Novel Multiple Targeting Monitoring by Label-Free Binding by Mass Spectrometry Directly in Tissue For Translational In Vitro Safety-Tox Studies.

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Polypharmacology describes the activity of compounds at multiple targets. In the drug development process, binding assays play a critical role in the assessment of the affinity of test compounds to specific targets. The "gold standard" for binding assay remains the use of radioligands. However, this approach does not allow for the monitoring of multiple targets simultaneously. Our aim was to determine the feasibility of using label-free multiple target monitoring (MTM) to analyze 10 receptors simultaneously. For each of the 10 receptors, a specific well documented ligand was selected. For the binding assays, the methodology was identical to that used for traditional "gold standard" radioligand binding assays. This included incubation of the ligand individually or a mixture of all 10 ligands (MTM) and the membrane fraction in the presence of individual test compounds, followed by a filtration and wash steps. The plates were subsequently dried and remaining bound ligands were eluted and quantified by UHPLC-MS/MS. The IC50 of each test compound was determined for each of the 10 targets individually or simultaneously. For the test compounds, 87% were found to have a binding affinity to at least one of the 10 targets. A high level of correlations of IC50 values were established between the individual binding by mass and the MTM binding for 27 targets with an IC50 value below 11 000 nM. Our work clearly demonstrates that using MTM label-free binding by mass spectrometry does allow for the monitoring of multiple targets simultaneously in complex biological matrices and might be an attractive approach for safety/toxicology studies related to polypharmacology issues.