Targeted Isolation™ of Impurities in Pharmaceutical Compounds Using SFC

AVERICA
Speeding Discovery into Development

Jeffrey P. Kiplinger, Paul M. Lefebvre, John H. Tipping; *Averica Discovery Services Inc., One Innovation Drive, Three Biotech, Worcester MA 01605 USA*

INTRODUCTION

Chromatographic isolation of impurities can be a painstaking process for the analytical laboratory. Generally a suite of analytical instrumentation and multiple chromatographic methods are required. Conventional preparative HPLC approaches result in large (often aqueous) fraction volumes, jeopardizing the stability of the collected fractions. Supercritical Fluid Chromatography (SFC) can help eliminate some of these problems. In our laboratory, we systematically approach SFC process development using an approach we term "Targeted Isolation".

In comparison with reversed-phase and normal phase HPLC, SFC offers rapid method development and high efficiency of preparative separations through increased loading capacity, faster linear solvent velocity, and drastically reduced solvent removal effort. The efficiency gains facilitate stepwise protocols that would be impractical using other chromatographies.

TARGETED ISOLATION™

Targeted Isolation is a problem-solving process in which we strategically design economical ways to isolate specific peaks of interest.

To a great extent, the source material for the impurity, whether API, drug substance, drug product, stress degradation sample, or an isolate such as a mother liquor determines the nature of the process. Once the source is understood, an isolation strategy is developed that leads to an enriched sample and finally a purified sample of the peak. Our strategy leverages the rapidity of method development in SFC, as methods for enrichment and isolation are often developed independently.

Two primary isolation strategies are used in our laboratory: **Enrichement** and **Direct Isolation.** The enrichment strategy involves removing the main peak, or isolating a fraction of the chromatogram containing the desired peak. Using direct isolation, a specific SFC method is developed that resolves the desired impurity peak, and the peak is collected as a purified isolate. These approaches are used alternatively and in tandem, depending on the nature of the sample and the problem.

Finally, the process developed is used to isolate sufficient material for "tube scale" NMR: 10 to 20 mg. This quantity is accessible using rapid processing of feed by SFC, and it greatly reduces resources consumed in structure elucidation

SOURCE OF IMPURITY

It is sometimes possible to identify a source for the desired impurity from which isolation is easier. Several examples are shown.

Forced Degradation

Figure 1 is an example in which the target impurity was enriched under acidic conditions.

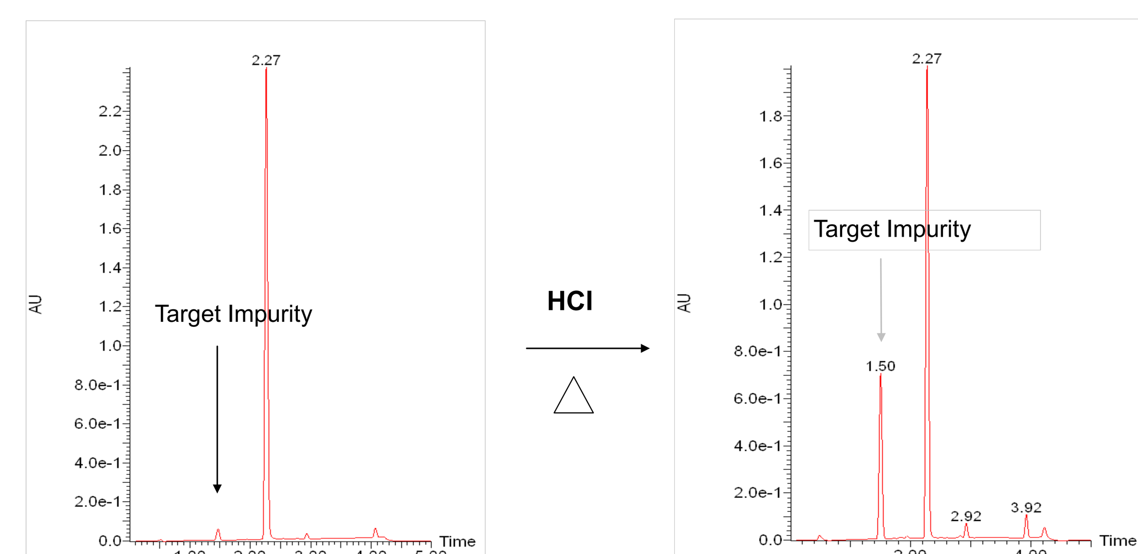

Figure 1: HPLC of the crude material , pre– and post degradation.

Mother Liquor

In another example, a finished API contained ~2% of a target impurity. The mother liquor from the final crystallization showed over 20% of the target impurity. (Figure 2)

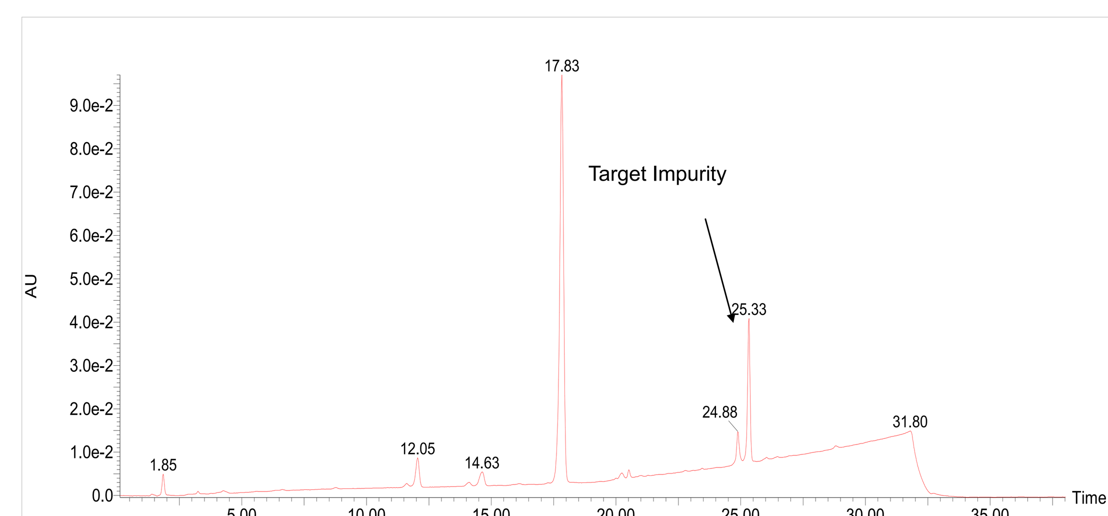

Figure 2: HPLC of the mother liquor from the final crystallization.

IMPURITY ENRICHMENT

Using the enrichment strategy, an SFC method is developed which appears to resolve the main peak (active ingredient) cleanly from neighboring peaks, and the main peak is captured and removed to produce an enriched fraction containing all other compounds, including the desired impurity(s). This approach takes advantage of the rapidity with which SFC can process a feedstock solution of the sample, and can be used even when impurities are not visible (below LOD) under preparative chromatography loads.

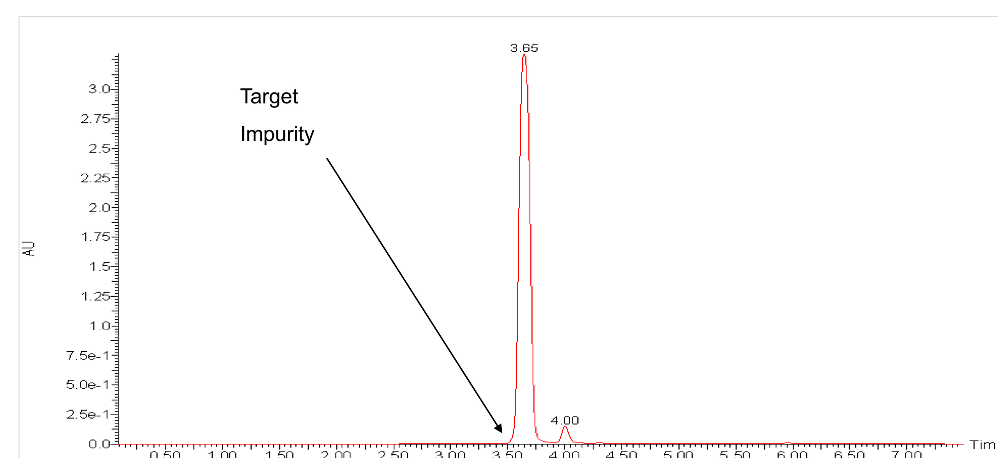

Figure 3: RP-HPLC of the crude material . The target impurity signal at 3.6 minutes, RRT = 0.97, is difficult to detect as a low concentration shoulder peak in the crude sample.

For Targeted Isolation of the RRT=0.97 peak, an isocratic, normal phase SFC method was developed which eluted the main peak at k'>2 with relatively sharp bandshape, and this method was scaled for preparative chromatography. Fractions were collected in the region of the main peak and assayed for the target impurity using the HPLC method from Figure 3.

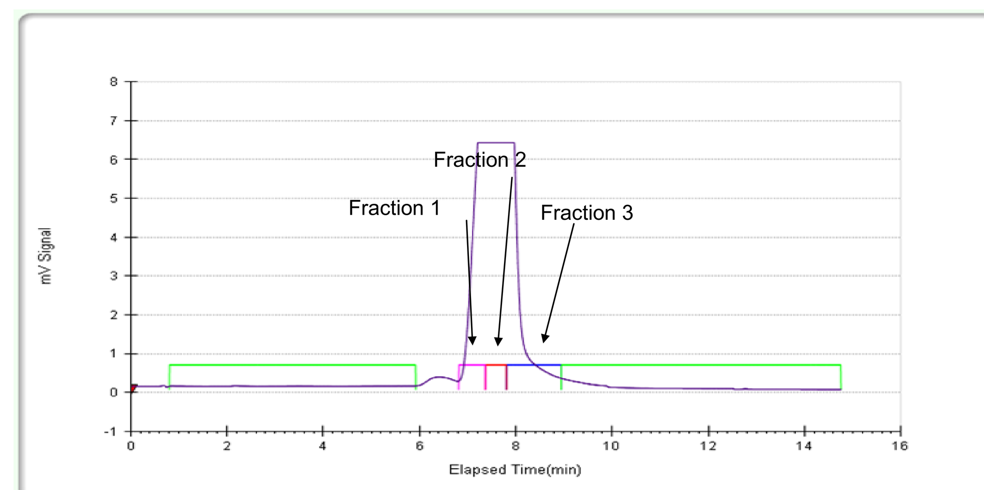

Figure 4: SFC preparative scale scouting injection. The main peak and the RRT=1.1 impurity are visible; the RRT=0.97 impurity is not.

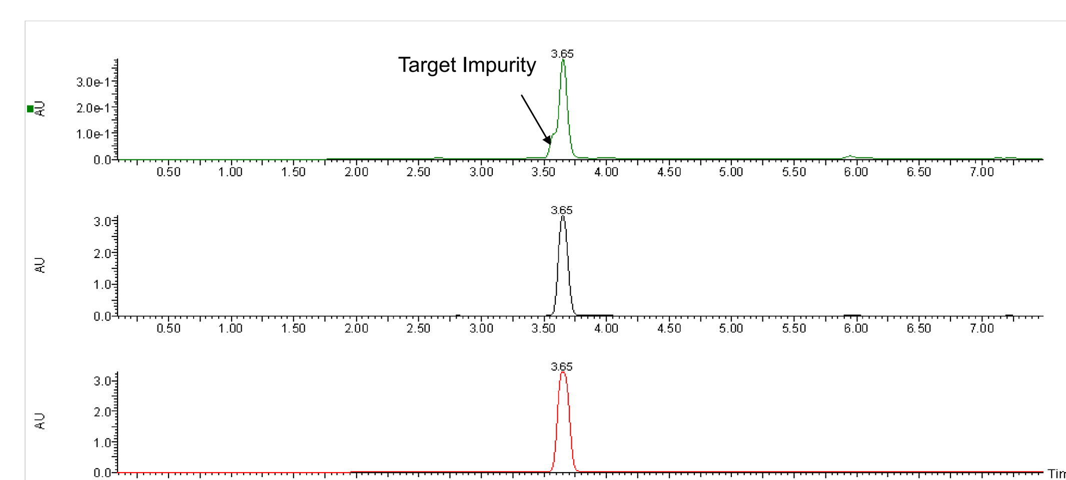

Figure 5: HPLC evaluation of collected fractions F1-F3. The desired impurity is observed in F1.

The SFC method from Figure 4 was used to process 20g of crude material in ~ 5 hours to obtain 100 mg of an enriched impurity fraction. Using an improved RP-HPLC method that successfully resolved the leading shoulder, the isolate was evaluated as enriched in the impurity to approximately 15% (by relative UV absorbance).

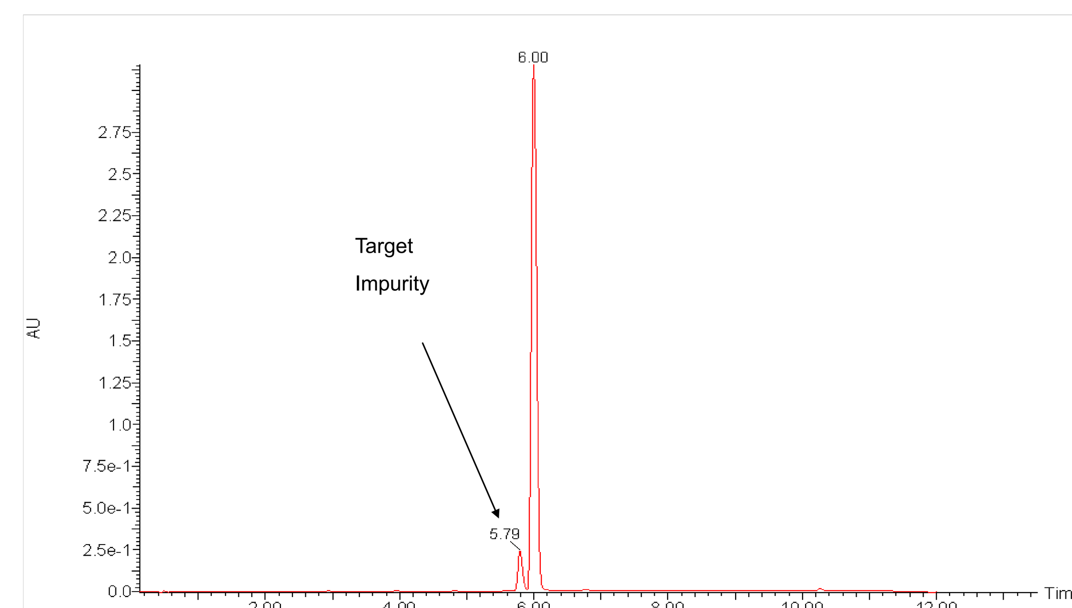

Figure 6: RP-HPLC of the enriched isolate, using the modified method.

DIRECT ISOLATION

Using direct isolation, a specific SFC method is developed that resolves the desired impurity peak. This approach is useful when the peak of interest is visible, or has a specific detection signature - e.g. a specific UV absorbance wavelength or a mass not shared by other compounds in the mixture.

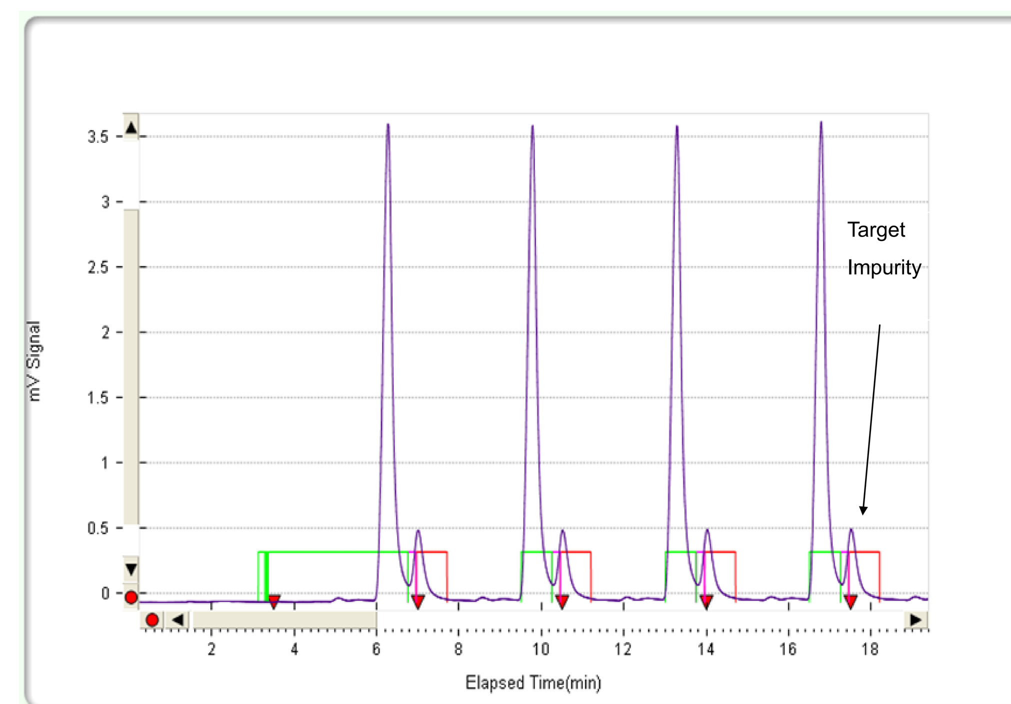

Figure 7: Optimized Prep SFC stacked injections of the enriched impurity.

Eleven milligrams of target impurity (UV_{260} purity = 97%) was obtained. The entire isolation consumed only two days of laboratory effort.

OTHER ENRICHMENT STRATEGIES

Often the iterative process of isolation and analysis suggests alternate strategies to speed up the isolation process. In the example shown in Figures 8 and 9, a sample containing three peaks, one of which was the target of interest, was obtained following enrichment of the sample impurities by SFC main peak removal.

RP-HPLC analysis of the mixture using an aqueous formic acid gradient distorted one peak and failed to produce an MH+ ion from the target with ESI -MS detection. Use of a pH9 buffered ammonium bicarbonate gradient resulted in retention time shifts for the two non-targets, but not for the target peak.

After concluding that the target peak was likely the only neutral species in the isolate, purification was completed using liquid-liquid extraction (LLE).

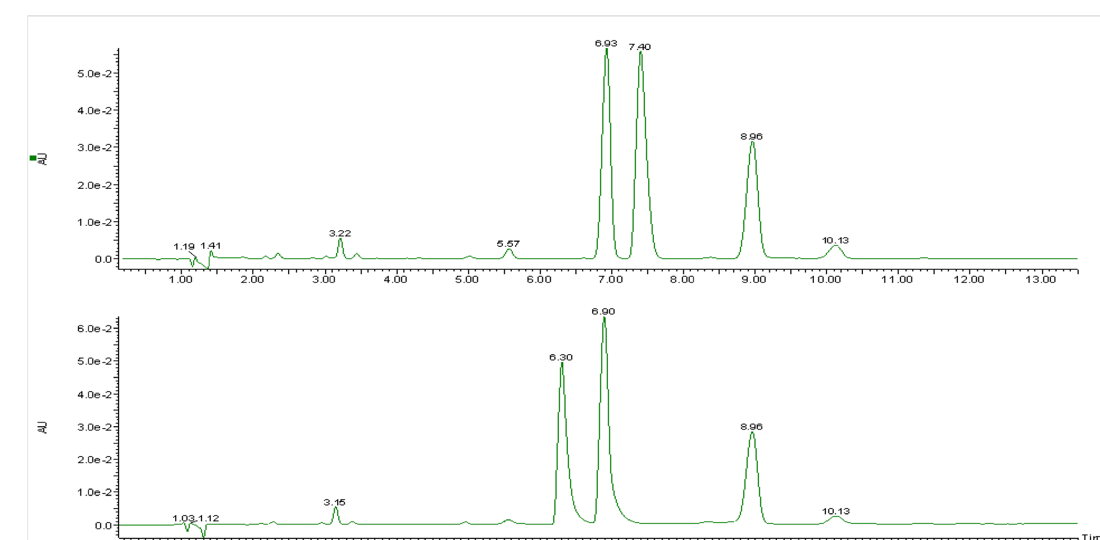

Figure 8: RP-HPLC analysis of enriched isolate using acidic (top) and basic (bottom) gradients showing retention time shifts in all components except the target impurity peak at 8.9 minutes.

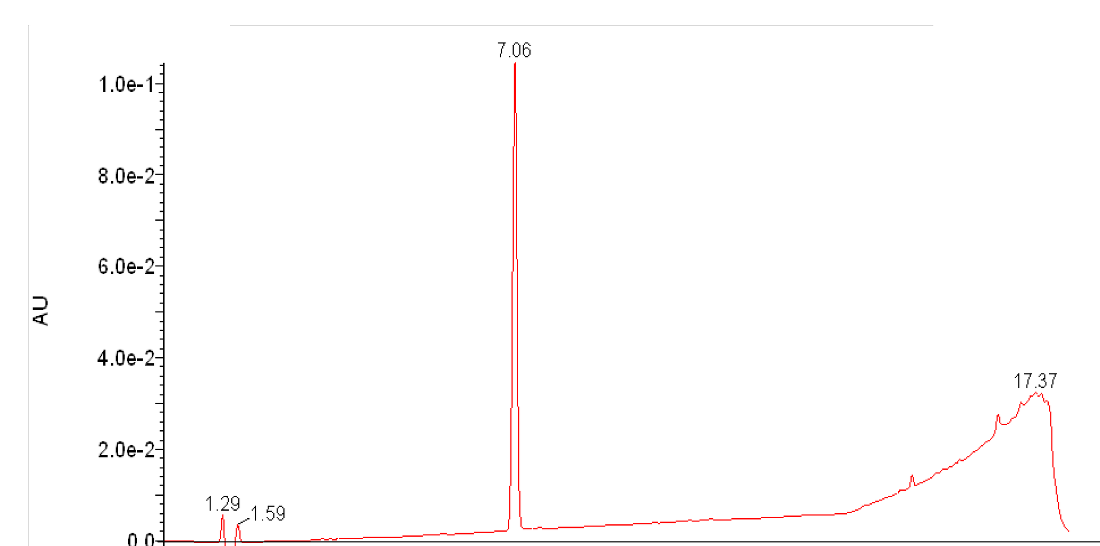

Figure 9: Target impurity isolated after liquid extraction from the enriched sample in Fig. 8. The RP-HPLC method used was supplied by the study sponsor.

SIMULTANEOUS CAPTURE OF MULTIPLE IMPURITIES

Often isolation and analysis of more than one impurity is required. By developing an SFC process aimed at removal of the main peak, enriched samples are often isolated from which more than one impurity may be isolated.

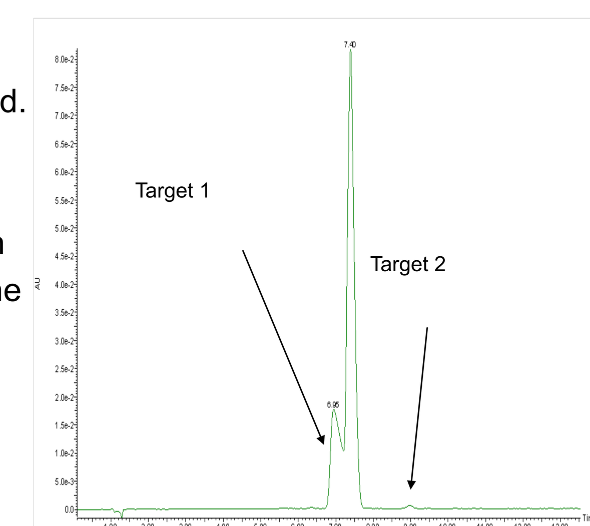

Figure 10: RP- HPLC of crude material.

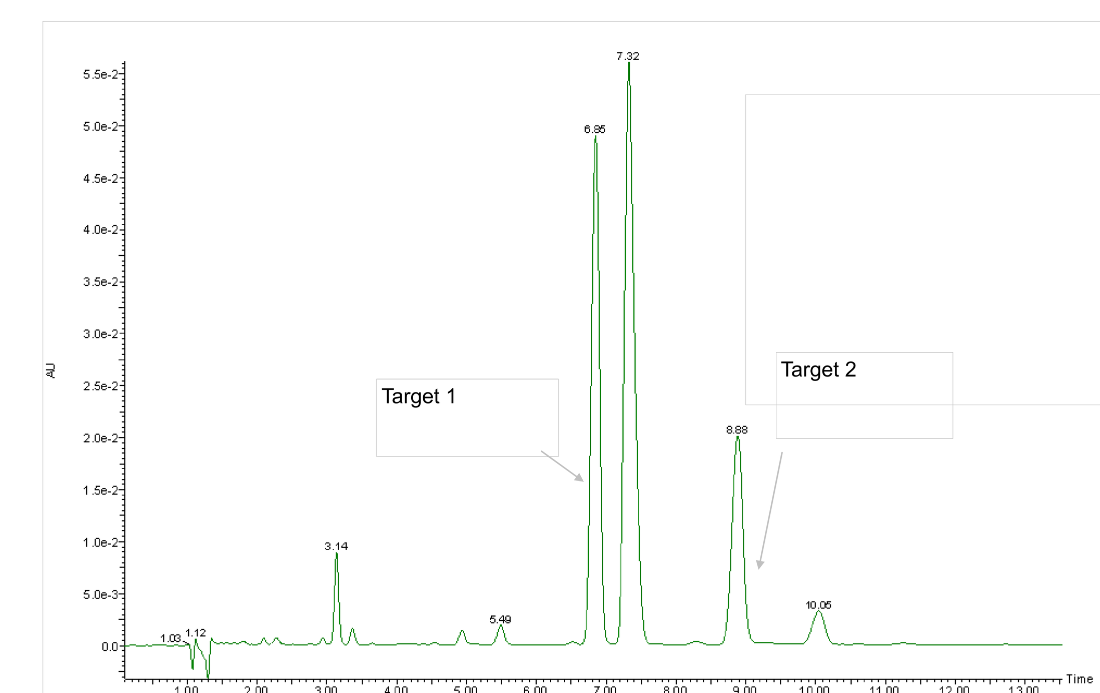

Figure 11: Impurity enriched fraction. Developing an SFC method for isolation of both targets can be achieved based on relative peak area alone.

CONCLUSIONS

Targeted Isolation™ is a process wherein information fed back from iterative HPLC analysis and SFC isolation steps guides the development of the isolation process. HPLC analysis is a desirable component due to its familiarity and simplicity -- the methods we use are frequently supplied by the project sponsor.

In the SFC isolation steps, we leverage the key performance advantages of SFC chromatography, including rapidity of method development and of processing feedstock. Indirectly, we often gain advantage from SFC's orthogonality to HPLC and from the low liabilities incurred using common SFC solvents (rapid removal, low reactivity, and low residual content). The objective, 10 milligrams of pure isolated unknown impurity, is achievable and limits the difficulties in structural analysis.