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Bioanalysis of Hydroxyl-Dendrimer Therapeutics Using LC-MS/MS with In-Source Fragmentation

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Introduction

Hydroxyl dendrimer therapeutics (HDTs) are a new precision nanomedicine technology selectively targeting reactive microglia and macrophages, and hypertrophic retinal pigment epithelial cells. Because of their highly branched and organized structure with water-like surface properties, HDTs are non-toxic and metabolically stable molecules that can easily cross tissue barriers. The unique characteristics of HDTs also mean they provide unique analytical challenges. The relatively large size (between 12 to <100 kD) and water-like surface of HDTs causes poor retention and peak shape using reverse phase-chromatography, and the size is too large for intact MS/MS measurement. Here, we describe a robust strategy for quantification of HDTs by inducing fragmentation of the HDT in-source.

Methods

Unique parent fragments are determined by direct infusion experiments comparing HDTs to the dendrimer component of HDTs, using high declustering potentials to induce in-source fragmentation. Plasma and urine samples are prepared for LC-MS/MS by protein precipitation using acetonitrile and methanol. The extracts are dried under nitrogen and reconstituted in aqueous solutions. Extracts containing HDTs are separated chromatographically using ion-pairing chromatography with trifluoroacetic acid (TFA) and large pore size (300 Å) columns before MRM of the in-source fragment and detection on AB Sciex 6500+ triple quad mass spectrometers. A body paragraph in which details the methods for your topic.

Preliminary Data

We have successfully validated methods for quantification of HDTs in both animal and human plasma, as well as human urine using 25-100 µL of starting material with LLOQs of 100-200 ng/mL. Calibrator and QC results showed good quantitation for typical regulated LC-MS/MS methods per FDA guidance. The back-calculated accuracy of the calibrators and QCs was within 15% of the nominal concentration (20% at the LLOQ level), and the %CV was within 15% (20% at the LLOQ level).

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Novel Aspect

We used ion-pairing chromatography with wide-pore LC columns coupled with in-source fragmentation LC-MS/MS to quantify hydroxyl-dendrimer therapeutics in plasma and urine.