

## Project Requirement:

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Cyclic peptides were to be analyzed for Kinetic solubility, Chrom Log D, MDCK, PAMPA, & Caco2 experiments, while overcoming the challenges associated with the impact of cyclization of peptides on in vitro experiments and bioanalysis

## What were the challenges!

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1. Nonspecific Binding (NSB)
2. Precipitation of peptides while spiking DMSO stock to buffer
3. Impact of pH on the compounds
4. Low recovery of peptides
5. Proteolysis in buffer and cellular enzymes
6. Inconsistent data

### Actions for Troubleshooting

- Switched from plastic ware to glassware
- Precoated the plates with BSA
- Switched from lipophilic plates to Hydrophilic plates

- Buffer stability and Cell surface metabolism was evaluated prior to the experiment

- Vacuum drying DMSO prior to buffer addition

- FBS addition in incubation

- Protocol modifications: Incubation time, buffer & conc of compound

### Resolutions

- Nonspecific Binding was minimized

- Ruled out Stability issues with the peptides

- Vacuum drying DMSO prevented precipitation

- Improved Solubility & Compound Recovery

- Improved Compound Recovery & Assay Consistency