BioAssay Systems
3191 CORPORATE PLACE
HAYWARD, CA 94545. U. S. A
TEL: 510. 782. 9988
FAX: 510. 782. 1588
www.bioassaysys.com
info@bioassaysys.com

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

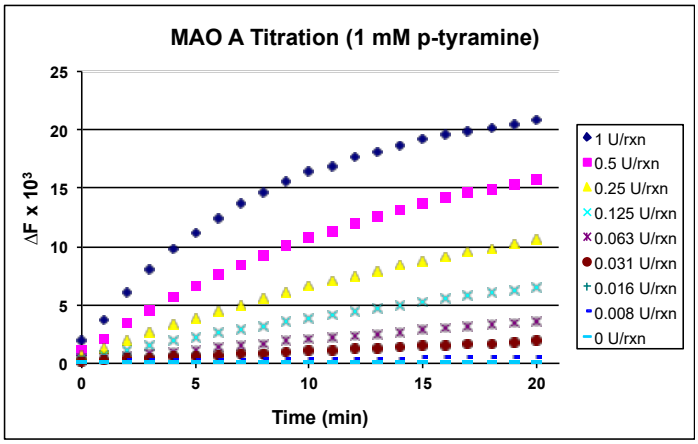
Summary

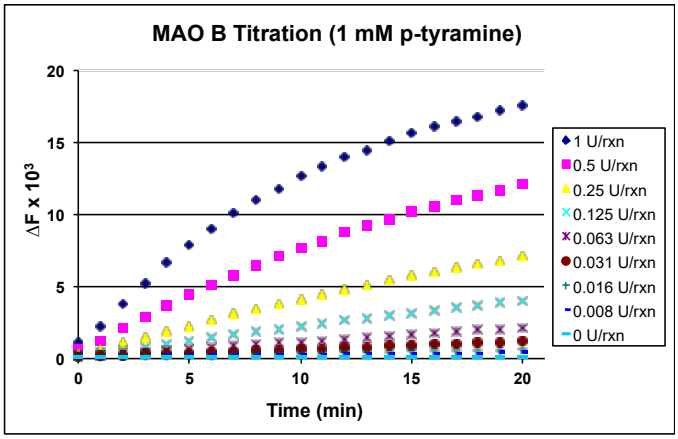
Before determining the IC50's for both Monoamine Oxidase A (MAO A) and Monoamine Oxidase B (MAO B) inhibitors, we performed some initial experiments to establish appropriate assay conditions. We assessed conditions for human MAO A and MAO B. First we titrated the MAO's using 1 mM p-tyramine as the substrate. 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 ± 3.7 mM for MAO A and 24.1 ± 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.





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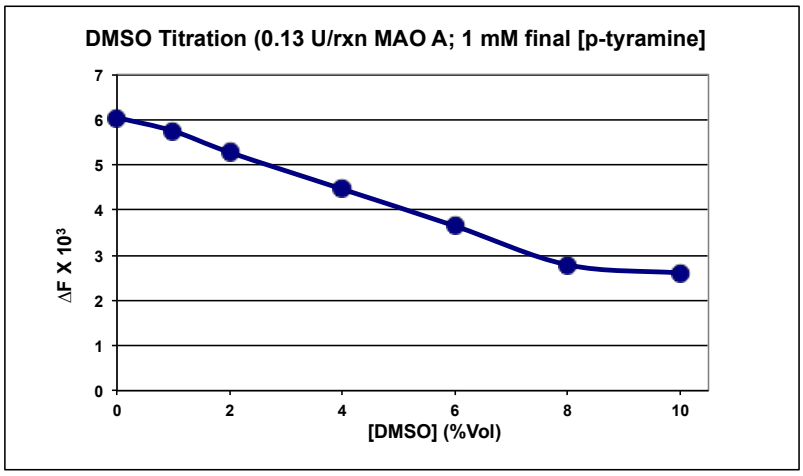
From these results we opted to use 0.13 U/rxn for the MAO A and 0.25 U/rxn for the MAO B. At these concentrations the reaction would remain linear for at least 20 mins.

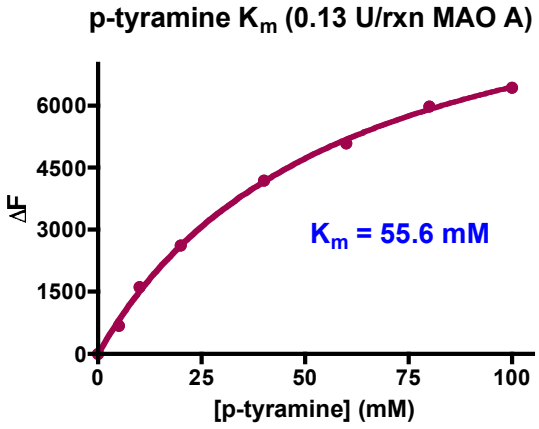
DMSO Titration

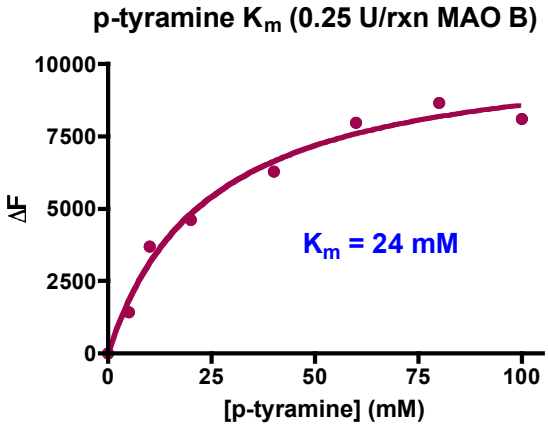
In order to determine tolerance for DMSO of the MAO, we ran DMSO titrations for MAO A from 0–10 v%. For these titrations we used 0.13U per reaction for the MAO A. The reaction was initiated by adding 1 mM final [p-tyramine] and was allowed to proceed for 20 mins. We then computed the ΔF ($F_{20min} - F_{0min}$), which is proportional to the rate of reaction, for each DMSO concentration. The ΔF 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 ΔF ($F_{20min} - F_{0min}$), which is proportional to the rate of reaction, for each p-tyramine concentration. The ΔF'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.





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Clorgyline and Pargyline IC50 Determination

The IC50 determination required two steps: 1) 15 min pre-incubation of 45 µL MAO (0.13 U/rxn MAO A or 0.25 U/rxm MAO B) with 5 µL clorgyline in 10% DMSO for MAO A and 5 µL 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 µM and computed the ΔF for each inhibitor concentration. The ΔF's were then plotted versus the inhibitor concentration and the IC50 was computed using Prism 4 (GraphPad Software Inc.). We observed an IC50 = 11 nM for MAO A and an IC50 = 404 nM for MAO B.