

PURIFICATION OF RADIOLIGAND PRECURSOR PSMA-617 FROM A FORCED DEGRADATION STUDY USING A WATERS MAXPEAK™ PREMIER OBD™ PREPARATIVE COLUMN

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INTRODUCTION

The targeted delivery of medicine using radioligand therapy kills cancer cells with precision.¹ Previous work discussed the forced degradation of the radioligand therapy precursor PSMA-617 and the development of a suitable UPLC-MS method to separate the resulting components in the mixture using MaxPeak High Performance (HPS) Technology.² HPS Technology³ mitigates the undesirable effects of compound to metal surface interactions with inert surfaces. Pharmaceuticals and their degradation products must be meticulously characterized before they can be approved as therapeutics. Typical forced degradation conditions, which include acidic, basic, oxidative, and thermal treatment, are useful for characterizing the stability and degradation pathways for drug candidates. As noted in previous work, PSMA-617, the precursor to PLUVICTO® from Novartis, was subjected to forced degradation conditions at a larger scale to produce enough sample to demonstrate the technical approach that might be used in a purification laboratory to isolate the PSMA-617 from its closely eluting contaminants. The sample mixture for these experiments was first analyzed using similar conditions which resulted from the systematic screening protocol used to speed up method development in the prior study, then additional adjustments were made to the analysis method for pre-prep optimization. The optimized analytical method was then scaled for the preparative isolation. A MaxPeak Premier OBD Preparative Column installed on a UV-directed AutoPurification System configured with a Timberline Column Heater⁴ was employed to perform the purification. The high purity of the recovered PSMA-617 precursor was the ideal outcome since the compound could be useful as a reference standard in subsequent studies. The short half-life of radionuclides used for targeted cancer therapy necessitate purification efficiency, which was achieved through direct scalability from small scale analysis to preparative isolation with a Waters Optimum Bed Density (OBD) Column rendered inert with HPS Technology.

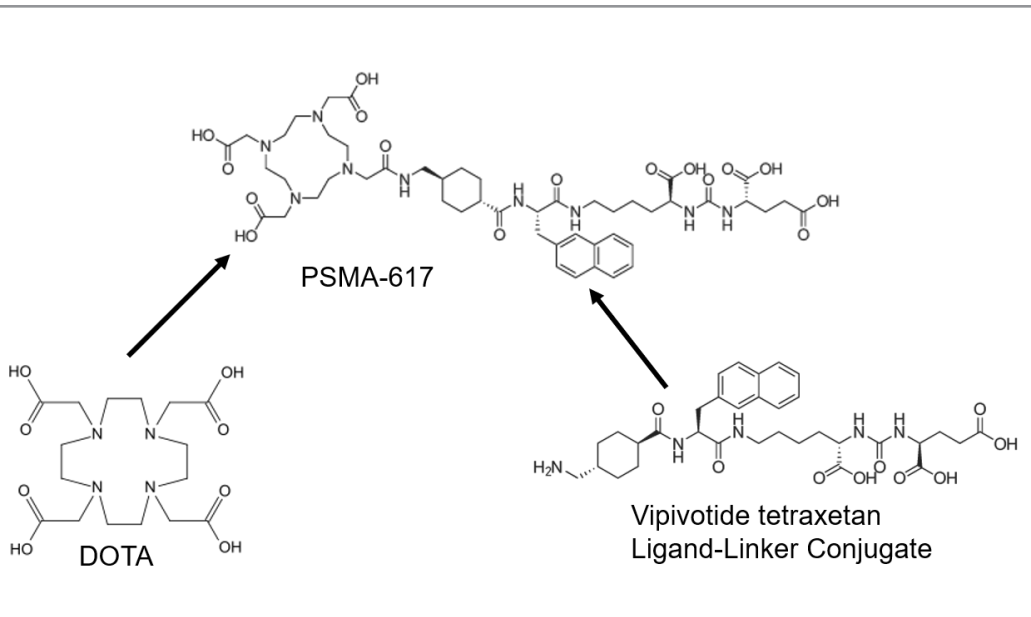


Figure 1. Chemical structure of PSMA-617 and two of the components used in its synthesis.

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METHODS

PSMA-617 and Impurities Isolation Conditions:

Analytical Column (inert) and flow rate: XSelect™ Premier CSH™ Phenyl-Hexyl Column; 2.5 µm, 2.1x100 mm; 0.35 mL/min

Prep Column and flow rate: XSelect Premier CSH Phenyl-Hexyl OBD Prep Column, 3.5 µm, 10x150 mm (custom inert column); 5.67 mL/min

Mobile phase A: Water with 0.1% Formic Acid

Mobile phase B: Methanol with 0.1% Formic Acid

Wavelengths: 254 nm

Injection volumes: Analytical 2 µL, 10 µL; Preparative 68 µL

Column temperature: 40 °C ± 5 °C

Sample temperature: Ambient

Sample: A stock solution of PSMA-617 was created at 5 mg/mL in water. Three separate 4 mL aliquots were removed and placed into polypropylene scintillation vials for degradation. To each vial, 400 µL of a catalyst was added. For acidic degradation, 400 µL of 1 N HCl was added, and the vial was held at 70 °C for 24 hours. For basic degradation, 400 µL of 1 N NaOH was added, and the vial was held at 70 °C for 24 hours. Lastly, for peroxide degradation, 400 µL of 3% H₂O₂ was added, and the vial was held at 70°C for 24 hours. All three vials were combined into a single 20 mL Nalgene vial for storage prior to analysis and purification.

Instrumentation

Analytical: ACQUITY UPLC H-Class System; Quaternary Solvent Manager, Sample Manager FTM-H, Column Manager, TUV Detector, ACQUITY QDa™ Detector

Prep: Waters AutoPurification™ System; 2545 Binary Gradient Module, 2767 Sample Manager, System Fluidics Organizer, 2998 Photodiode Array Detector, Timberline Column Heater; 500 µL stainless steel sample loop

Gradient Methods

Analytical Scouting Method: 5-95% B in 10 min, hold @ 95% B for 2 min, return to 5% B in 2 min, equilibrate for 6 min

Analytical Focused Method 1: 5-51% B in 1 min and hold @ 51% B for 2 min, 51-59% B in 4.47 min, 59-95% B in 0.53 min, hold @ 95% B for 1 min, return to 5% B in 1 min, equilibrate for 2 min

Analytical Focused Method 2: 5-45% B in 2 min and hold @ 45% B for 4 min, 45-54% B in 4.46 min, 54-95% B in 0.54 min, return to 5% B in 2 min, equilibrate for 7 min

Preparative Method: hold at 5% B for 1.29 min, 5-45% B in 4.2 min, hold @ 45% B for 8.4 min, 45-54% B in 9.37 min, 54-95% B in 1.13 min, return to 5% B in 4.2 min; equilibrate for 14.7 min

RESULTS AND DISCUSSION

PSMA-617, the radioligand precursor (Figure 1), was degraded using the forced stressor conditions outlined above. Because the chemical structure of PSMA-617 contains a chelating group (DOTA), this precursor was likely to have an affinity for adsorbing to metal surfaces, a phenomenon called non-specific adsorption (NSA). Negatively charged molecules can interact ionically with the positively charged metal oxide surfaces of the column and LC flow path in neutral and acidic pH environments. This non-specific adsorption occurs with stainless steel and titanium, as well as with other metal alloys. Metal sensitive compounds with strong acidic moieties like sulfates, phosphates, or carboxylates are prone to adsorption (Figure 2). Berthelette² *et al.* detailed the advantages of using an XSelect Premier CSH Phenyl-Hexyl Column with an inert surface for the separation of the PSMA-617 precursor from the degradant impurities. This stationary phase, manufactured on a hybrid particle, provides alternative selectivity to C₁₈ packings.⁵ Higher peak areas, lower USP tailing factors, and narrower peak widths were observed when a column with an inert surface was used.

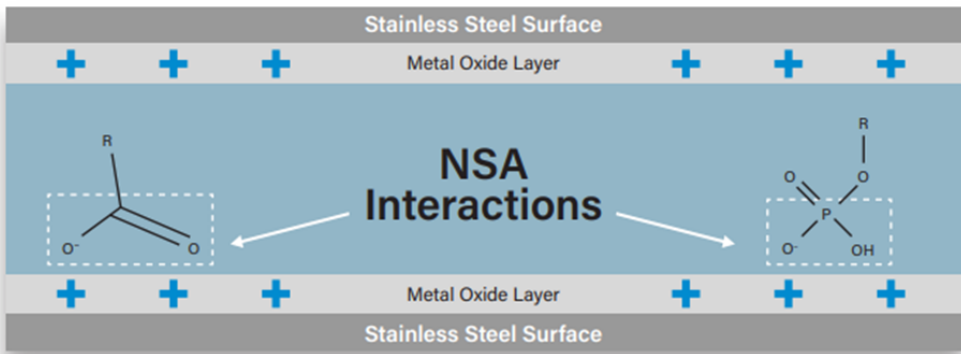


Figure 2. Non-Specific Adsorbers result in bad peak shapes and poor or inadequate fraction triggering, and can lead to compromised target purity and recovery.

For this ensuing study, all chromatography was performed using columns with inert hardware. The sample was first analyzed using a 5-95%B scouting gradient at room temperature. The chromatogram showed two contaminant peaks eluting very close to the PSMA-617 peak at ~7 min (Figure 3).

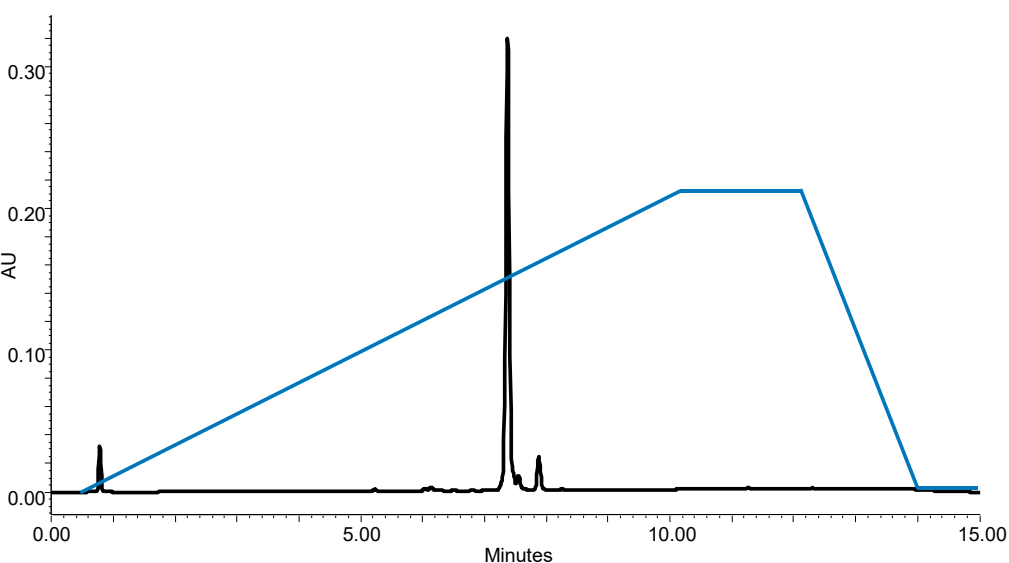


Figure 3. Analytical separation for degraded PSMA-617 using the 5-95% B scouting gradient; room temperature; XSelect Premier CSH Phenyl-Hexyl Column, 2.1x100 mm, 2.5 µm

A focused gradient was developed for improving the resolution between the main peak and the closely eluting contaminants.⁶ Because the 51-59% B gradient failed to separate the main peak from the peaks eluting immediately after it, the focused gradient was modified to run from a slightly lower starting percentage with an 8% range (45-54% B). This second focused gradient separated the two small peaks from the PSMA-617 and was subsequently scaled for the preparative isolation (Figure 4).

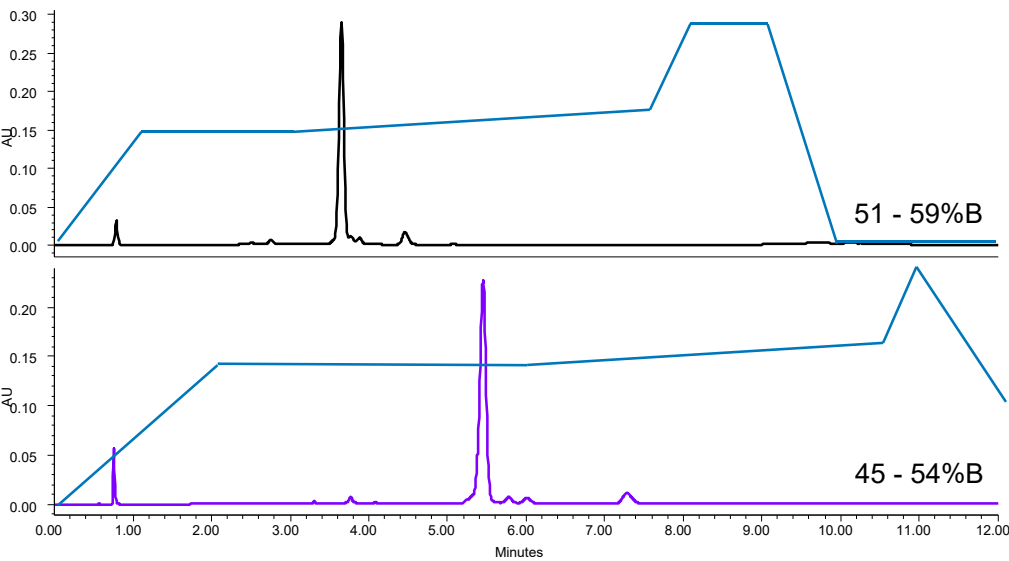
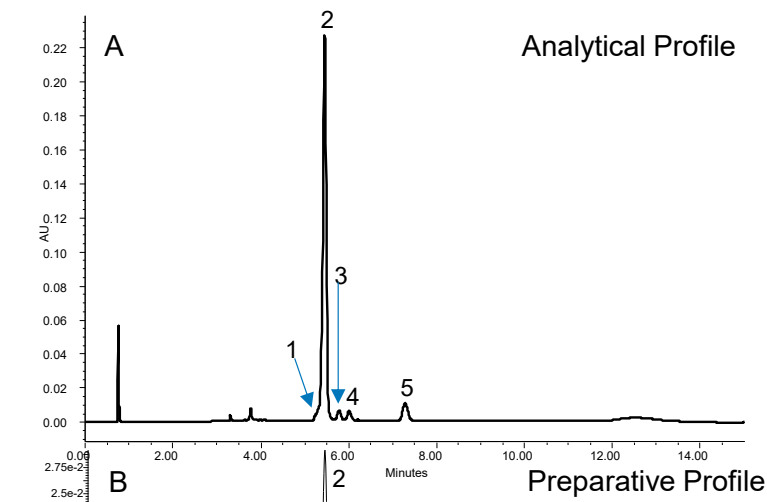


Figure 4. Analytical separation for degraded PSMA-617 using two focused gradients @ 40°C on the XSelect Premier CSH Phenyl-Hexyl Column, 2.1x100 mm, 2.5 µm

The L/d_p ratio (ratio of column length to the particle size) between two columns must be similar to maintain the separation when scaling.⁷ The L/d_p was 40,000 for the 2.5 µm 2.1x100 mm column and 42,857 for the 3.5 µm 10x150 mm custom packed preparative column. The preparative separation matched the analytical separation, which made peak identification and compound isolation straightforward (Figure 5). Peaks were identified using mass analysis in electrospray positive mode. Peak 1 contained the singly and doubly charged ions for PSMA-617 (*m/z* 1042 and 521, respectively) as well as two other degradants with *m/z* 371 and *m/z* 741. Peak 2 was identified as PSMA-617. Peaks 3, 4, and 5 all had the same two masses, *m/z* 1024 and *m/z* 512, most likely the singly and doubly charged ions for each of these three unknowns. Compound isolation was targeted for the main peak and the two closely eluting impurities after peak 2, so peak 5 was not collected.



Peak	Mass (m/z)	ID
1	371, 521, 741, 1042	PSMA-617 and degradants
2	521, 1042	PSMA-617
3	512, 1024	Impurity peak 1
4	512, 1024	Impurity peak 2
5	512, 1024	Impurity peak 3

Figure 5. Analytical and preparative separations for degraded PSMA-617 using the 45-54% B gradient on the A) XSelect Premier CSH Phenyl-Hexyl Column, 2.1x100 mm, 2.5 µm; 2 µL injection and B) XSelect CSH MaxPeak Premier Phenyl-Hexyl OBD Column, 10x150 mm, 3.5 µm; 68 µL injection.

Small particle preparative columns empower the purification scientist to isolate highly pure compounds faster at reduced scale and with lower volume fractions for faster dry-down times. In these experiments, the small particle preparative column effectively resolved the impurity eluting just before the PSMA-617 target peak. In addition, two contaminant peaks eluting just after the target peak were well separated and their isolated fractions were easily collected (Figure 6). To save time, the purification runs were terminated early to prepare the column for subsequent injections.

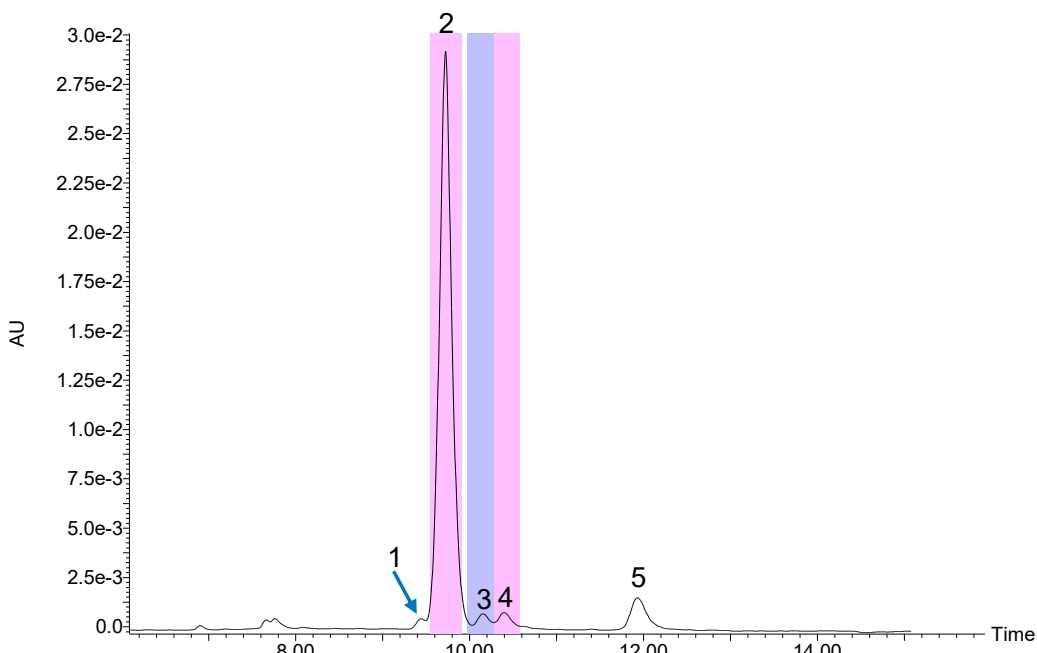


Figure 6. Preparative separation of PSMA-617 using the 45-54% B focused gradient @ 45°C on the 3.5 µm, 10x150 mm MaxPeak Premier XSelect CSH Phenyl-Hexyl OBD Column; 68 µL injection; 254 nm. Peaks were identified via ESI+MS. 1) PSMA-617 and degradants 2) PSMA-617 3) Impurity peak 1 4) Impurity peak 2 5) Impurity peak 3 (not collected)

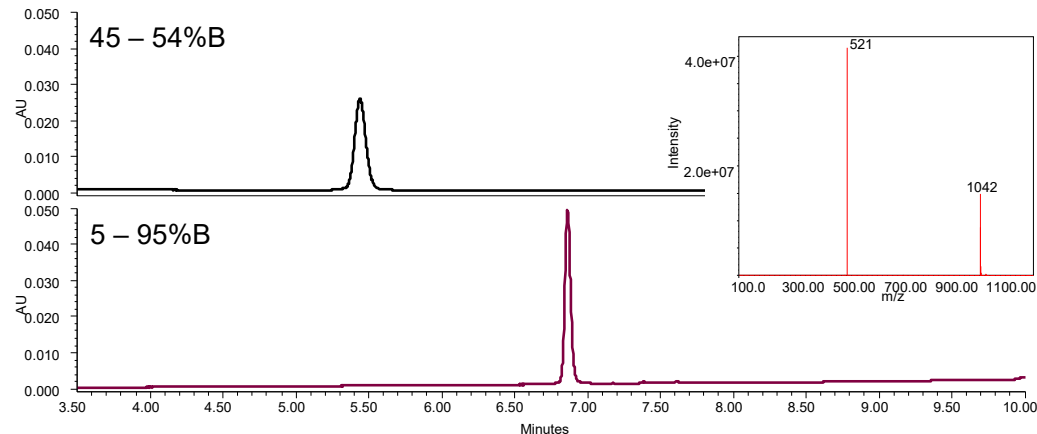


Figure 7. Analysis of the PSMA-617 fraction pool from 4 preparative runs gave a single UV peak with the MS scan showing only the singly and doubly charged PSMA-617, confirming the purity achieved during isolation. Analysis performed on the 2.5 µm 2.1x100 mm XSelect Premier CSH Phenyl-Hexyl Column; 10 µL injection; 40°C; 254 nm; 5.44 min retention time.

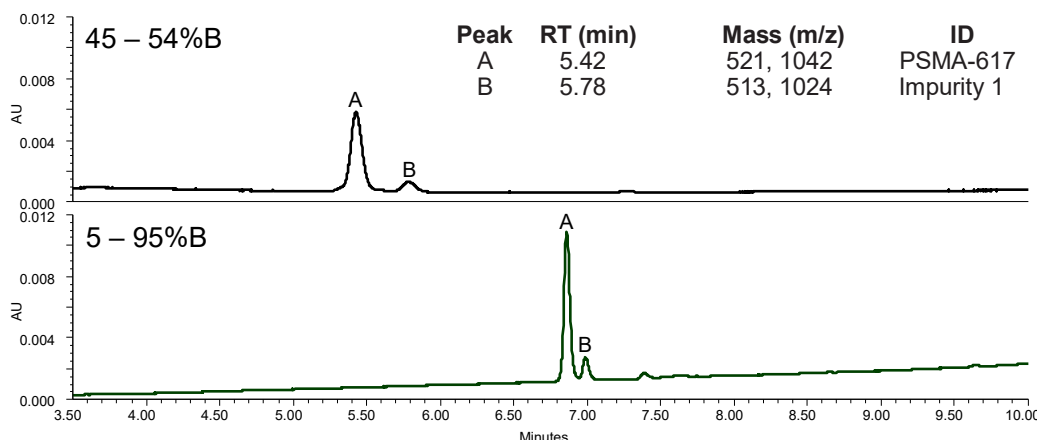


Figure 8. Analytical separation of Impurity 1 fraction pool

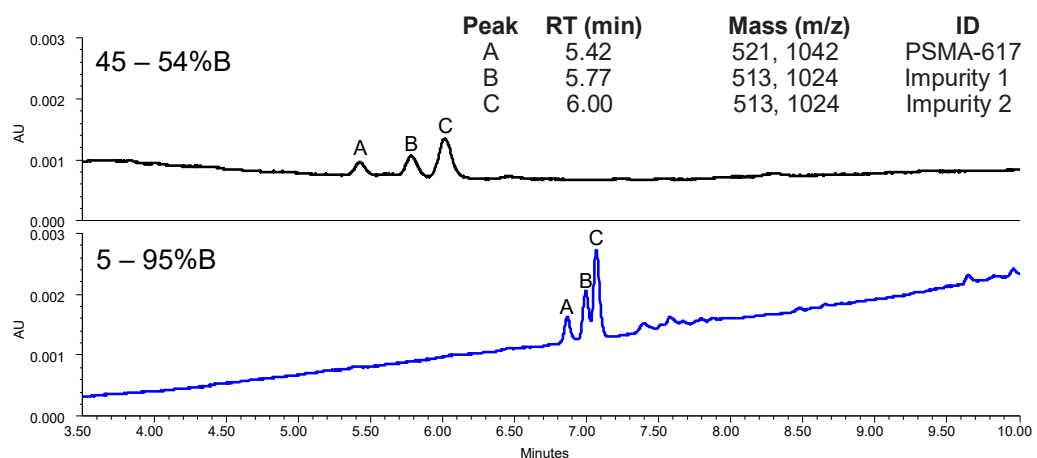


Figure 9. Analytical separation of Impurity 2 fraction pool

Although UV and mass analyses of the fraction pools of the two impurities (Figures 8 and 9; analyses performed on the 2.5 µm, 2.1x100 mm XSelect Premier CSH Phenyl-Hexyl Column; 10 µL injection; 40°C; 254 nm) indicated that these degradants were not pure, they were target-enriched. Enrichment simplifies the subsequent purification step by producing more material for the ensuing workup. The chromatography of the impurity pools suggested that another isolation using the same focused gradient would most likely produce pure fractions since the peaks in each fraction pool were very well resolved. Scientists who purify and characterize targets, as well as the degradants or impurities in the crude sample mixture, usually enrich the minor components and then perform another purification. Known also as a polishing step, this approach often produces enough material for full structural elucidation and component identification, a crucial step in the drug development process.

CONCLUSIONS

- The high-performance surface technology used in MaxPeak Premier Columns produced higher peak areas, lower USP tailing factors and narrower peak widths for the radioligand precursor PSMA-617 and its degradants during analytical method development, with XSelect CSH Phenyl-Hexyl stationary phase giving the best chromatographic resolution.
- Predictable scaling from UHPLC to prep was achieved by employing columns with inert hardware and using a focused gradient for the isolation of PSMA-617 from the impurities in the forced degradation sample mixture, which simplified the purification process and generated pure target.
- Two impurities were collected and enriched and analysis indicated that a final polishing step would most likely produce pure compounds for characterization, a critical step in the drug development process.
- Inert column hardware, OBD Technology, and small particle preparative columns are well-suited for the demands of the purification laboratory, generating favorable outcomes that improve target isolation while saving method and fraction drying time and promoting sustainability by reducing solvent usage.

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