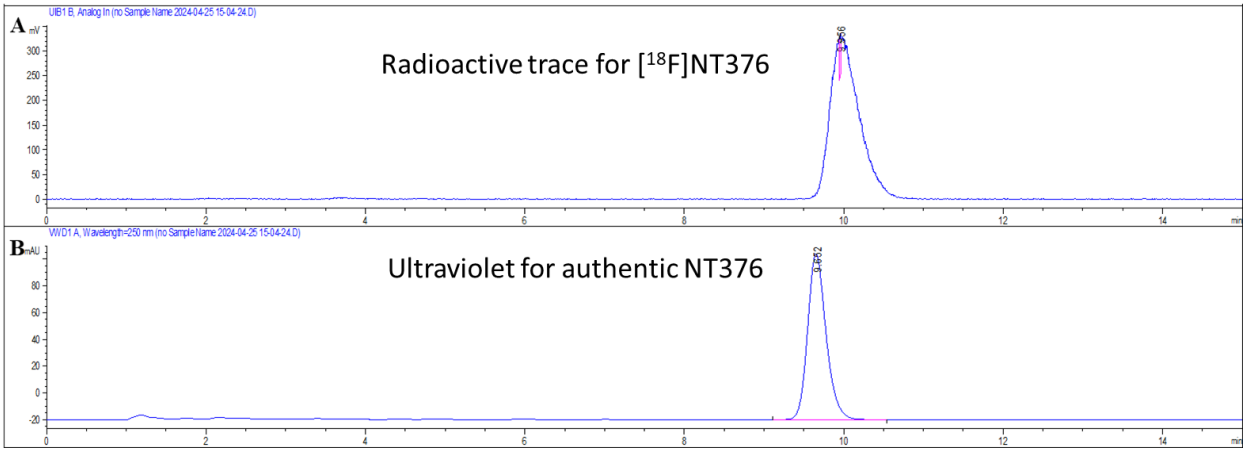

Figure 8. Analytical radio-HPLC chromatograms of quality control for [^{18}F]NT376: A) radioactive trace and B) co-injection with authentic NT376. Samples were eluted with 70% acetonitrile/30% ammonium acetate buffer (20.0 mM) at a flow rate of 1.0 mL/min.

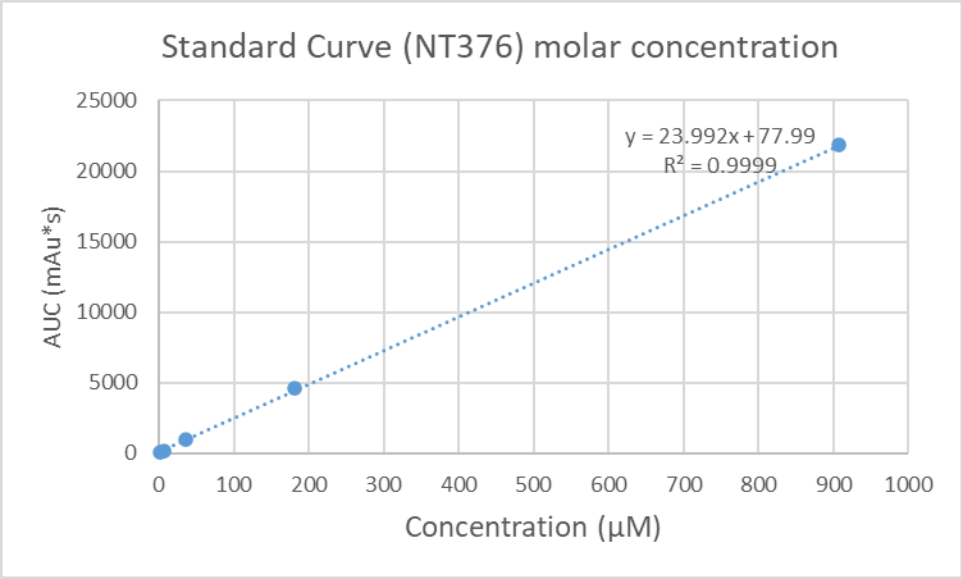

Figure 9. Standard curve obtained for NT376 and was used to determine the molar activity.

(1) Turkman, N.; Xu, S.; Huang, C. H.; Eyermann, C.; Salino, J.; Khan, P. High-Contrast PET Imaging with [(18)F]NT160, a Class-IIa Histone Deacetylase Probe for In Vivo Imaging of Epigenetic Machinery in the Central Nervous System. *J Med Chem* **2023**, *66* (8), 5611-5621. DOI: 10.1021/acs.jmedchem.2c02064.

(2) Turkman, N.; Liu, D.; Pirola, I. Design, synthesis, biochemical evaluation, radiolabeling and in vivo imaging with high affinity class-IIa histone deacetylase inhibitor for molecular imaging and targeted therapy. *Eur J Med Chem* **2022**, *228*, 114011. DOI: 10.1016/j.ejmech.2021.114011.

(3) Turkman, N.; Liu, D.; Pirola, I. Novel late-stage radiosynthesis of 5-[18F]-trifluoromethyl-1,2,4-oxadiazole (TFMO) containing molecules for PET imaging. *Sci Rep* **2021**, *11* (1), 10668. DOI: 10.1038/s41598-021-90069-x.