Identification and Quantitation of Low-Level Dendrimer (10183 Da) in Rat Plasma Using HRAM-Mass Spectrometry

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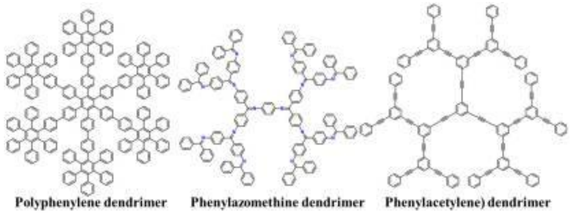
Introduction

Dendrimers are highly branched macromolecules. These nano-objects have been proven to play an important therapeutic role due to their efficient delivery, absorption, and mode of action. Because of their high molecular weight, it is challenging to develop a method for identification and quantitation at low concentration in biological matrixes, which is required for the PK studies. Current methods generally utilize mass spectrometry to detect and quantitate macromolecules, but to date, there is very little literature to support the use of mass spectrometry to quantitate highly charged dendrimers. Our research identified a reliable method to quantify the presence of a 10183 Da dendrimer using HRAM-MS present in rat plasma at low levels (200 ng/mL).

Method Qualification

Analyte calibrators and QCs were prepared in the range of 200 to 200,000 ng/mL in rat plasma K_2EDTA. 30 µL sample aliquots were extracted using a liquid-liquid extraction of basic buffer and phenol-chloroform, the aqueous phase was evaporated, and reconstituted with 1% HFIP, 0.1% DIPEA and 10 µM EDTA in water. Masses from the most predominant charge states 11 and 12 were used for quantitation. The assay achieved an acceptable accuracy and precision quantitation down to 200 ng/mL for non-regulated bioanalysis.

Dendrimer Example



Polyphenylene dendrimer Phenylazomethine dendrimer Phenylacetylene) dendrimer

Pérez-Ferreiro M, M Abelairas A, Criado A, Gómez IJ, Mosquera J. Dendrimers: Exploring Their Wide Structural Variety and Applications.Polymers (Basel). 2023 Nov 9;15(22):4369. doi: 10.3390/polym15224369. PMID: 38006093; PMCID: PMC10674315.

Extraction and Quantitation Method Development

A phenol-chloroform-isoamyl alcohol extraction commonly used for oligonucleotides was optimized to isolate the analyte from rat plasma. After being mixed and separated twice, the aqueous phase was collected, the samples were dried under nitrogen and reconstituted with 1% HFIP & 0.1%DIPEA and 10 µM EDTA in Water.

The samples were further analyzed using UPLC system in tandem with a Thermo Q Exactive™ mass spectrometer. Using a DNAPac™ 2.1x 50 mm, 4 µm particle size. The LC method use a gradient from 20% Organic to 40% Organic mobile phase in 1.6 minutes.
The mass spectrometer was set to use the HESI- full scan mode. With a resolution of 70,000, an injection time of 120 ms and an AGC of 1e6. The source temperature was set to 350°C with a capillary temperature of 375°C.
Data was imported to Watson LIMS and regressed quadratic $1/x^2$.

Chromatography

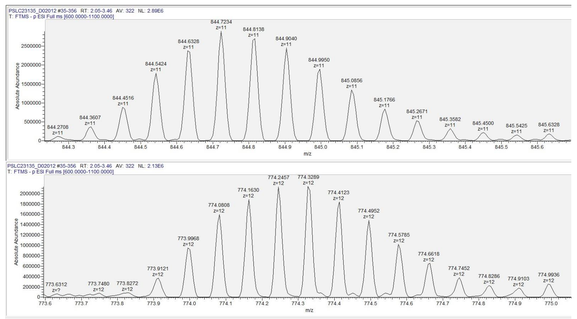


Figure 2. Tuning of the dendrimer on a Q Exactive™. Masses from charges z=11 and 12 were selected and further used for identification and quantitation of the dendrimer in rat plasma samples.

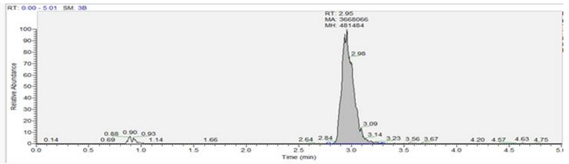


Figure 3.Neat samples were subjected to a gradient for UPLC method optimization. The masses obtained during tuning were used for processing the samples.

Qualification Design

The Qualification for this method was performed in a non-regulated manner, including an eight-point calibration curve from a range of 200 to 200,000 ng/mL and a set of quality controls at Low (600 ng/mL) and High (160,000 ng/mL) concentrations.

The qualification of the method was bracketed with the calibrators and QC's in between n=6.

See Tables 1 and 2 for Accuracy and Precision in Rat Plasma, K2EDTA.
See Figure 7 for Internal Standard contribution.
The acceptance criteria for this method is presented in the table below.

Results of the Method Development Qualification

LC-HRMS Parameter	Acceptance Criteria
Carryover:	≤20% of LLOQ
Concentration Range:	See method
CAL Precision & Accuracy:	≥75% of STDS within ±20% (±25% LLOQ)
QC Precision and Accuracy:	≥67% of QCs within ±20%
QC Precision and Accuracy:	≥50% at each level pass
QC Levels:	See method

All concentrations are expressed as ng/mL.

Run Date	Run Number	200	400	2000	10000	60000	120000	180000	200000
05-Aug-2023	1	231	423	2020	9600	56700	125000	182000	*142000
		160	410	2060	10800	*75700	105000	199000	188000
17-Aug-2023	2	180	399	1790	11100	*102000	122000	146000	*346000
		218	420	1850	10400	*97900	117000	207000	204000
Mean		197	413	1930	10500	56700	117000	184000	196000
S.D.		32.9	10.9	130	650	ISD	8810	27100	ISD
%CV		16.7	2.6	6.7	6.2	ISD	7.5	14.7	ISD
%Bias		-1.5	3.3	-3.5	5.0	-5.5	-2.5	2.2	-2.0
n		4	4	4	4	1	4	4	2

* Standard calibrator outside acceptance criteria and excluded from regression
ISD = Insufficient data points for statistical calculations

Table 2. Quality Control Samples Data for IMD-026 in Rat Plasma

Run Date	Run Number	LQC 600 ng/mL	HQC 160000 ng/mL
05-Aug-2023	1	608	152000
		553	147000
		622	138000
17-Aug-2023	2	702	184000
		713	**327000
Mean		640	190000
S.D.		67.2	78700
%CV		10.5	41.4
%Theoretical		106.7	118.8
%Bias		6.7	18.8
n		5	5

** > ±20% deviation from theoretical

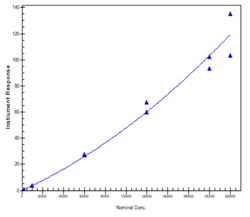


Figure 4.Quadratic Regression Concentration (ng/mL) vs. Intensity (area counts)

Representative Chromatograms

Figure 5. Representative Chromatogram for the LLOQ (200 ng/mL)

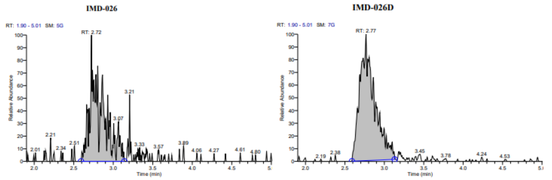


Figure 6. Representative Chromatogram for the ULOQ (200000 ng/mL) and Associated Internal Standard (IMD-026D)

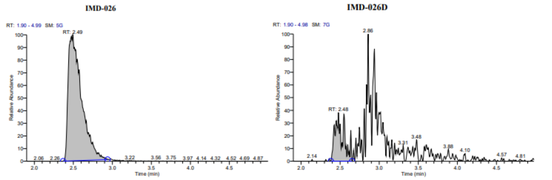
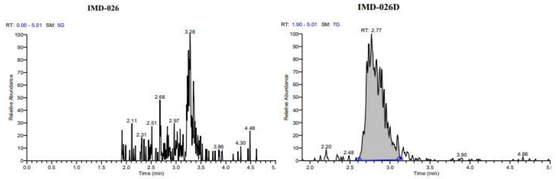


Figure 7. Representative Chromatogram for the Blank Matrix and Associated Internal Standard (IMD-026D)



Conclusion and Future Work

The method presented will require additional optimization in order to meet GLP acceptance criteria. One area of optimization is determining the stability of the organic mobile phase and its impact on chromatography quality. It has been noted that the organic mobile phase (acetonitrile) performs appropriately when used within 1 week of the preparation. This will make this assay robust enough to meet GLP acceptance criteria.
In addition, future work must be done to improve the instrument response at the lower level of the range to regress the data in a linear plot. However, it is a promising start in the identification and quantitation of dendrimers in biological matrices using QExactive.