



## Title: MAO Activity Assay Control Tests: $K_m$ for MAO A and MAO B and IC50 Determination for Clorgyline and Pargyline

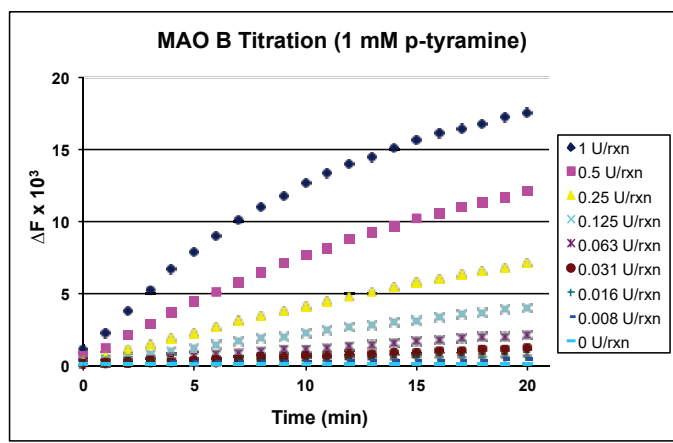
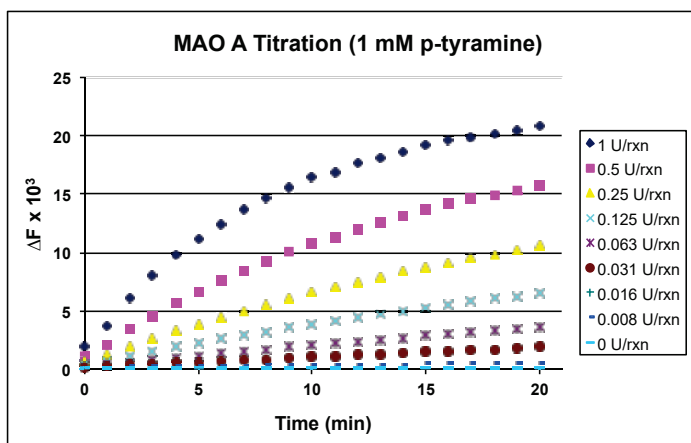
### Summary

Before determining the IC50's for both Monoamine Oxidase A (MAO A) and Monoamine Oxidase B (MAO B) inhibitors, we performed some initial experiments to establish appropriate assay conditions. We assessed conditions for human MAO A and MAO B. First we titrated the MAO's using 1 mM p-tyramine as the substrate. From these titrations we decided to use 0.13 U/reaction for the MAO A and 0.25 U/reaction for the MAO B. We next titrated DMSO to determine the highest concentration that would be tolerated by each MAO. The  $K_m$  of p-tyramine for each MAO was then measured in the presence of the highest tolerable DMSO concentration. Finally, the IC50 for a known MAO A inhibitor, clorgyline, and MAO B inhibitor, pargyline was measured for each MAO. For the MAO A, we determined the maximum DMSO concentration for the test compounds to be 10 v% (final reaction concentration of 0.5 v%). We determined the p-tyramine  $K_m$  to be  $55.6 \pm 3.7$  mM for MAO A and  $24.1 \pm 4.8$  mM for MAO B. Using 55 mM p-tyramine, we observed an IC50 = 11 nM for clorgyline for the MAO A. Using 24 mM p-tyramine, we observed an IC50 = 404 nM for pargyline for the MAO B.

### Results

#### MAO A and MAO B Titrations

In order to determine an appropriate amount of MAO to use for the IC50 determinations, we titrated each MAO. The MAO A and MAO B were titrated from 1 U/reaction. Both titrations were performed using 1 mM final [p-tyramine] as the substrate. The MAO's titration was performed in a 96 black well plate.

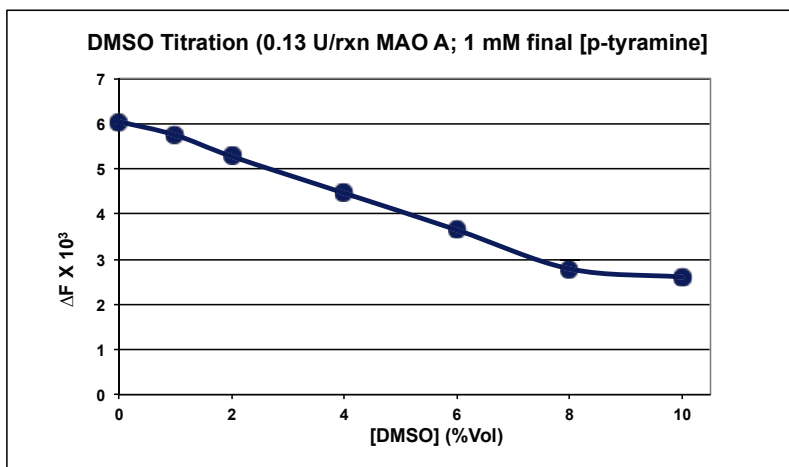




From these results we opted to use 0.13 U/rxn for the MAO A and 0.25 U/rxn for the MAO B. At these concentrations the reaction would remain linear for at least 20 mins.

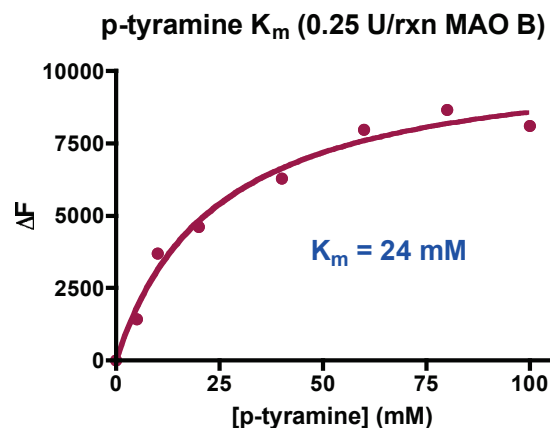
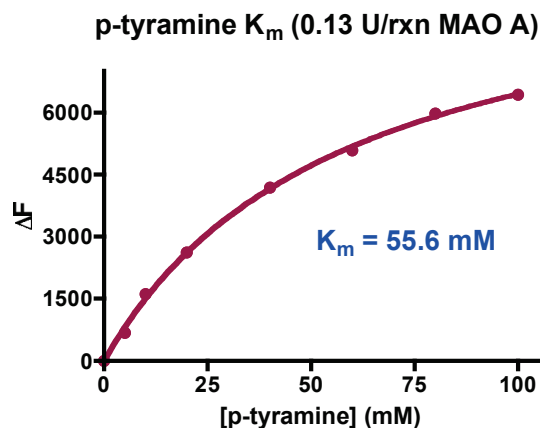
### DMSO Titration

In order to determine tolerance for DMSO of the MAO, we ran DMSO titrations for MAO A from 0–10 v%. For these titrations we used 0.13U per reaction for the MAO A. The reaction was initiated by adding 1 mM final [p-tyramine] and was allowed to proceed for 20 mins. We then computed the  $\Delta F$  ( $F_{20\text{min}} - F_{0\text{min}}$ ), which is proportional to the rate of reaction, for each DMSO concentration. The  $\Delta F$  was then plotted versus DMSO concentration. From these result we discovered that DMSO actually inhibited the activity of MAO. For the MAO A, we decided to move forward with <10 v% DMSO to used to dilute the inhibitors (final <0.5 v/v% per reaction)



### $K_m$ of MAO A and MAO B

In order to determine the  $K_m$  of p-tyramine for MAO A and B, we titrated p-tyramine from 0–100 mM with either 0.13 U/rxn MAO A or 0.25 U/rxn MAO B and computed the  $\Delta F$  ( $F_{20\text{min}} - F_{0\text{min}}$ ), which is proportional to the rate of reaction, for each p-tyramine concentration. The  $\Delta F$ 's were then plotted versus p-tyramine and the  $K_m$  was computed using Prism 4 (GraphPad Software Inc.). The MAO A and MAO B  $K_m$  determination was performed in the presence of 0.5 v% DMSO (final reaction concentration). We found the  $K_m = 55.6$  mM for the MAO A and the  $K_m = 24$  mM for the MAO B.





### Clorgyline and Pargyline IC50 Determination

The IC<sub>50</sub> determination required two steps: 1) 15 min pre-incubation of 45  $\mu$ L MAO (0.13 U/rxn MAO A or 0.25 U/rxm MAO B) with 5  $\mu$ L clorgyline in 10% DMSO for MAO A and 5  $\mu$ L pargyline in 10% DMSO for MAO B, and 2) monoamine oxidase reaction with 0.53 mM final [p-tyramine] for MAO A and 0.23 mM for MAO B for 20 min at 25°C. We titrated clorgyline from 0-0.025 mM and pargyline inhibitor from 0-10  $\mu$ M and computed the  $\Delta F$  for each inhibitor concentration. The  $\Delta F$ 's were then plotted versus the inhibitor concentration and the IC<sub>50</sub> was computed using Prism 4 (GraphPad Software Inc.). We observed an IC<sub>50</sub> = 11 nM for MAO A and an IC<sub>50</sub> = 404 nM for MAO B.

