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Development of medium throughput muscarinic receptor binding assay

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A filtration based competitive radioligand muscarinic receptor binding assay suitable for medium throughput screening has been developed. In this assay, 96-well GF/C filter plate was adapted and integrated with FilterMate cell harvester and TopCount NXT Microplate Scintillation and Luminescence Counter. Using rat brain as the source of muscarinic receptor, a linear relationship of protein concentration and radioligand binding was established up to a protein concentration of 133 μg of protein/well. The parameters investigated include radioligand concentration, pH, temperature, incubation time, and number of washings. In general, the optimum protocol contained 36 μg of protein/well, 0.5 nM [^{3}H] N-methylscopolamine ([^{3}H]NMS) and 0.05 M Tris HCl buffer pH 7.4. The receptor-radioligand equilibration was reached after 90 min incubation at 21^{0}C. Saturation analysis of [^{3}H]NMS gave B_{max} of 293 fmol/mg protein and K_D of 0.059 nM. The B_{max} value shows the muscarinic receptor density is high whilst K_D value suggests [^{3}H]NMS has high affinity towards the receptor and is a suitable radioligand source for filtration based assay. The linear Rosenthal plot suggests a single site binding for this receptor-radioligand interaction. K_i obtained from competition experiments with known muscarinic receptor ligands are 0.30 nM (atropine), 0.052 nM (scopolamine) and 1.6 nM (dicyclomine). Low inter-plate and intra-plate variability (CV, 4.9% and 5.6% respectively) and Z' factor of 0.8 for intra-plate, clearly show that this assay protocol is robust and reliable as a medium throughput screening assay for the detection of potential muscarinic receptor active compounds in chemicals or natural products libraries.