There is no simple answer to this question as it is matrix, eluent, column phase, and target dependent. Here are some things to consider:

1. Peak separation. You may be able to get 150–250 mg loading under good conditions, but it may drop to 50 mg or less if you have closely eluting peaks. The reason for this is because peaks begin to broaden significantly at higher loading, and this may result in unacceptable resolution if the peaks are close together and begin to overlap. Now it becomes a question of how much overlap you are willing to accept.

2. Solubility. At the semi-prep level, **analyte** solubility may become the limiting factor on loading **capacity**. This can be more significant in reversed phase compared to ANP or normal phase. Every compound has a different solubility profile in different solvents so it is impossible to give a maximum limit in general cases. To determine the extent of its contribution to loading **capacity**, you will need to look up your **analyte**'s solubility in your chosen solvent.

3. Chromatographic mode. Normal phase compounds tend to elute much more critically in narrow polarity **band** than in reversed phase. Whereas in reversed phase, many compounds all may be starting to move at same time, thereby overloading column, but you will have much more sequential movement in ANP and can load more on column.

4. ***Injection volume.*** As a general rule to thumb, you should be able to use about 4.5 X the injection volume as on a 4.6mm ID analytical column. Again, this may vary depending on concentration, which would determine total sample load in terms of mass.

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