Pre-Purify Peptides with Flash Chromatography to Increase Purification Throughput

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Abstract

Peptides are often used as active site models in drug discovery and as Active Pharmaceutical Ingredients (APIs). The increased use of peptides necessitates improved purification techniques. Purification of synthesized peptides is a bottleneck. Impurities, and the injection solvent required to dissolve the sample and impurities, limit the amount of the desired peptide that can be loaded in a single purification run. Pre-purification of peptides by flash chromatography improves throughput by reducing impurities, that have significantly different polarities when compared to the target compound, loaded on the preparative column. Removal of these impurities also allows a more appropriate injection solvent that results in sharper peaks permitting an even higher sample loading.
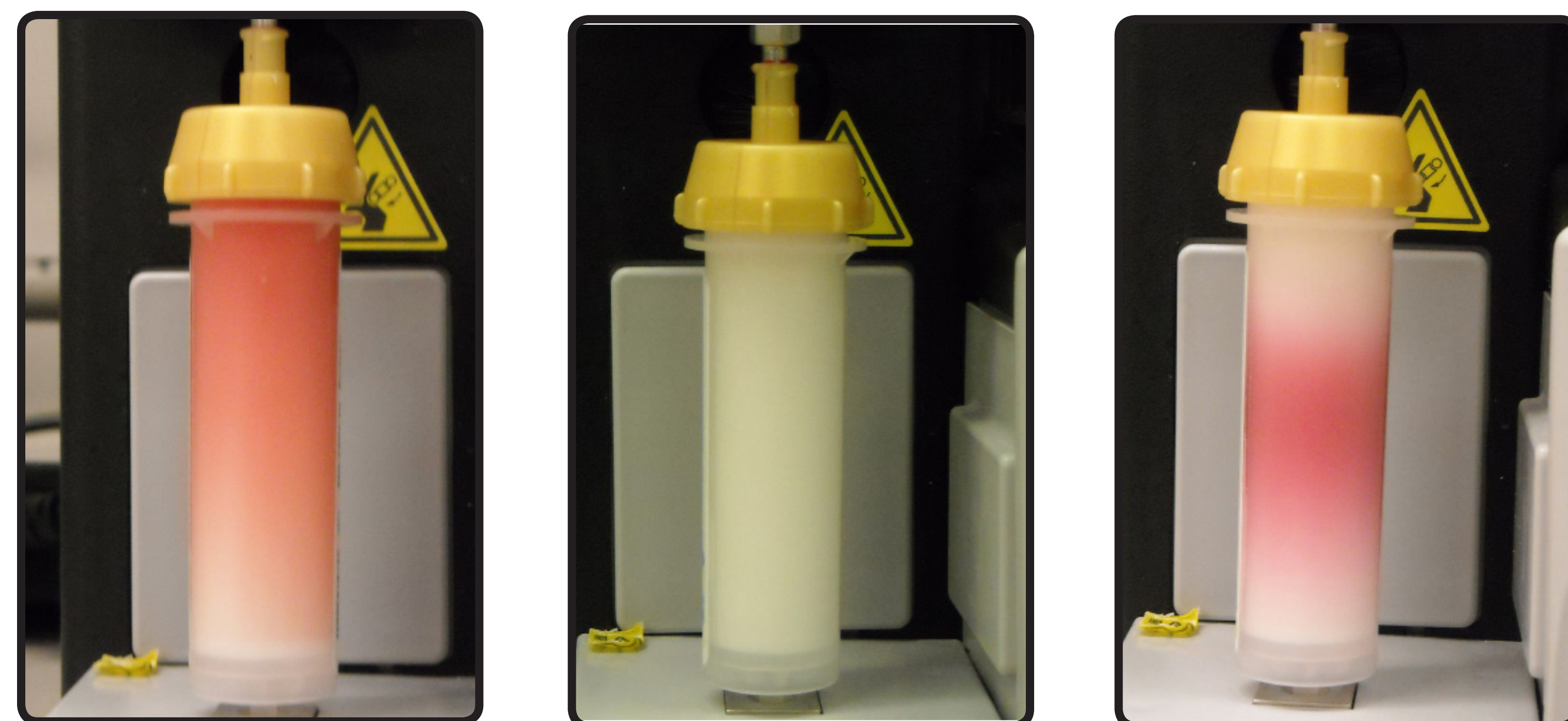
Background

Peptides are challenging to purify because of impurities closely related to the final product. These impurities include deletion errors due to incomplete deprotection during synthesis, and side reactions. As the deletions can occur at anytime during the synthesis, there are a number of closely related compounds produced. The closely related compounds have little difference in resolution during chromatography which limits the sample loading and throughput for purifying peptides.

Another factor which limits resolution and sample load during preparative chromatography is the solvent used to dissolve the crude peptide. The side reactions create compounds which are both more polar and less polar than the desired peptide, causing limited solubility in the solvent system used to elute the peptide. To avoid dissolution problems, DMSO (diethyl sulfoxide) or DMF (dimethylformamide) are commonly used to dissolve peptide samples. However, this introduces problems in that the solvent used is chromatographically "stronger" than water and can lead to reduced resolution at increased loading as the sample becomes "dragged" down the column.
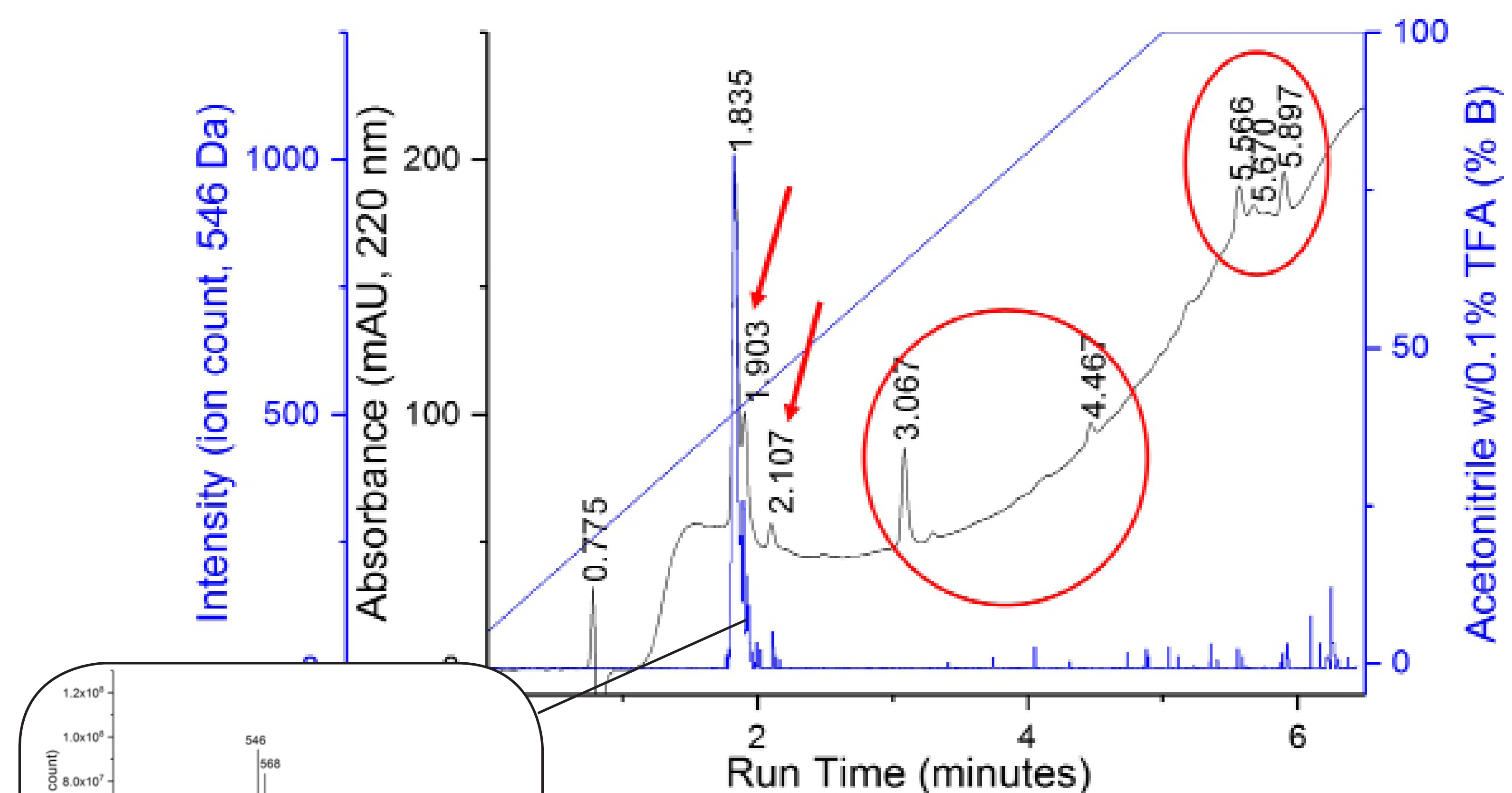
Experimental

Experimental details are described in each section. All solvents are ACS grade except where specified.

DMSO, 1.0 mL **DMSO, 2.5 mL**

Water, 2.5 mL **Water, 4.0 mL**

- The effects of dissolution solvent on preperative HPLC loading capacity are demonstrated in the traces above
- Samples are catechol and resorcinol, 10.0 mg/mL each, dissolved in the DMSO or water. Injection volume listed in each trace
- Solvent is water/methanol both with 0.1 % formic acid. Column is a RediSep® Prep C18 column (20x150 mm, 100 Å, 5 μ, PN 69-2203-826)
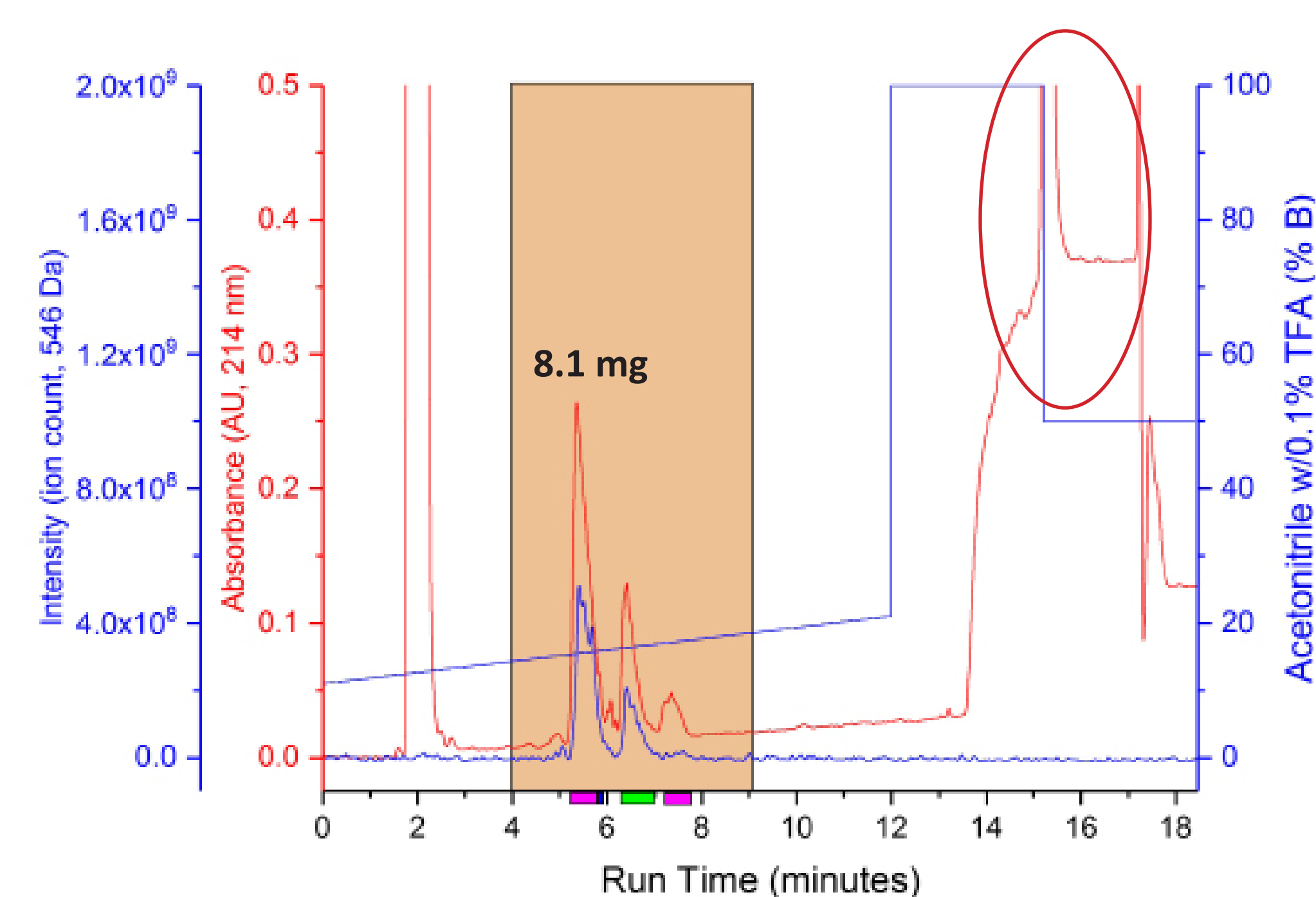

- Flash column compares chromatography of a sample dissolved in strong solvent to that dissolved in a weak solvent
- Image on left shows sample dissolved in strong solvent (1:1 methanol: water) after 1 column volume elution time
- Elution of sample dissolved in weak solvent (water) shown in images in the center (1 column volume elution) and right image
- Sample is Vitamin B12 run on a RediSep Rf Gold C18 column (PN 69-2203-334) run on a CombiFlash® Rf+ (PN 68-5230-021)
- Dissolution in weak solvent greatly improves peak shape

The peptide HNWYPAAPH, synthesized by a local peptide synthesis lab, was studied as an ACE inhibitor[1] and is considered typical of a peptide that could be purified.


- Peptide was evaluated with mass spectrometer (Purlon L, Teledyne Isco, PN 68-5237-084).
- The base peak used for detection was the $[M + 2H]^{2+}$ ion at 546 Da
- Analytical scouting run was performed on an experimental RediSep C18 column (2x50 mm, 100 Å, 2 μ) in 5->100% acetonitrile in water, both containing 0.1% triflouroacetic acid (TFA), LC/MS grade solvents.
- Arrows denote closely eluting impurities, circles denote non-polar impurities.
- Desired compound elutes in less than 2 minutes, should be water soluble
- Crude peptide not water soluable, DMSO required to dissolve sample due to non-polar impurities


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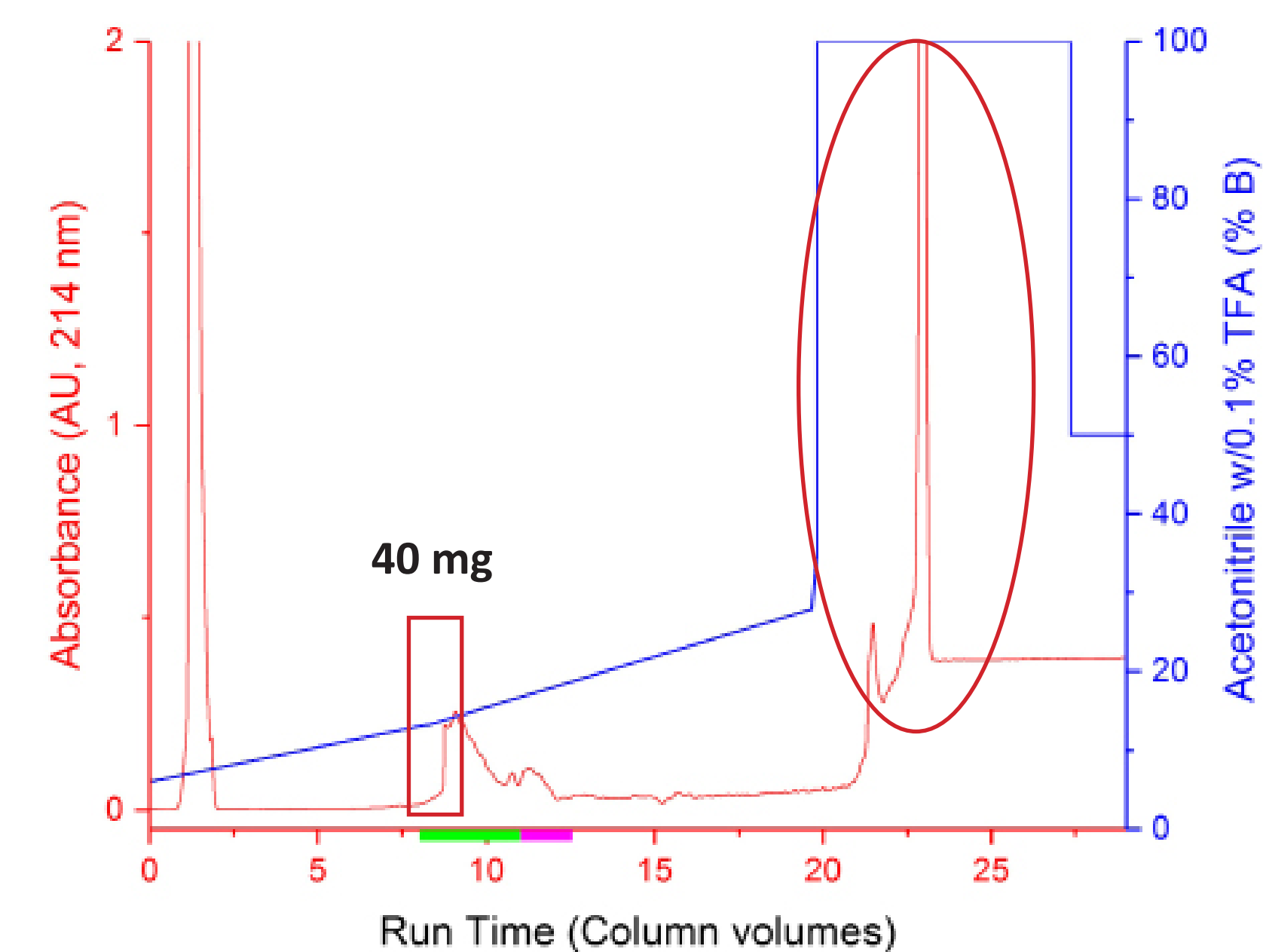
- Sample dissolved in DMSO, run on 50 g RediSep Rf Gold C18 column (PN 69-2203-336) with a CombiFlash Rf+ (PN 68-5230-021)
- Gradient similar as prep HPLC same solvents (ACS grade)
- 100 mg loaded
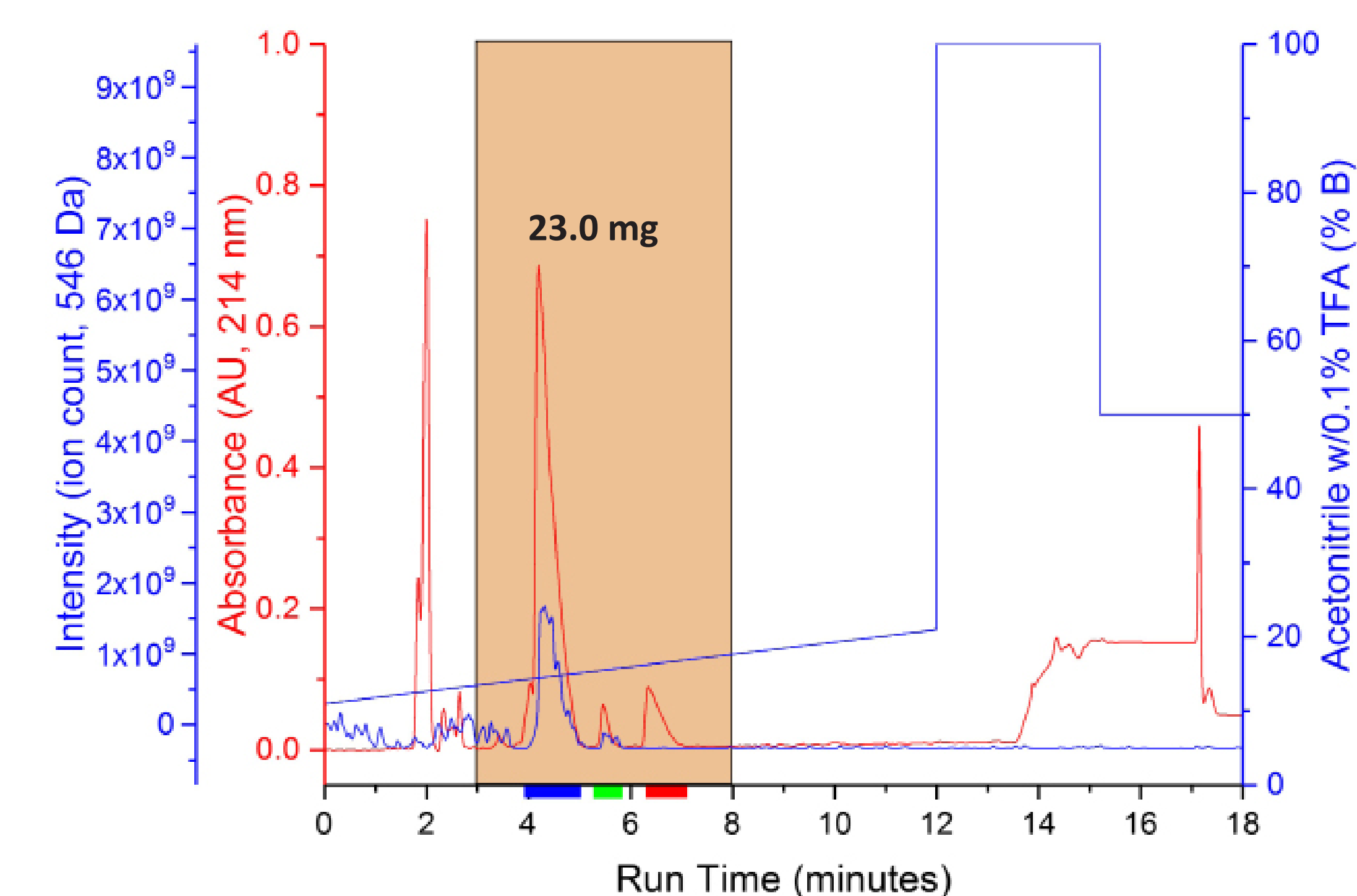
- Gradient same as previous run
- 30 mg loaded sample soluable in water/dissolved in water
- Flash column acted as "pre-column-wash step no longer needed
- Run could be ended after 8 minutes
- 3x loading and 1/2 run time mean 6x improved throughput!
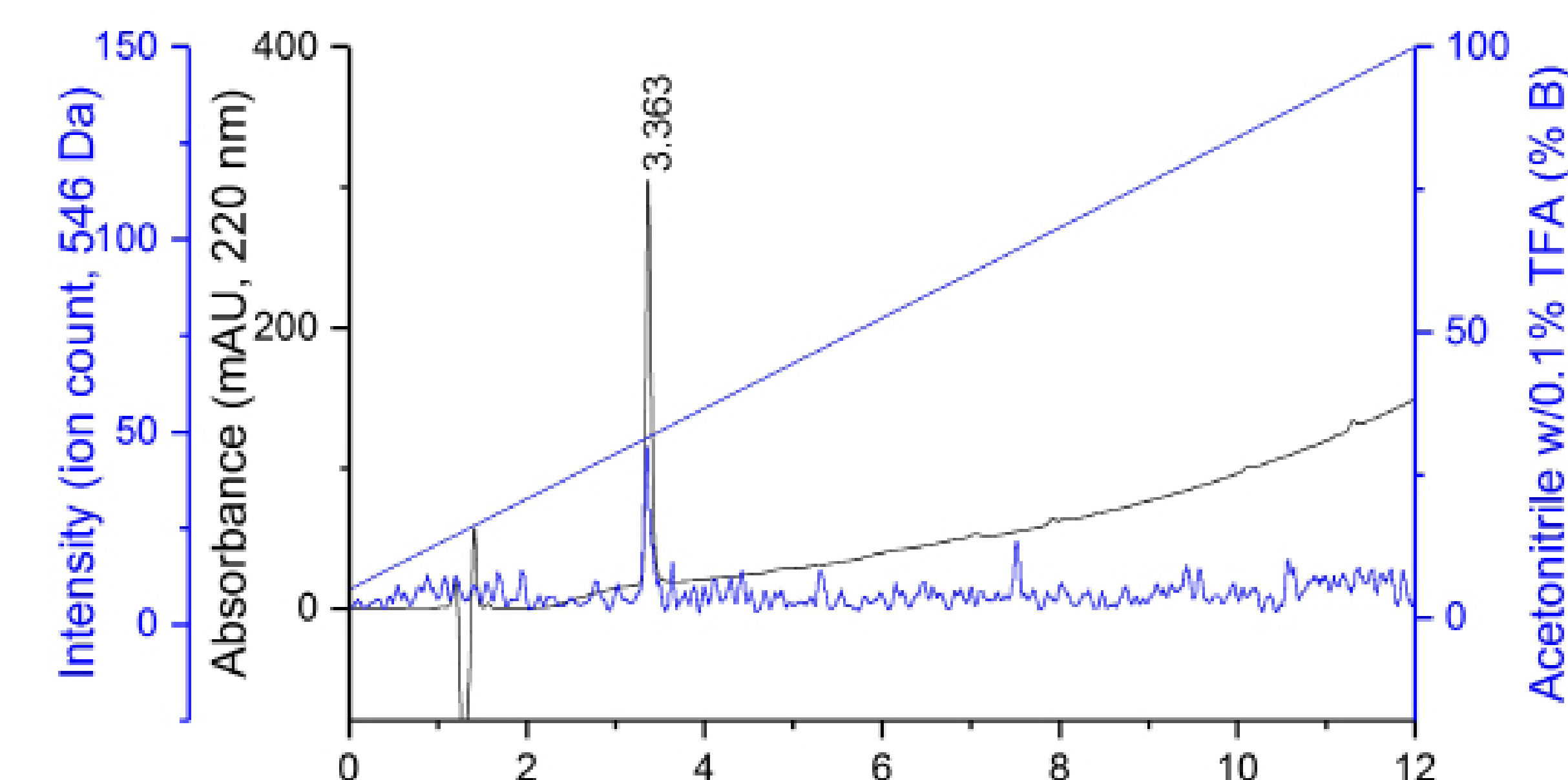
LC-MS shows purified peptide as a clean, single peak.

Conclusion

- Flash Chromatography is a useful tool to pre-purify peptides
- Removal of impurities that reduce sample solubility allow in optimal injection solvent (weakest possible solvent)
- Optimal injection solvent allows maximum resolution, allowing increased loading
- Enrichment of desired compound; closely eluting impurities reduced
- Elimination of late eluting impurities on the prep HPLC column
- At least 6x improvement in prep through-put
- Although flash run is an extra step, the time required for this run is more than offset by the increased throughput

[1] Lee,S-J; Kim, Y-S; Kim, S-E; Kim, E-K; Hwang, J-W; Park, T-K; Kim, B;K; Moon, S-H; Jeon, B-T; Jeon, Y-J; Ahn, C-B; Je, J-Y; Park, P-J. Purification and Characterization of a Novel Angiotensin I-Converting Enzyme Inhibitory Peptide Derived from an Enzymatic Hydrolysate of Duck Skin Byproducts. *J. Agric. Food Chem.* 2012, **60**, 10035-10040

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