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Note d'application

Analysis of Radioligand Therapy Components Using Reversed-Phase and HILIC Columns

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Abstract

Radiotherapy techniques have evolved over the years. While traditional radiotherapy is still used heavily to treat cancer, some forms of the disease require more targeted approaches. One of the most recent advances is radioligand therapy, which employs a radioisotope bound to a targeting ligand.

This application note focuses on the analysis of the components of Lutetium (177 Lu) vipivotide tetraxetan, marketed as PLUVICTO®. Three analytes were examined: the main compound, the linker conjugate, and the chelator. Reversed-phase chromatography using an ACQUITY Premier HSS T3 Column worked well for the main compound and the linker conjugate but failed to retain the chelator. However, by using Hydrophilic Interaction Chromatography (HILIC) with an Atlantis Premier BEH Z-HILIC column, all three compounds could be retained and separated.

Benefits

- Comparison of reversed-phase and HILIC approaches
- Good separation of the main compound (vipivotide tetraxetan) from the ligand-linker conjugate and the chelator using HILIC
- Improved recovery and detection of the main compound and chelator compound when using MaxPeak

Premier Columns compared to a conventional stainless-steel column

Introduction

Advancements in radiotherapy treatments have led to increased challenges in analysis and quality control. As the therapies become more complex, so too must the quality control measures that are put in place to ensure patient safety. One advancement in radiotherapy treatments includes the use of radioligand compounds that are designed to target specific cell lines to combat cancer growth. One such compound is PLUVICTO, also called Lutetium (177 Lu) vipivotide tetraxetan. This compound was designed to bind to prostate-specific membrane antigen (PSMA) on the outside of certain cancer cells. After binding, PLUVICTO is taken up by the cell through endocytosis where the radioisotope 177 Lu releases beta-radiation causing cell death without affecting surrounding cells.[1] The structure of this treatment includes the ligand-linker conjugate which is the part of the compound which attaches to the PSMA protein, and then a chelator which carries the radioisotope into the cell. The synthesis of this compound is complicated, involving seven-steps.[2] The final step, attachment of the 177 Lu, must be done close to the time of administration as the radioisotope has a half-life of approximately 6.6 days.[3] Because of the short half-life of the radioisotope, the compound needs to be tested prior to the final synthesis step. This means that the final compound, without the attached metal would be analyzed for purity and to ensure it was synthesized properly. Monitoring of the synthesis reaction is also crucial in understanding the process of creating the final compound, as well as ensuring that no side impurities are formed. As such, it is often prudent to analyze the precursors in a chemical reaction as well as the final product.

This application note examines the analysis of three compounds, vipivotide tetraxetan (PSMA-617), the chelator compound 1,4,7,10-tetraazacyclododecane-N,N′,N″,N‴-tetraacetic acid (DOTA), and the ligand-linker conjugate. This analysis would simulate not only reaction monitoring for the synthesis of PSMA-617 but also monitoring of the chelator as a part of DMPK studies.

Experimental

Sample Description

Stock solutions of all three compounds were made at a concentration of 1 mg/mL. The combined sample contained 0.1 mg/mL of each compound with a final solvent composition of 97:3 v/v water:acetonitrile.

Reversed Phase LC Conditions

LC system:	ACQUITY Premier Binary Solvent Manager System with PDA Detector
Detection:	SIRs and MS Full scan (ESI+)
Columns:	Atlantis Premier BEH C_{18} AX, 2.1 x 50 mm, 1.7 µm (p/n: 186009366) or ACQUITY Premier HSS T3, 2.1 x 50 mm, 1.8 µm (p/n: 186009467)
Column temperature:	30 °C
Sample temperature:	10 °C
Injection volume:	1.0 µL
Flow rate:	0.5 mL/min
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in methanol
Gradient conditions:	Isocratic hold at 0% B for 2.00 minutes. Linear ramp to 95% B in 6.00 minutes. Hold at 95% B for 1.15 minutes. Return to starting conditions in 0.02 minutes. Re-equilibrate at 0% B for 1.16 minutes. Total run time: 10.33 minutes

HILIC LC Conditions

LC system:	ACQUITY Premier Binary Solvent Manager System with PDA Detector
Detection:	SIRs and MS Full scan (ESI+)
Column:	Atlantis Premier BEH Z-HILIC, 2.1 x 50 mm, 1.7 µm (p/n: 186009978) or stainless steel 2.1 x 50 mm column packed with BEH Z-HILIC 1.7 µm particles
Column temperature:	30 °C
Sample temperature:	10 °C
Injection volume:	1.0 µL
Flow rate:	0.5 mL/min
Mobile phase A:	95:5 Acetonitrile:Water (v/v) with 10 mM ammonium formate pH 3.0
Mobile phase B:	50:50 Acetonitrile:Water (v/v) with 10 mM ammonium formate pH 3.0
Gradient conditions:	Initial conditions of 0% B. Linear ramp to 95% B in 6.00 minutes. Hold at 95% B for 1.15 minutes. Return to starting conditions and hold for 1.17 minutes. Total run time: 8.33 minutes.

MS Conditions

MS system:	Xevo TQ-S Micro
Detection:	SIRs and MS Full Scan (ESI+)
Full scan range:	10–1500 m/z
SIRs:	See Table 1
Capillary voltage (kV):	3
Cone voltage (V):	20
Desolvation temperature:	350 °C
Desolvation gas flow:	650 L/hr
Cone gas flow:	0 L/hr

Data Management

Chromatography software:	Masslynx V4.1

Results and Discussion

The radioligand therapy drug PLUVICTO, also known as Lutetium (177 Lu) vipivotide tetraxetan, is a combination of a ligand-linker conjugate and a chelator group. The chelator, DOTA, is attached to the ligand-linker conjugate so that a radioisotope, in this case Lutetium-177, can be attached to deliver the radiation to the target cells. Figure 1 shows the chemical structures of the three analytes tested and Table 1 highlights some of the chemical properties that are of import for this work.

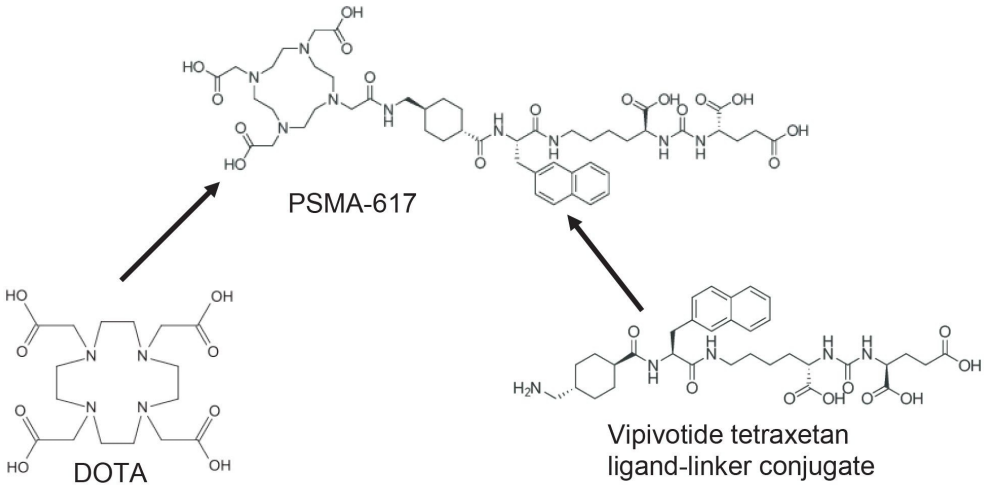

Figure 1. Chemical structures of PSMA-617 as well as the two major components of its synthesis.

Compound	Formula	pKa	logP	M+H
Vipivotide tetraxetan ligand-linker conjugate	$C_{33}H_{45}N_5O_9$	3.36	-1.178	656.329
DOTA	$C_{16}H_{28}N_4O_8$	1.09	-6.782	405.198
PSMA-617 (Vipivotide Tetraxetan)	$C_{49}H_{71}N_9O_{16}$	1.14	-3.435	1042.510

Table 1. Compounds tested in this application note, their molecular formula, strongest acidic pKa, logP values, and M+H values used for SIR detection. Full scan ESI+ was also used for detection in the event of adduct formation.

The logP values, or log of the partition coefficients, indicate that all three components are polar. DOTA is the most polar with a logP value of -6.782, which is expected given the presence of four carboxylic acid groups as well as four tertiary amines. Due to their polarity, analysis of these compounds in reversed-phase liquid chromatography may require the use of columns specifically designed for retention of polar compounds. One such column, the ACQUITY HSS T3 Column contains a stationary phase with a lower bonding coverage than

traditional C_{18} bonded phases. This allows more interaction between polar analytes and the silica surface, increasing retention, particularly for basic compounds.[4-6]

Another problem that these compounds present is their acidic nature. Both DOTA and PSMA-617 have at least one negatively charged group at pH 3. This means that the compounds may adsorb on the metal surfaces present in the LC column and system leading to poor recovery and poor peak shape. This adsorption can be mitigated by using inert hardware, such as MaxPeak High Performance Surfaces (HPS) Technology.[7-11] This technology utilizes a covalently bonded hybrid organic/inorganic surface on both the LC column hardware and system tubing. This technology has been shown to improve recovery of metal-sensitive analytes in LC and LC/MS applications.[7-11] In order to get the best performance for the radioligand therapy analytes, inert hardware was used. The ACQUITY Premier HSS T3 Column was tried first, as it offers good retention for polar analytes, while also employing the MaxPeak HPS Technology to improve recovery of the acidic analytes. Figure 2 shows the chromatograms for the three analytes using this column.

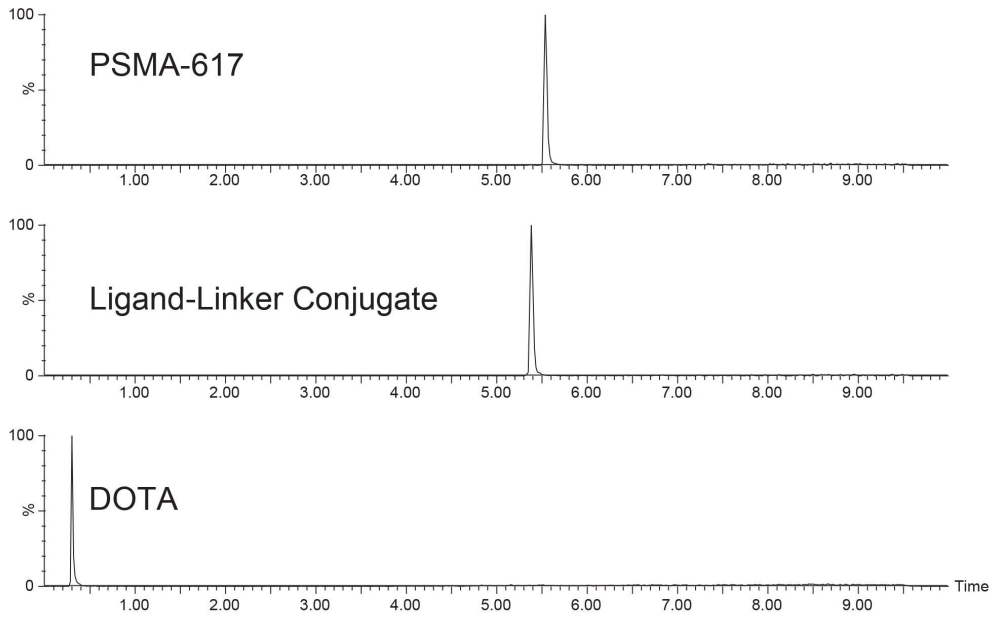

Figure 2. SIRs of the three components tested using the ACQUITY Premier HSS T3 Column.

The ACQUITY Premier HSS T3 Column shows good retention and separation for the ligand-linker conjugate and

PSMA-617. If the intent of a method was to separate only these two compounds, then the current method and column would be sufficient, with the potential for optimization to improve throughput. However, if the method being developed requires retaining DOTA as well, the ACQUITY Premier HSS T3 Column does not achieve that goal. An alternative column to try would be a mixed-mode reversed-phase anion exchange column to retain the compound via ionic interactions along with the reversed-phase mechanism. The Atlantis Premier BEH C_{18} AX Column employs an anion exchange functional group specifically designed to retain acidic probes.[9,12] Figure 3 shows the chromatograms obtained using the Atlantis Premier BEH C_{18} AX Column, which can retain analytes by both reversed-phase (RP) and anion exchange (AEX) mechanisms.

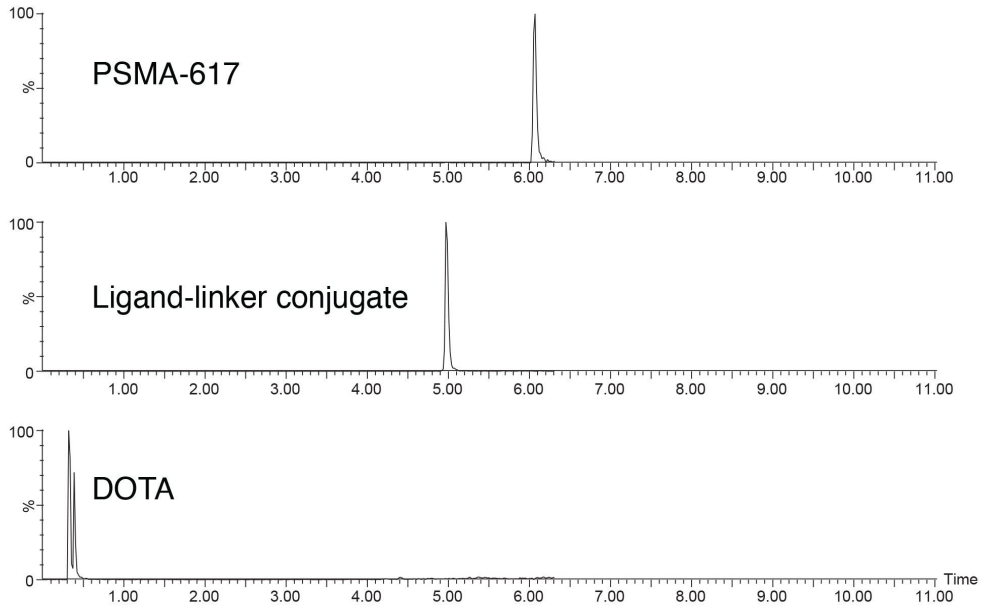

Figure 3. SIRs of the three components tested using the Atlantis Premier BEH C_{18} AX Column.

The Atlantis Premier BEH C_{18} AX Column improves the overall separation quality by increasing the resolution between the ligand-linker conjugate and PSMA-617. However, the DOTA peak is still poorly retained, albeit with a new issue arising. On the mixed-mode stationary phase the peak is split. This could be caused by a mismatch between the sample solvent and the initial mobile phase. In any event, the DOTA peak is still poorly retained indicating that, in order to get retention of this analyte, a different analytical technique is required.

HILIC, or hydrophilic interaction liquid chromatography, is a more specialized analytical technique that retains

polar analytes better than reversed-phase. The improved retention of polar analytes is achieved by using a polar stationary phase which strongly attracts water to the surface of the sorbent. This adsorbed aqueous layer allows for polar analytes to partition into and out of the layer based on their affinity to an aqueous environment.[13] More polar analytes, or those with lower logP values, are retained more than analytes with higher logP values. HILIC columns can also retain analytes through secondary interactions which are not as dominant in reversed-phase, mainly ionic interactions, hydrogen bonding, and adsorption.[14] These secondary retention mechanisms not only improve overall retention but can also play a large role in selectivity between stationary phases in HILIC.

To assess retention in HILIC, the Atlantis Premier BEH Z-HILIC Column was selected for use. This stationary phase uses the same hybrid BEH particle that is seen in other columns, but with a slightly smaller pore size. The change in pore size helps drive retention by increasing the surface area of the particle.[15] Next, this particle is bonded with a zwitterionic ligand which helps draw water to the surface of the sorbent, increasing the adsorbed aqueous layer thickness and therefore retention for neutral analytes. The zwitterionic ligand can also interact via ionic interactions with both acidic and basic analytes further improving retention. Figure 4 shows the separation of the three analytes using the Atlantis Premier BEH Z-HILIC Column and a typical HILIC screening gradient.

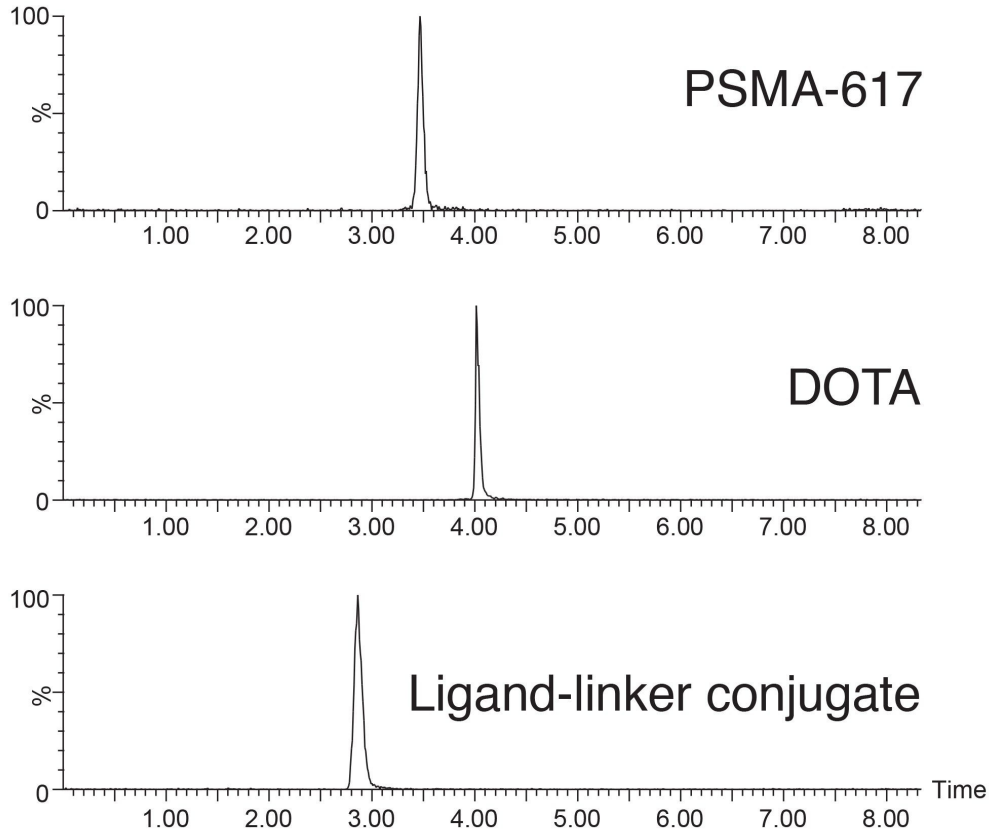

Figure 4. SIRs of the three components tested using the Atlantis Premier BEH Z-HILIC Column.

As expected, the elution order of the components is different than what is seen with reversed-phase columns, most notably, the DOTA peak is eluting last instead of first. It is retained out to approximately 4 minutes using the generic screening gradient of 5–50% aqueous with a constant 10 mM buffer. The ligand-linker conjugate is still eluted before the PSMA-617 peak. It should be noted that the elution order of the components aligns with the logP values for the compounds, with the compound with the highest logP value eluting first and the lowest logP, or most polar, compound eluting last. Good peak shape is achieved for these compounds as well under these conditions. Further optimization of the method could be performed if desired to improve cycle time or resolution. In order to fully appreciate the benefits of using inert hardware columns for these analytes, a comparison was made between the inert Atlantis Premier BEH Z-HILIC Column and a stainless-steel column packed with the same batch of sorbent. Figure 5 shows the comparison of the two columns.

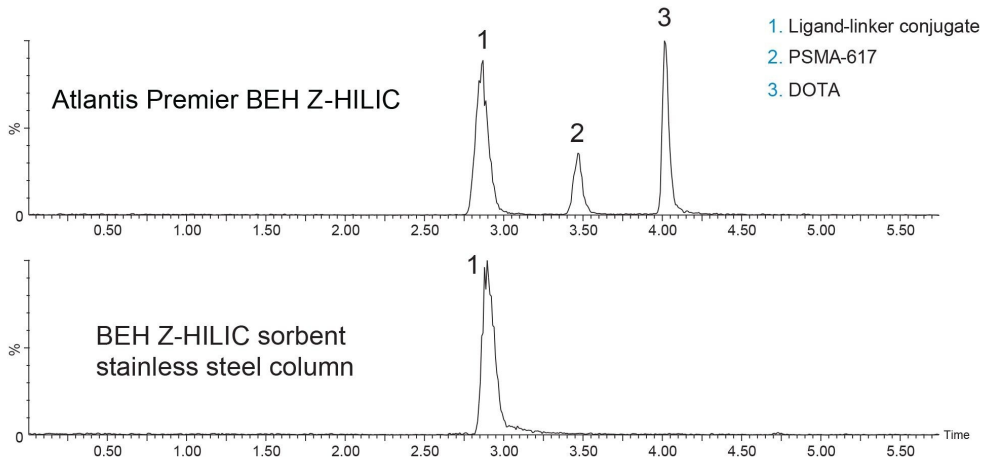

Figure 5. SIRs of the three components tested using the Atlantis Premier BEH Z-HILIC Column (right) and a stainless-steel column packed with the same sorbent (left).

The stainless-steel column packed with the BEH Z-HILIC sorbent shows comparable results for the ligand-linker conjugate, but both the DOTA and PSMA-617 peaks have disappeared. This could be caused by adsorption of these analytes onto the metal surface of the stainless-steel column but could also be caused by adduct formation with iron. The formation of adducts would confound the MS detector by shifting the mass of the analytes, making the use of SIRs, or even MRMs more complicated. Additionally, if these adducts are not constant, or complete, then variability in detection would occur making final analysis results potentially unreliable. Figures 6, 7, and 8 shows mass spectra for the DOTA, ligand-linker conjugate, and PSMA-617 peaks respectively, obtained on the BEH Z-HILIC sorbent packed into stainless hardware.

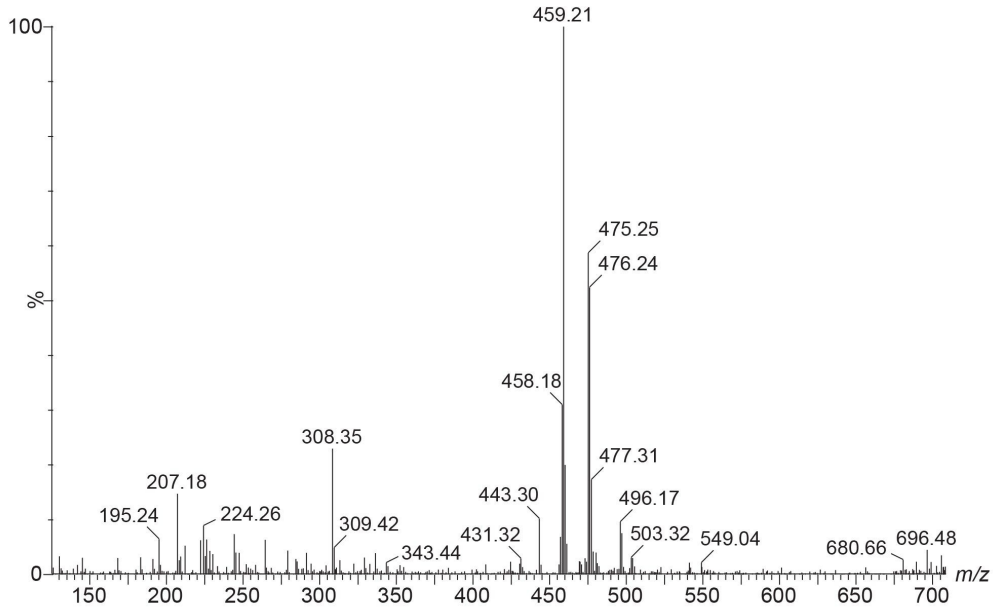

Figure 6. Mass spectra of the DOTA peak on the BEH Z-HILIC sorbent packed into stainless-steel hardware.

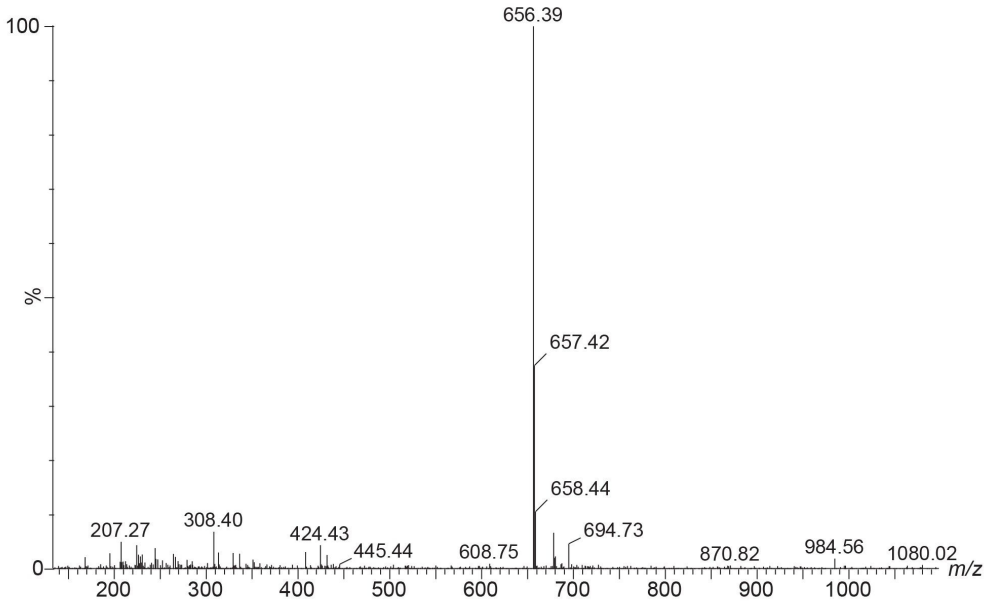

Figure 7. Mass spectra of the ligand-linker conjugate peak on the BEH Z-HILIC sorbent packed into stainless-steel hardware.

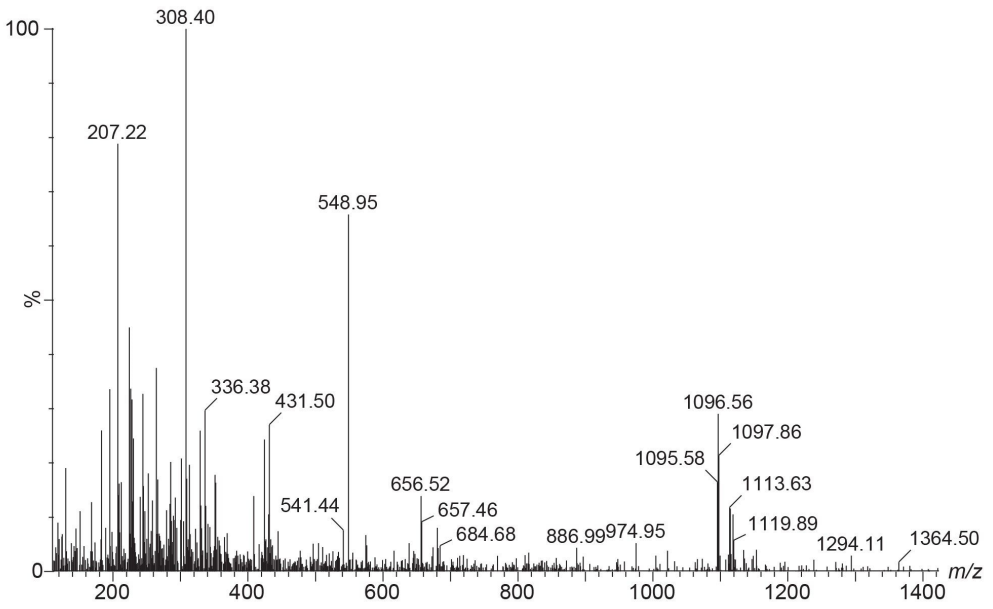

Figure 8. Mass spectra of the PSMA-617 peak on the BEH Z-HILIC sorbent packed into stainless-steel hardware.

In examining the full scan ESI+ results for the injections, it was determined that both DOTA and PSMA-617 are forming iron adducts while the ligand-linker conjugate does not form any iron adducts. Figures 6 and 8 show complete adduct formation for the DOTA and PSMA-617 peaks as neither show any of the M+H ions in the mass spectra. However, over time as the column and system hardware become passivated, the adduct formation may be reduced causing variable detection of these analytes. This does not even address the potential issue of adsorption of these analytes to the metal surfaces reducing peak areas and lowering overall recoveries. However, by using inert hardware, like MaxPeak Premier Columns, the LC-MS analysis of metal sensitive compounds like PSMA-617 and DOTA can be greatly improved. The use of this hardware also eliminates the need for mobile phase additives like EDTA, which have been used in the past for the analysis of these types of compounds.[16]

Conclusion

Radioligand therapies are on the rise, with the technique having already been approved by the FDA with the

drug PLUVICTO. These drugs employ a targeting ligand which interacts with certain proteins or cells and then delivers radiation therapy directly to the cancerous cells with minimal negative impact on the surrounding, healthy cells. Analysis of these compounds can be complicated, however, as the chelating group of the drugs can interact with metal surfaces in an LC system or column. Using inert hardware, like MaxPeak Premier Columns, mitigates those interactions improving overall separation quality and detection.

This application note examines the drug PLUVICTO and the two components of the drug, namely the chelator and ligand-linker conjugate. In order to retain all of these compounds, an Atlantis Premier BEH Z-HILIC Column was required, as the chelator is poorly retained in reversed-phase LC, even when using sorbents designed to retain polar acidic analytes. Comparisons between the inert hardware and stainless-steel hardware were performed once final method conditions were established.

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