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Validation of a Label-Free Mass Spectrometry-based Binding Assay for the Gamma-aminobutyric Acid B 1b Receptor

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The binding of a ligand to a specific target is an essential requirement for endogenous messengers as well as for exogenous molecules to exert physiologically or pharmacologically relevant effects. In the drug development process, binding assays play a critical role in the assessment of the affinity of test compounds to specific targets. Traditionally, binding assays have been performed using either radioactive, fluorescent, or luminescence-labeled ligands. Our aim was to determine the feasibility of using a mass spectrometry-based readout in order to study the binding kinetics of the gamma-aminobutyric acid (GABA) $_{B1b}$ receptor using an unlabeled ligand.

$GABA_B$ receptors are the G-protein-coupled receptors (GPCRs) for GABA, the main inhibitory neurotransmitter in the central nervous system. The $GABA_B$ receptor is one of the most important therapeutic targets in the treatment for spasticity.

The membrane fraction of a Chinese hamster ovary (CHO) cell stably expressing the $GABA_{B1b}$ subunit was isolated. The potent, selective antagonist CGP 54626 was selected as the specific ligand. The detection of CGP 54626 was optimized by liquid chromatography coupled to mass spectrometry (LC-MS), an ultra-high performance liquid chromatography (Infinity 1290 and CTC autosampler, Agilent) using a C18 column which was coupled to a triple quadrupole linear ion traps (QTRAP 5500, Sciex). As per "gold standard" radioligand binding assays, identical steps were followed which included incubation of ligand and receptor (in the presence or absence of test compound), followed by afiltration and wash steps, the plates were subsequently dried and remaining CGP 54626 bound to the GABA $_{B1b}$ receptor was eluted and quantified by LC-MS. IC_{50}'s of Baclofen, CGP 52432 and GABA were determined and conformed with data generated by in-house radioligand binding assays. Finally, the equilibrium dissociation constant K_D of CGP 52432 was determined. The K_{off} of CGP 52432 was determined by both a displacer technique and dilution method.

Our work clearly demonstrates that mass spectrometry-based binding assays are a viable alternative to traditionally labeled binding assays and will allow for the development of novel binding assays for targets for which no labeled ligands exists, as well as to determine the K_D's.