

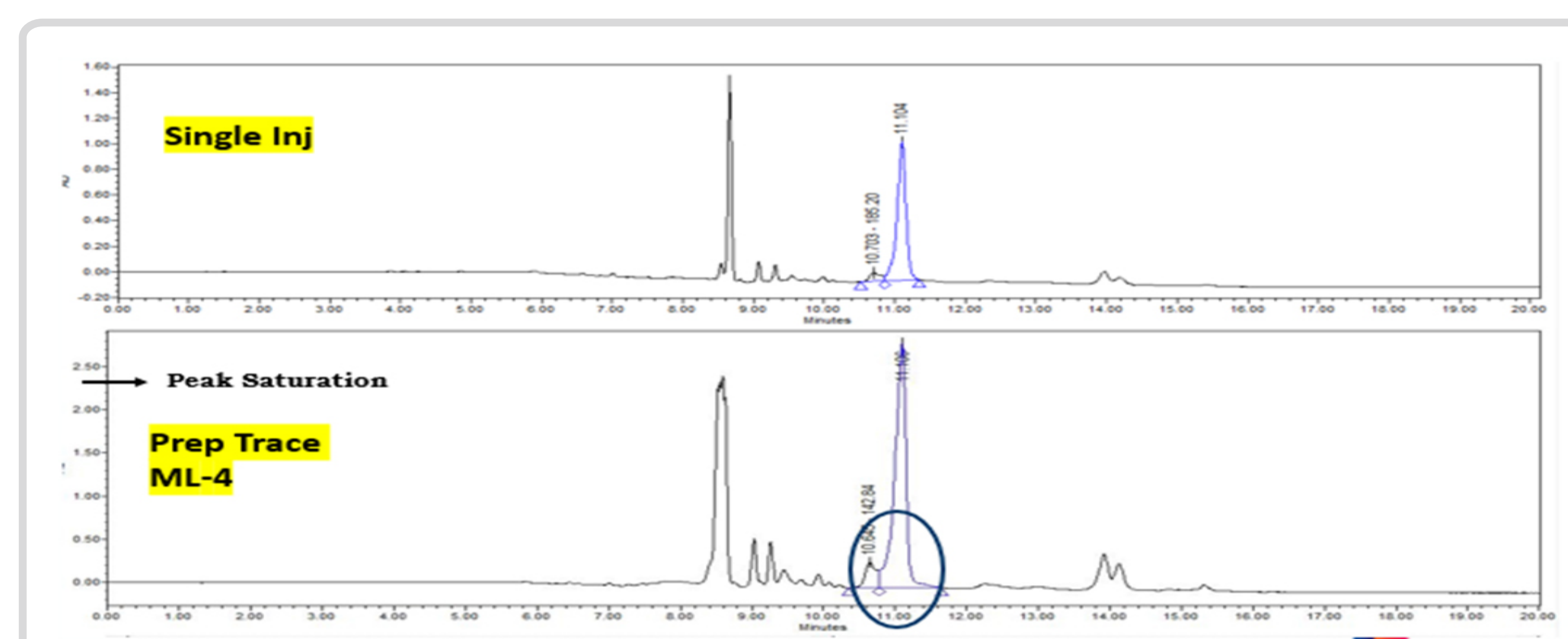
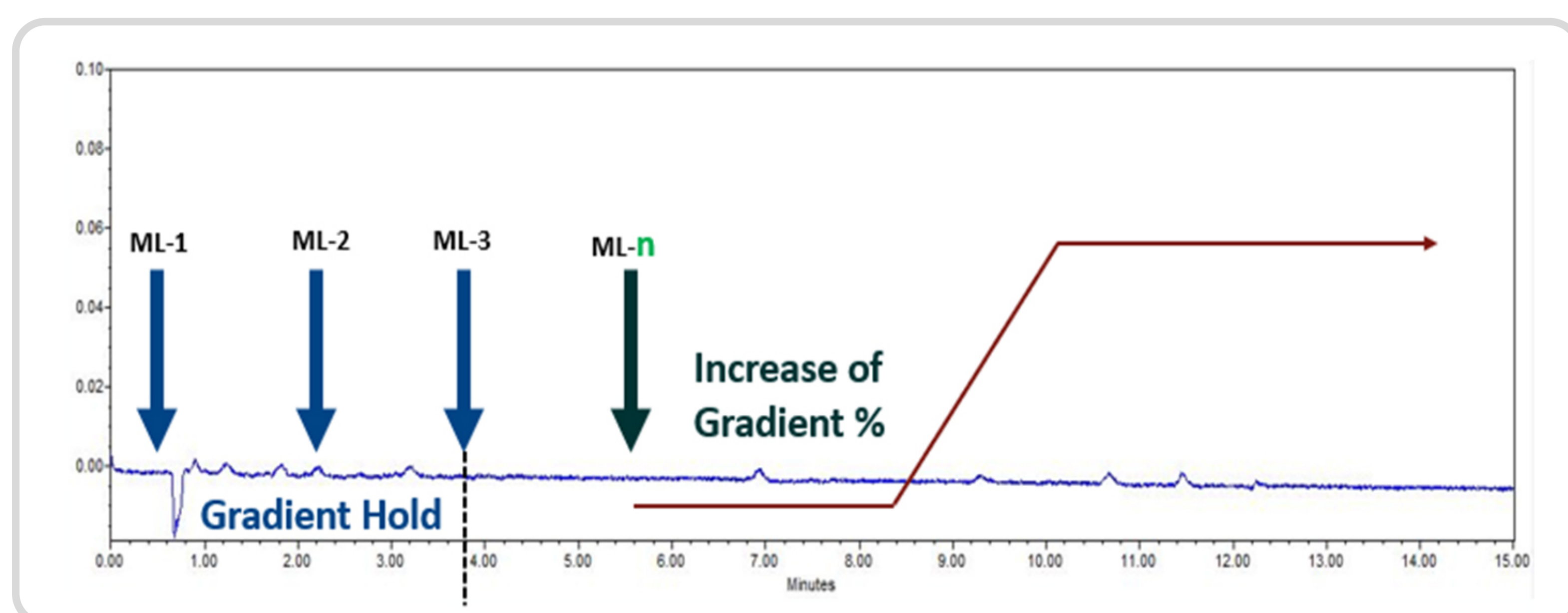
Column Head Loading in SFC - A unique sample injection technique for polar heterocycles and oligopeptides

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Abstract

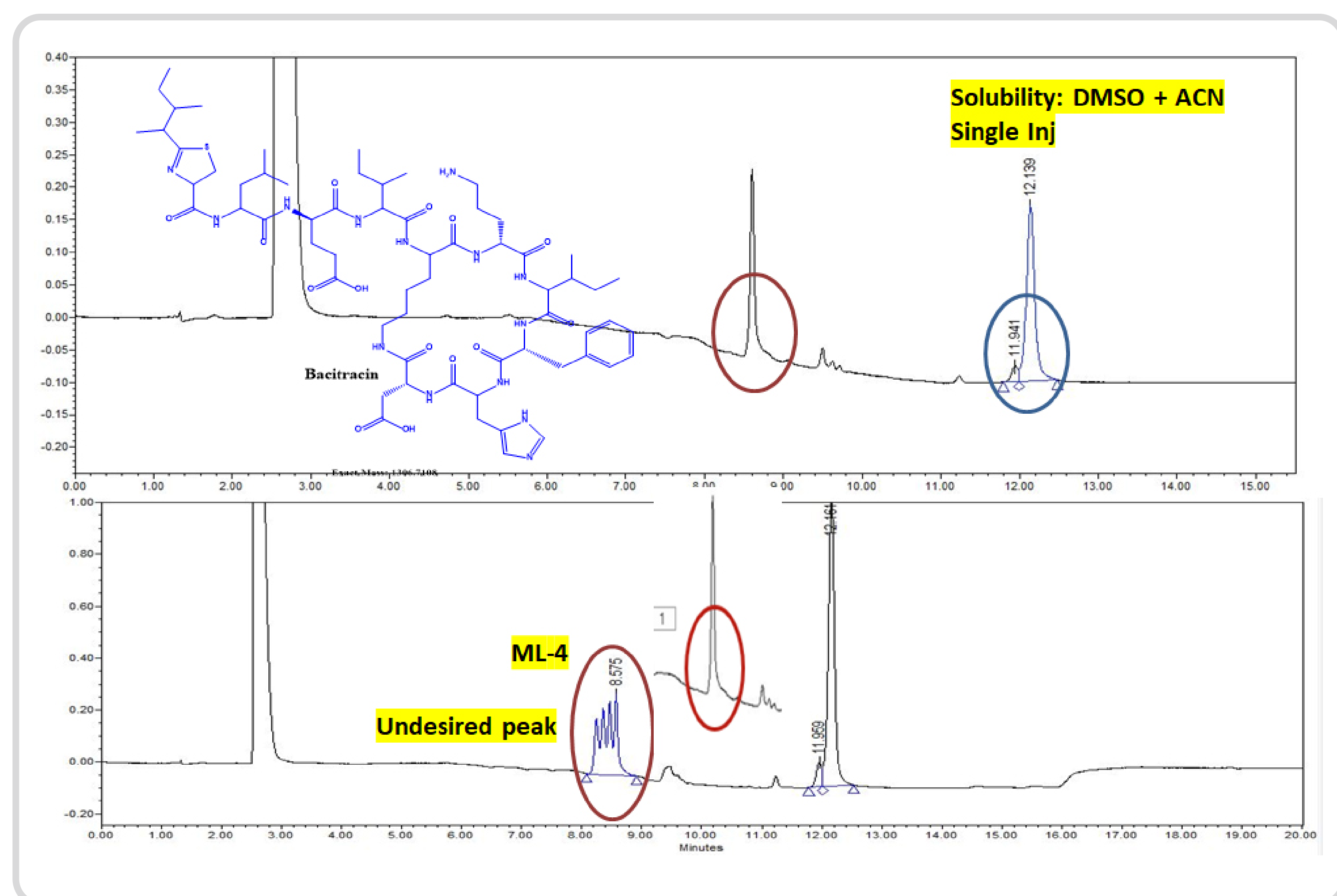
SFC has become an imperative purification technique. Purification throughput depends on the compound solubility as poor solubility induces viscous fingering and plug effect, higher injection volumes with fixed injector loop size is a limitation. Since feed solubility cannot be altered, 'column head loading with multiple injection approach' is proposed as an alternative. This approach would minimise the plug effect as it accumulates analyte bands on column head and distributing the bands more uniformly than in single injection. This methodology helps to purify poorly soluble heterocyclic compounds & oligopeptides using SFC.

Column Head Loading - Multiple Injections in a single gradient_ Proof of concept



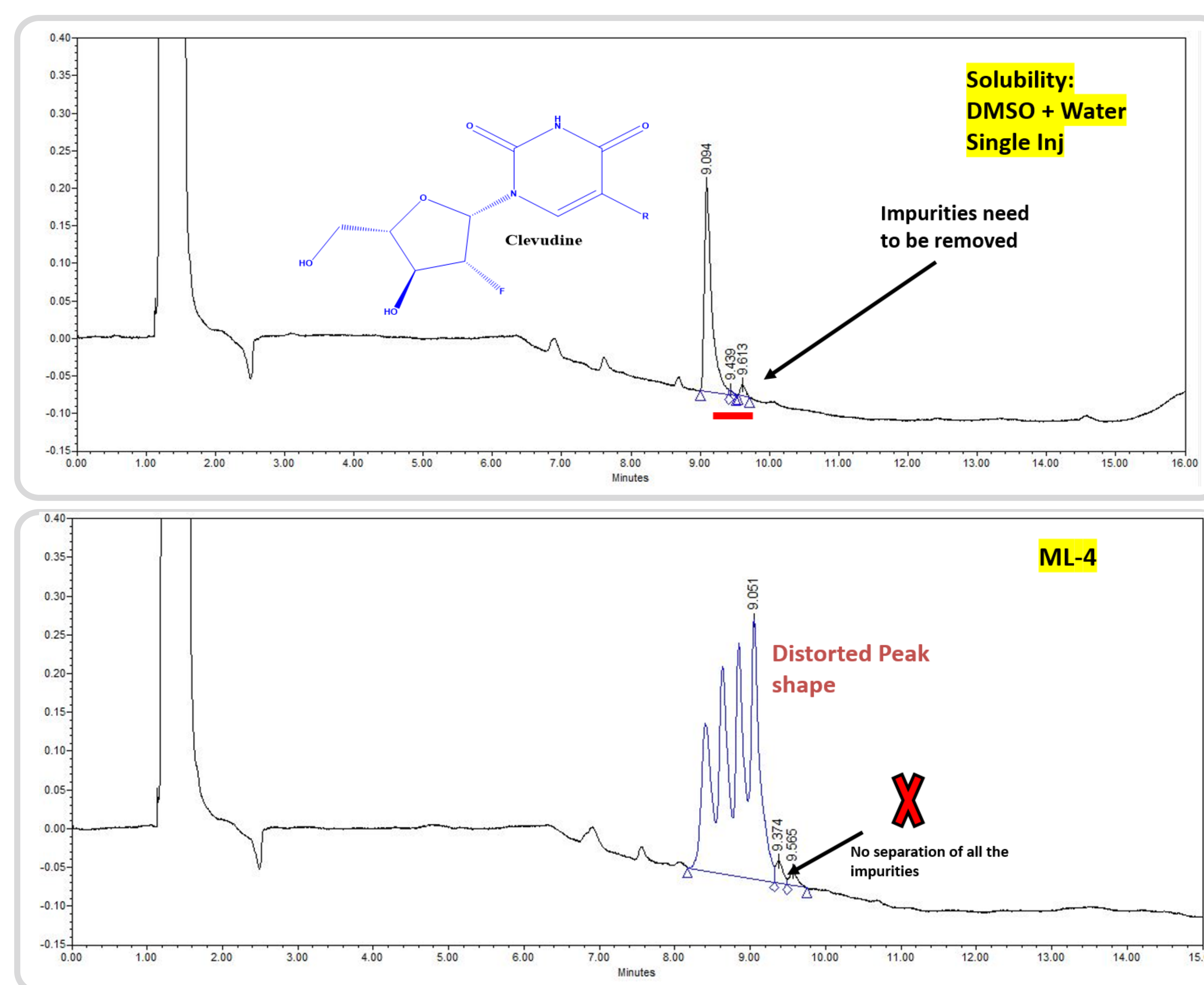
Applications of column head loading Case Study - 1_Hydrophobic Cyclic Peptide

After establishing the proof of concept with heterocyclic compounds, the methodology was extended to hydrophobic peptides. A cyclic hydrophobic peptide was chosen with good solubility in DMSO and limited solubility in chromatographic compatible solvents. The column headload helped in increasing the purification throughput by 4-fold without any compromise in the loss of resolution. The undesired peak was observed to be water soluble in nature. To investigate this splitting pattern, further experimentations were conducted with hydrophilic compound.



Case Study - 2_Nucleoside

To understand the splitting behaviour of hydrophilic compound under gradient elution, a water-soluble nucleoside was selected. The compound solubility was performed in DMSO + water and taken up for purification. As the compound was water soluble, distorted peaks were observed with coelution of impurities.



To address this peak distortion and poor resolution for compound with water solubility, 4% of water is added into SFC modifiers to enhance the distribution and propagation of accumulated analyte bands (multiple injections) more uniformly. This assisted in better purification performance and resolution of peaks. A partially water-soluble cyclic peptide was chosen to explain the usefulness of water in SFC modifiers.

Observations & Conclusions

- Multiple injection approach decreases purification time, improves loadability and purification throughput - Cycle time improvement & better productivity.
- This methodology was explored on various organic compounds viz heterocycles, peptides, nucleosides with high water solubility.
- The approach will be further investigated on molecules with >1500 Da and ionic compounds.

