Capillary-Scale Radiopharmaceutical Analysis using the Axcend Focus LC®

By Sam Foster[1], Richard Coelho[2], Milton Lee[1]

[1]Axcend, Lehi, UT 84043, United States [2]Perceptive, Durham, NC 27709, United States

Abstract

The production and validation of radiopharmaceutical compounds result in generation of radioactive solvent waste. These waste streams must be stored for a sufficient time to allow for decay to occur (often several years of storage) before disposal. This leads to significant costs in waste storage and disposal, as well as risks to human exposure and other environmental impacts. In this application note, the analyses of several small radio-labeled compounds (representative of radiopharmaceuticals) using capillary liquid chromatography (LC) are demonstrated. The considerable reduction in mobile phase consumption inherent to capillary LC (µL/min vs mL/min) offer much reduced expense and greener alternatives to existing analytical LC workflows. Small molecules labeled with Lu-177, At-211, or I-125 radionuclides were used to demonstrate the analysis of compounds containing isotopes of varying half-life and emission energy. This application note demonstrates the significant advantage of using capillary-scale LC to reduce hazardous solvent generation in radiopharmaceutical analytical workflows. Mobile phase cost savings were reduced from ~$123 per day on the analytical scale, to ~$0.25 per day on the capillary scale.

Introduction

Radiopharmaceuticals are drugs labeled with radionuclides that are used in medical applications ranging from imaging of different organs, such as brain, heart, kidney and bone, to treatment of cancer and hyperthyroidism. These drugs must be labeled days to hours before administration due to continual radioactive decay. Liquid chromatography (LC) can be used to determine the strength, yield, and purity of the drugs. Waste solvents involved in LC analysis must be held for a minimum of

10 half-lives before disposal to allow for sufficient radioactive decay. Depending on the radionuclide, this storage time can range from months to years leading to high storage and disposal costs. Capillary-scale LC can greatly attenuate this waste problem because it operates at flow rates orders of magnitude below the more traditional analytical-scale LC systems. To demonstrate this, small compounds labeled with radionuclides Lu-177, At-211, and I-125 were analyzed using the Axcend Focus LC. These three selected radionuclides offer a broad range of energy levels and half-lives to cover a wide range of relevant radiopharmaceutical treatments. The sensitivity and repeatability of the capillary LC system for these analyses are reported.

Materials and Methods

A LabLogic Dual Scan-RAM radio detector was coupled to the Axcend Focus LC® for radio-LC analysis. All samples were labeled the day of analysis to avoid complications due to radioactive decay. The labeled analytes are proprietary small molecules and, therefore, their structures are not available. Samples were measured across a 5-injection repeatability sequence, and a calibration curve was generated for each. The chromatographic conditions were as follows:

Chromatographic Conditions

Column	100 x 0.15 mm column, 1.8 µm Waters HSS T3 particles
Mobile Phase	A) Water with 0.1% formic acid B) Acetonitrile
Gradient	Isocratic hold at 5% B for 0.5 min followed by a linear gradient to 95% B over 7.5 min, with a 1-min isocratic hold at 95% B
Flow Rate	2 µL/min
Injection Volume	40 nL
Temperature	Ambient
Detector Settings	Dwell (s): 1 Shift (s): 0 Efficiency (%): 100 Spill (%): 0.0 Lower Limit: 450 Upper Limit: 4095 High Voltage/VBias (V): 920

Results and Discussion

Capillary LC with the radio detector was used to analyze each of the radio-labeled analytes to demonstrate the viability of capillary-scale separations for radiopharmaceutical detection. The Lu-177 isotope is a radionuclide with a multi-day half-life and a moderate emission energy, making it representative of a baseline sample for system validation. A chromatogram of a Lu-177-labeled compound is shown in Figure 1A. Across a 7-injection sequence, the peak area and retention time

had relative standard deviation (RSD) values of 2.99% and 0.60%, respectively. Due to the decreased flow rates inherent in capillary-scale LC, each injection required only ~24 µL of mobile phase. A four-point calibration curve from 0.28 to 2.33 mCi/mL was generated with each sample being analyzed in triplicate. This calibration curve is shown in Figure 1B and represents the label intensity standard working range with a limit of detection (LOD) of 0.07879 mCi/mL (3 nCi on column) and limit of quantification (LOQ) of 0.2627 mCi/mL (10 nCi on column), which meets the requirements of radio-HPLC analysis.

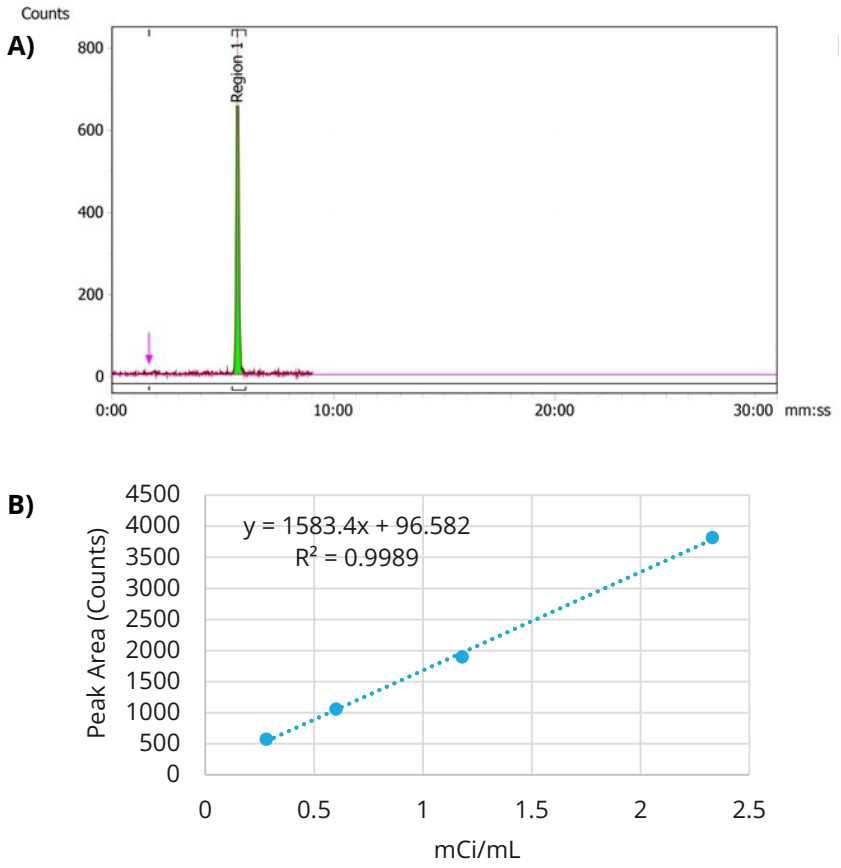

Figure 1. (A) Chromatogram and (B) calibration curve of Lu-177 labeled analyte.

The At-211 radionuclide was selected for its short (~7 h) half-life. This short half-life poses challenges to obtaining peak area repeatability and linear calibration curve since, within a single workday, nearly 50% of the label will degrade. Across a 7-injection sequence, the peak area RSD was 3.76% and the retention time RSD was 0.272%. A representative chromatogram of the labeled At-211 compound is shown in Figure 2A. A five-point calibration curve was generated between intensities of 0.126-2.032 mCi/mL, with each intensity being run in triplicate. This calibration curve is shown in Figure 2B with LOD of 0.07368 mCi/mL (2.95 nCi on-column) and LOQ of 0.2456 mCi/mL (9.82 nCi on-

column). The short half-life resulted in increased peak area fluctuations and decreased linearity; however, the results fell within acceptable levels, demonstrating capillary-radio-LC as a suitable analytical method for this radionuclide-labeled compound.

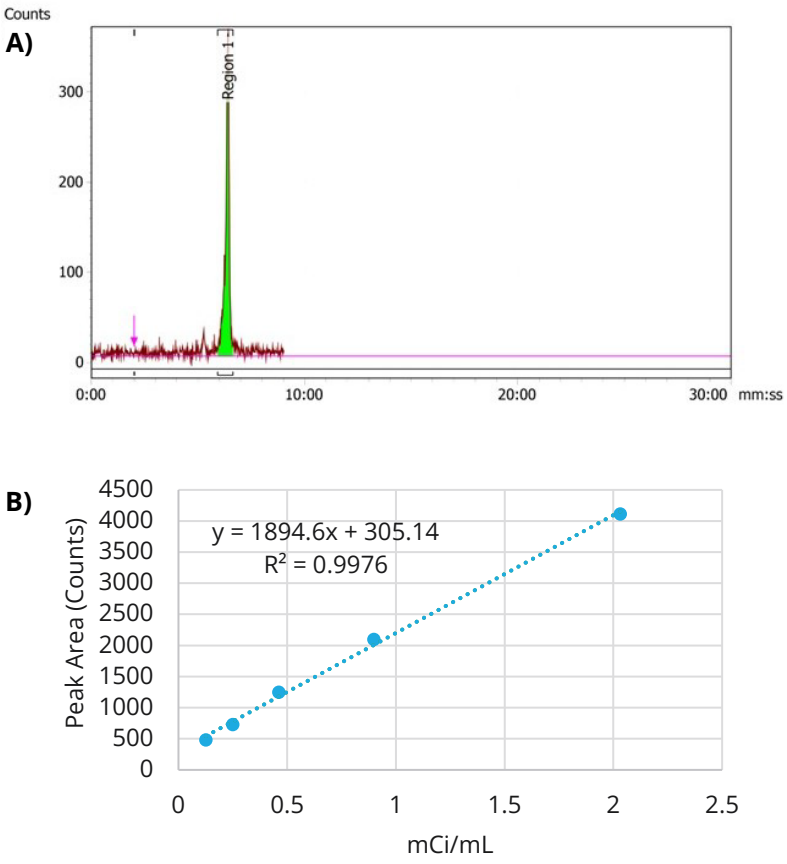

Figure 2. (A) Chromatogram and (B) calibration curve of At-211 labeled analyte.

The I-125 radionuclide was selected due to its low energy intensity, making detector sensitivity a key concern. Due to the acceptable repeatability of the other two radionuclide-labeled compounds, a repeatability study was not performed for this analyte. A 3-point calibration curve was generated for intensities of 0.475-1.715 mCi/mL. A representative chromatogram at the highest intensity is shown in Figure 3A and a calibration curve is shown in Figure 3B. Sufficient sensitivity and linearity across a relevant intensity range was obtained. The LOD was calculated as 0.1880 mCi/mL (7.52 nCi on-column) and the LOQ as 0.6267 mCi/mL (25.06 nCi on-column). Despite the low emission energy, the radionuclide was detected at sufficient levels for use in standard workflows, indicating that capillary-radio-LC is again a suitable alternative to existing analytical-scale methods.

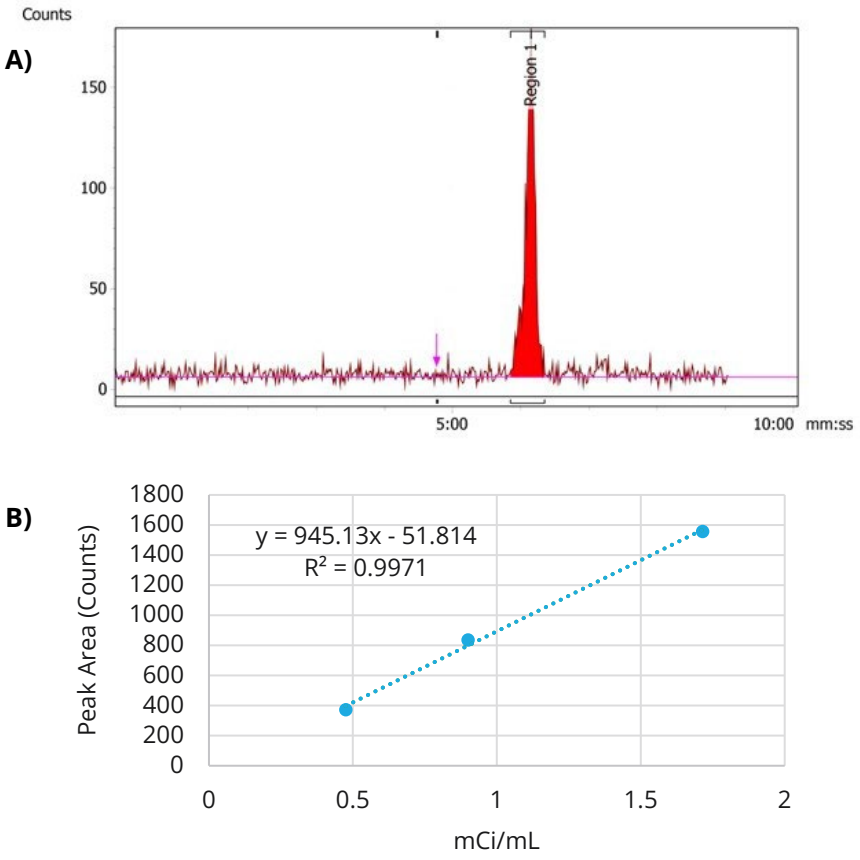

Figure 3. A) Chromatogram and (B) calibration curve of I-125 labeled analyte.

Conclusions

The use of radiopharmaceuticals in both imaging and therapeutic applications is expanding. From the production and use of radiopharmaceuticals, radioactive waste is generated, which must be stored for considerable periods of time before disposal. By reducing the solvent consumption required for validation and purification of these analytes, hazardous waste generation and disposal costs can be reduced by orders of magnitude. The Axcend Focus LC, a compact capillary-scale LC, was used for the analysis of three radionuclide-labeled compounds to demonstrate that acceptable LOD, LOQ, repeatability, and linearity values can be easily obtained using capillary-scale LC while, concurrently, reducing toxic mobile phase waste generation by orders of magnitude.