Characterization, HPLC Method Development, and Impurity Identification for 3,4,3-LI(1,2-HOPO): A Potent Actinide Chelator for Radionuclide Decorporation
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Purpose
3,4,3-LI(1,2-HOPO), a potent octadentate chelator of actinides, is being developed as a decorporation drug for internal contamination with radionuclides. As part of the pre-clinical program sponsored by NIH-RAID, physico-chemical characterization, HPLC method development/validation and shelf-life evaluation have been undertaken. Existing HPLC methods exhibited speciation peaks and bridging, likely due to complexation with residual metallic ions in the eluent. Derivatization of the target ligand in situ with Fe(III) chloride provided a single homogeneous iron-complex which can readily be detected and analyzed.

Methods
Test samples were prepared at 0.25 mg/mL in diluent containing 0.3 mg/mL FeCl3. The HPLC method used an Agilent Eclipse XDB-C18 column (150 mm x 4.6 mm, 5 µm) at 25 °C with UV detection at 280 nm. A gradient elution, with acetonitrile (11% to 100%)/buffer mobile phase, was developed for impurity profiling. The buffer consisted of 0.02% formic acid and 10 mM ammonium formate at pH 4.6. An Agilent 1200 LC-6530 Q-TOF/MS system was used to characterize the [Fe(III)-3,4,3-LI(1,2-HOPO)] derivative and the impurities.

Results
Samples were prepared at a 5.5:1 molar ratio of FeCl3 to fully chelate 3,4,3-LI(1,2-HOPO) during the chromatographic process. Specificity was demonstrated by resolution of impurities and forced decomposition products. Linearity was validated in 0.13-0.35 mg/mL range (r = 0.9999). Accuracy was demonstrated with 98.3-103.3% recoveries. Precision for multiple preparations was 1.6% RSD. The LC/HRMS revealed that the derivative was a complex consisting of one 3,4,3-LI(1,2-HOPO) molecule, one hydroxyl group and two iron atoms. Impurities were also identified with LC/HRMS. The validated HPLC method was used in the shelf-life evaluation studies which showed that the API remained unchanged for one year at 25°C/60% RH.

Conclusion
A specific, linear, accurate, and precise HPLC method was developed and validated for assay and impurity determination of 3,4,3-LI(1,2-HOPO). The method was utilized in the shelf-life evaluation studies which showed that the API remained unchanged for one year at 25°C/60% RH.

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