HPLC Validation and Q/A of $^{62}$Cu-PTSM, $^{62}$Cu-ATSM, and $^{62}$Cu-ETS Synthesized by $^{62}$Zn/$^{62}$Cu Microgenerator Kit

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Introduction

- $^{62}\text{Cu}$ labeled bis(thiosemicarbazone) PET agents
  - $^{62}\text{CuPTSM}$
  - $^{62}\text{CuETS}$
  - $^{62}\text{CuATSM}$

- PTI’s $^{62}\text{Cu}/^{62}\text{Zn}$ microgenerator with interchangeable instant synthesis kit
Structure Identity Validation using HPLC

• Media selection
  – Common reverse phase C-18 column
    • Good separation
    • Irreversible binding of Ionic $^{62}\text{Cu}^{2+}$ to column media
  – Reverse phase Oasis® HLB column (Waters™)
    • Good separation
    • Much less binding of Ionic $^{62}\text{Cu}^{2+}$ to column
Structure Identity Validation using HPLC

HPLC conditions:
Shimadzu Vp system, with radiation detector, 35%ACN/ 65% 25mM NaOAc buffer (pH=4.7), Isocratic mode (1 mL/min), 40°C,
Amount injected:
Cu-ETS 0.20 µg
Cu-PTSM 0.20 µg
Cu-ATSM 0.20 µg
$^{62}\text{Cu}$-ETS 10.0 µCi
$^{62}\text{Cu}$-PTSM 10.0 µCi
$^{62}\text{Cu}$-ATSM 10.0 µCi

Reverse Phase: Oasis® HLB column

UV/Vis Chromatogram –462nm

Radiation Chromatogram

Cu-ETS
Cu-PTSM
Cu-ATSM

$^{62}\text{Cu}$-ETS
$^{62}\text{Cu}$-PTSM
$^{62}\text{Cu}$-ATSM
Structure Identity Validation using HPLC

UV/Vis Chromatogram –462nm

- Cu-ATSM
- Cu-PTSM
- Cu-ETS
- Solvent front

Radiation Chromatogram

- $^{62}$Cu-ATSM
- $^{62}$Cu-PTSM
- $^{62}$Cu-ETS

HPLC conditions:
Shimadzu Vp system, with radiation detector, 20%EtOH/ 80% hexane, Isocratic mode (1 mL/min), 40°C
Amount injected:
Cu-ETS 0.23 µg
Cu-PTSM 0.20 µg
Cu-ATSM 0.20 µg
$^{62}$Cu-ETS 3.0 µCi
$^{62}$Cu-PTSM 1.0 µCi
$^{62}$Cu-ATSM 2.0 µCi

Normal Phase:
Nova-Pak® column

Sample Preparation:
Octanol:Hexane extraction
Radiochemical Purity Determination

• Activity recovery in HPLC column eluate

<table>
<thead>
<tr>
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<th>Recovery % (Germanium detector)</th>
<th>Recovery % (Well detector)</th>
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<tbody>
<tr>
<td>$^{62}\text{Cu-ETS}$</td>
<td>101.8 ± 2.3</td>
<td>–</td>
</tr>
<tr>
<td>$^{62}\text{Cu-PTSM}$</td>
<td>99.7 ± 1.5</td>
<td>99.2 ± 2.5</td>
</tr>
<tr>
<td>$^{62}\text{Cu-ATSM}$</td>
<td>98.8 ± 1.3</td>
<td>101.4 ± 1.0</td>
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Radiochemical Purity Determination

- Calibration of Radioflow detector (Berthold) attached with Cerenkov counting cell
  - Dead time correction: paralyzable model
    \[ m_{\text{observed}} = n_{\text{true}} \cdot \exp(-n_{\text{true}} \cdot t_{\text{au}}) \]
  - Better linearity of detector response to dose injected
  - Higher method sensitivity

\[ R^2 = 0.99839 \] without dead time correction
\[ R^2 = 0.99995 \] with dead time (\( \tau = 2.8 \mu \text{s} \)) correction
Radiochemical Purity Determination

- Radiochemical purity for $^{62}\text{Cu}$ PET agents produced via PTI instant synthesis Kit is usually measured >95%

**Assay Conditions:**
- Reverse Phase Oasis® HLB column
- Shimadzu Vp system
- Calibrated Radioflow detector
- 25% ACN/75% 25mM NaOAc buffer for $^{62}\text{Cu}$-ETS
- 35% ACN/65% 25mM NaOAc buffer for $^{62}\text{Cu}$-PTSM
- 45% ACN/55% 25mM NaOAc buffer for $^{62}\text{Cu}$-ATSM
- Isocratic mode (1 mL/min) at 40°C
- $^{62}\text{Cu}$ injected: <2.5Ci

Representative decay corrected radiochromatogram for micro-generator produced $^{62}\text{Cu}$-ETS
Radiochemical Purity Determination

- HPLC performance under extreme circumstances
  - Reproducible retention times
  - Reliable separation performance

- 62Cu-ETS
- 62Cu-PTSM
- 62Cu-ATSM
Radiochemical Purity Determination

- Comparison of HPLC and Rapid Cartridge Assay

\[ y = 1.002x \quad R^2 = 0.973 \]

\[ y = 1.001x \quad R^2 = 0.960 \]

\[ y = 1.006x \quad R^2 = 0.988 \]
Conclusion

- Results from two HPLC separation modes confirm that $^{62}$Cu labeled bis(thiosemicarbazone) compounds synthesized via PTI instant synthesis kit have the same molecular structures as the cold references.
- HPLC assay of radiochemical purity is accurate and reliable.
- Rapid Oasis® cartridge assay is competent for Quality Assurance at clinic site.