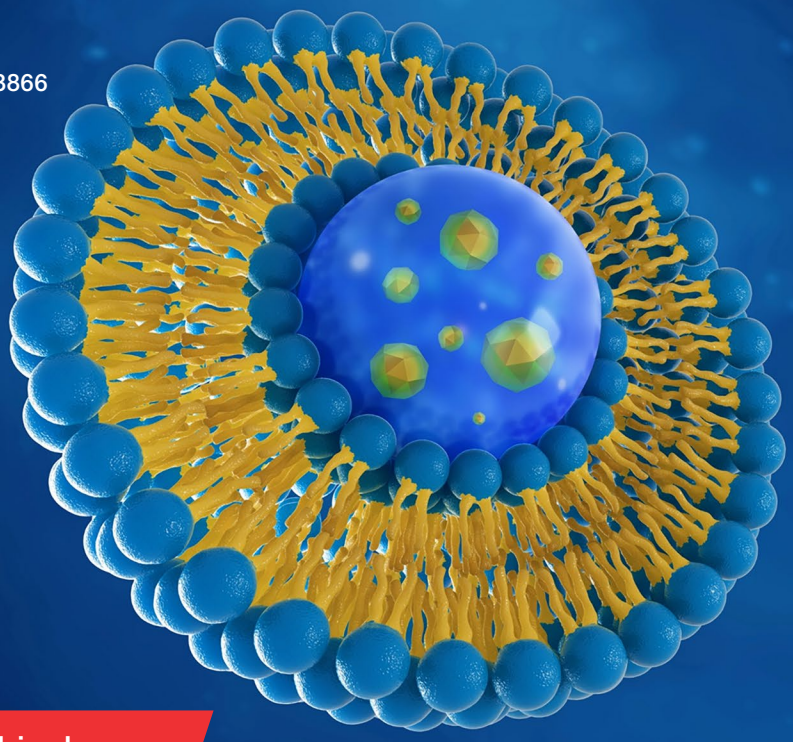
Application brief | 003866



Pharma and biopharma

Sensitive cationic lipids impurities analysis with quantitation by charged aerosol detection and simultaneous mass confirmation by MS

Authors

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Keywords

Cationic lipid, ionizable lipid, lipid nanoparticle (LNP), Hypersil GOLD C8 column, Vanquish Charged Aerosol Detector HP, single quadrupole mass spectrometer (ISQ-MS), ISQ EM mass spectrometer

Application benefits

Highlight the sensitivity and repeatability of the LC-CAD-MS inverse gradient method.

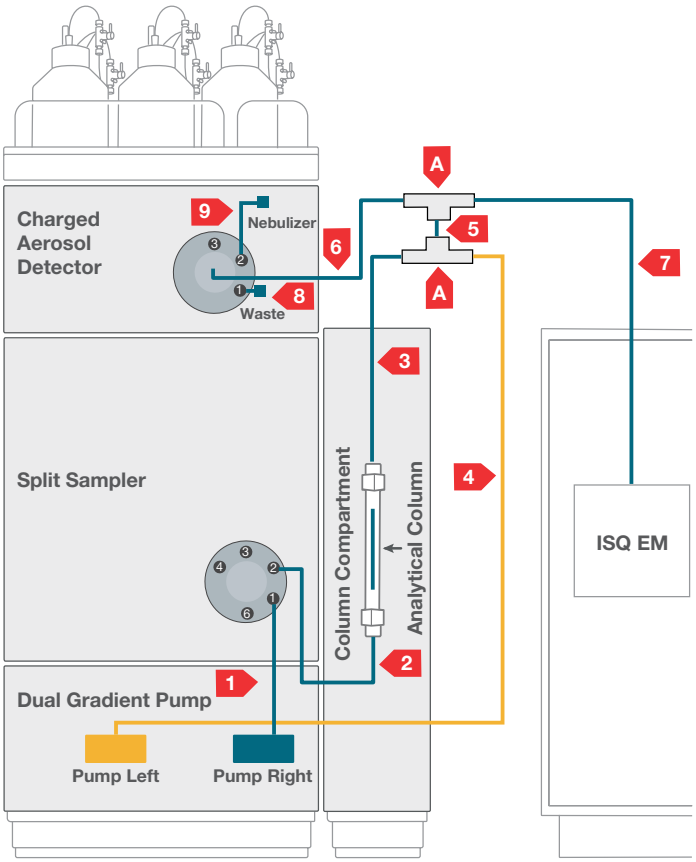
Goal

Using the inverse gradient method from Application Note 003384,[1] to demonstrate impurity analysis of cationic lipids on the Thermo Scientific™ Vanquish™ Charged Aerosol Detector HP in the Thermo Scientific™ Vanquish™ Flex Inverse Gradient UHPLC System, with parallel mass confirmation by the Thermo Scientific™ ISQ™ EM Single Quadrupole Mass Spectrometer.

Introduction

A cationic lipid is a critical component in lipid nanoparticle formulations for delivering nucleic acids such as mRNA, siRNA, and antisense oligonucleotides.[2] Cationic lipids are amphiphilic molecules that possess a hydrophilic region, a hydrophobic region, and a linker structure connecting the two.[1] Charged aerosol detectors (CAD) and evaporative light scattering detectors (ELSD) are preferred for characterizing lipid nanoparticle components, with CAD being the better choice for impurity quantification due to its higher sensitivity.[3] In a previous study, an inverse gradient method was developed to quantify impurities in cationic lipids raw materials.[1]

To demonstrate the inverse gradient method on the Vanquish CAD HP, the same cationic lipids—R-DOTAP, DLin-KC2-DMA and ALC-0315—were measured in this experiment as in Application Note 003384.[1] New diverter valve and software features were showcased, along with method's signal-to-noise ratio and repeatability.



No.	Connection between	Description
1	Pump right outlet - Injection valve port 1	Viper capillary, ID x L 0.10 × 350 mm, MP35N, P/N 6042.2340
2	Injection valve port 2 – Column inlet	Active pre-heater, 0.10 × 380 mm, MP35N, P/N 6732.0110
3	Column outlet – T-piece	Viper capillary, ID × L 0.10 × 450 mm, MP35N, P/N 6042.2360
4	Pump left outlet – T-piece	Viper capillary, ID × L 0.10 × 950 mm, MP35N, P/N 6042.2395
5	T-piece -T-piece	Viper capillary mixer, 25 µL, MP35N, P/N 6042.3020
6	T-piece – Charged aerosol detector inlet	Viper capillary, ID × L 0.10 × 550 mm, MP35N, P/N 6042.2360
7	T-piece – MS inlet	Viper capillary, ID × L 0.10 × 350 mm, MP35N, P/N 6042.2340
8	Diverter valve port 1 to waste	Viper SST, ID × L 0.18 × 250 mm, P/N 6040.2385
9	Diverter valve port 2 to nebulizer	Viper capillary, ID × L 0.10 × 150 mm, MP35N, P/N 6042.2320
No.	**Additional part**	**Description**
A	T-piece	Standard 500 µm ID, P/N 6263.0035

Figure 1. Schematic diagram of inverse gradient setup with the Vanquish CAD HP switched to the nebulizer

Experimental

Chemicals

- Fisher Chemical™ Water, Optima™ LC/MS grade (P/N W64)
- Fisher Chemical™ Acetonitrile, Optima™ LC/MS grade (P/N A461-4)
- Fisher Chemical™ Methanol, Optima™ LC/MS grade (P/N A456-4)
- Ammonium formate 10 M in H_2O, BioUltra, Sigma-Aldrich (P/N 78314)

Sample handling

- Fisher Scientific™ Fisherbrand™ Disposable Controlled Drop Pipets (P/N 13-678-30)
- Fisher Scientific™ Fisherbrand™ Pipets (P/N 13-678-25D)
- Fisherbrand™ Mini Vortex Mixer (P/N 14-955-151)
- Thermo Scientific™ SureSTART™ 2 mL GOLD-Grade Glass Screw Top Autosampler Vials (P/N 6PSV9-1PG)
- Thermo Scientific™ SureSTART™ 9 mm Screw Caps (P/N 6PSC9ST1)

Instrumentation

- Thermo Scientific™ Vanquish™ Flex UHPLC system with the ISQ EM MS consisting of:
  - System Base Vanquish Horizon/ Flex (P/N VF-S01-A-02)
  - Vanquish Dual Pump F (P/N VF-P32-A-01)
  - Vanquish Split Sampler FT (P/N VF-A10-A-02)
  - Vanquish Column Compartment H (P/N VH-C10-A-03)
  - Vanquish Charged Aerosol Detector HP (P/N VH-D21-A-01)
  - ISQ EM Mass Spectrometer (P/N ISQEM-ESI)
  - Vanquish Inverse Gradient Kit (P/N 6036.2010)

Sample preparation

Lipid standards were purchased from Cayman Chemicals. R-DOTAP standard was dissolved in 100% methanol and vortexed. The Dlin-KC2-DMA and ALC-0315 standards, which came in ethanol solutions, were diluted in 100% methanol and vortexed. All lipid standards were prepared to a total concentration of approximately 1 mg/mL.

Chromatographic conditions

Table 1. Chromatographic conditions

Parameter	Value
Column	Hypersil GOLD C8 column, 3.0 × 100 mm, 3 µm, P/N 25203-103030
Mobile phase	A: 5 mM ammonium formate in 50% acetonitrile/50% water B: 5 mM ammonium formate in 100% methanol
Analytical gradient	Time (min) % A % B 0.0 70 30 1.0 50 50 6.0 10 90 10.0 1 99 12.0 1 99 12.1 70 30 15.0 70 30
Inverse gradient	Time (min) % A % B 0.829 1 99 1.829 21 79 6.829 61 39 10.829 70 30 12.829 70 30 12.929 1 99 15.0 1 99
Flow rate	0.7 mL/min
Column temperature	50 °C with active pre-heater at 50 °C
Autosampler temperature	8 °C
Autosampler wash solvent	Solvent B of mobile phase
Injection volume	1 µL
CAD detector settings	Power value: 1.5 Evaporator temperature: 35 °C Data rate: 10 Hz Filter: 5.0 s Diverter valve position: Nebulizer

MS settings

Table 2. Instrument and scan settings for the mass spectrometer

Parameter	Value
Method source type	HESI
Polarity (Spray voltage)	Positive (+3,000 V)
Method type	Scan mode
Spectrum type	Profile
Dwell time	0.2 s
Mass range	*m/z* 300–900
Vaporizer temperature	338 °C
Ion transfer tube temperature	300 °C
Gas flow pressure	Sheath gas: 56.9 psig Auxilliary gas: 6.5 psig Sweep gas: 0.5 psig

Chromatography Data System

The Thermo Scientific™ Chromeleon™ 7.2.10 Chromatography Data System (CDS) was used for data acquisition and analysis.

Results and discussion

Six consecutive injections were conducted using the method for impurity analysis of cationic lipids as described in Application Note 003384.[1] The Vanquish CAD HP settings are listed in Table 1. The integrated diverter valve in the CAD allows guiding of the flow into the nebulizer, waste, or bypass. Using the diverter valve to divert everything to waste except the compounds of interest increases the detector up time. For example, the flow can be directed to waste at the beginning of the injection due to sample matrix effects or toward the end of the run for column reconditioning. Since clean standard was used here, the diverter valve was set to the nebulizer position, and the flow diagram was updated as shown in Figure 1. The power value was set to 1.5, which is equivalent to a power function value of 1.0 on previous CAD models. For the ISQ EM mass spectrometer settings (Table 2), in addition to monitoring for no co-elution of impurity peaks, a slightly narrower mass range was used to boost intensity of the impurities, as shown in the extracted mass channel in Figure 2. Example chromatograms of R-DOTAP (A), ALC 0315 (B), and Dlin-KC2-DMA (C) and their impurities with spectra are also shown in Figure 2. In the R-DOTAP figure, for the lowest abundance peak impurity III, the relative peak area is 2.31% and has a signal-to-noise ratio (S/N) of 10.4, indicating that this impurity on the Vanquish CAD HP is at the LOQ limit, as S/N ≥ 10 is generally required for LOQ. The calculated amount of impurity III is 0.023 mg/mL. As demonstrated previously,[1] the estimated quantitation without reference standard is accurate using the Vanquish Inverse Gradient LC setup. Meanwhile, the extracted MS (ISQ EM mass spectrometer) channel for the same impurity has a S/N ratio of 54, making its LOQ level well above that of the LOQ on the Vanquish CAD HP. Depending on the different regulatory impurity thresholds, the Vanquish CAD HP, ISQ EM mass spectrometer, or both can be chosen for impurity quantification.

To determine the repeatability of the method, six replicate injections were made. Relative standard deviations (%RSD) for peak area and height are 2% and below, while the %RSD of retention time is only 0.03% and below, demonstrating superior repeatability of retention times and peak areas/heights of the impurities (Table 3).

The ISQ EM mass spectrometer confirms the unit mass of both the lipid standard and its associated impurities across a wide mass range using the correlation of CAD and single quadrupole MS channels. Additionally, it demonstrates excellent repeatability, particularly for peak area and retention time (Table 4).

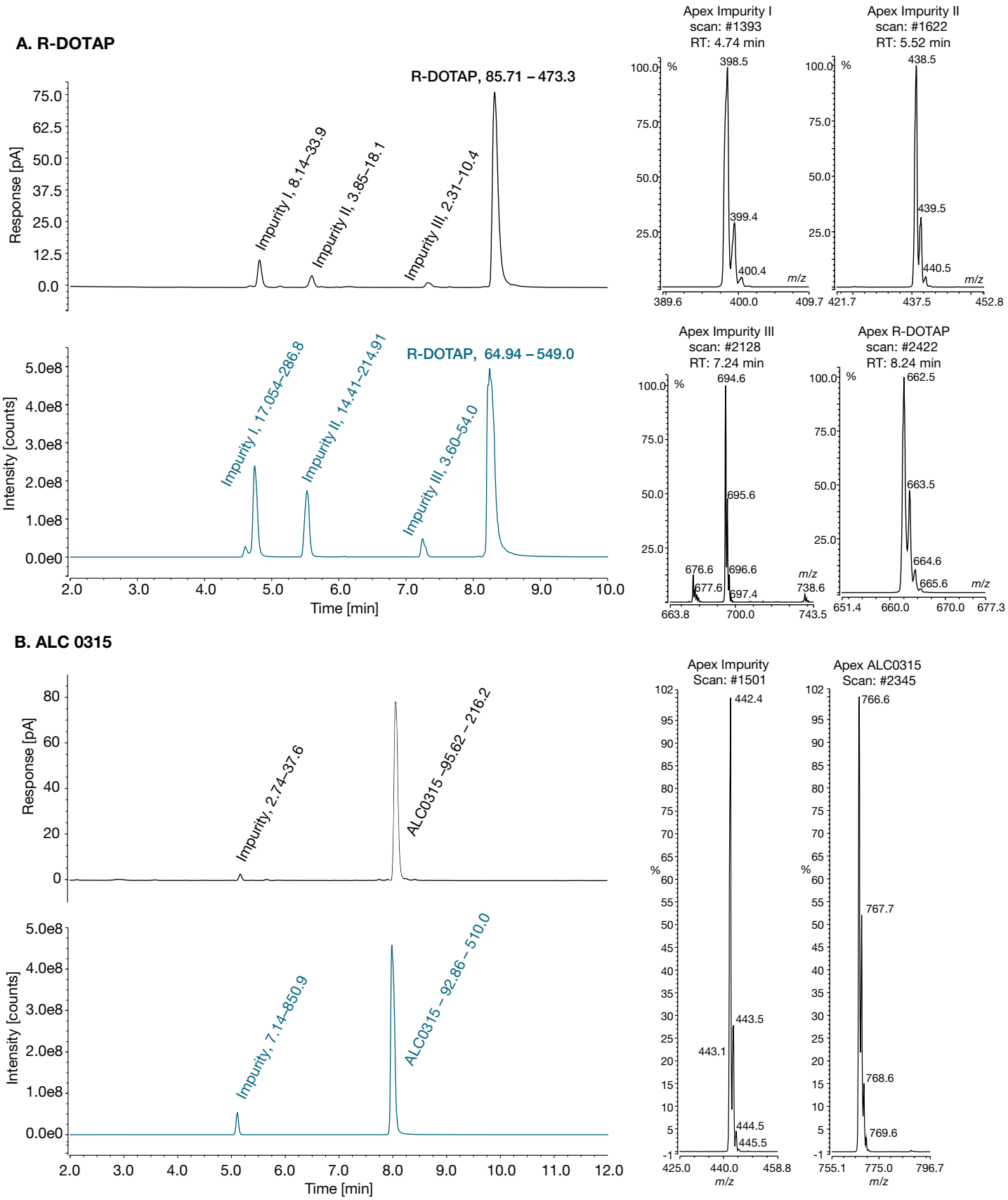


Figure 2 A–B. Example chromatograms and spectra of R-DOTAP, ALC0315, and their impurities. The peak label includes the peak name followed by the relative area percentage and S/N. All concentrations of the lipid standards were around 1 mg/mL.

C. DLin-KC2-DMA

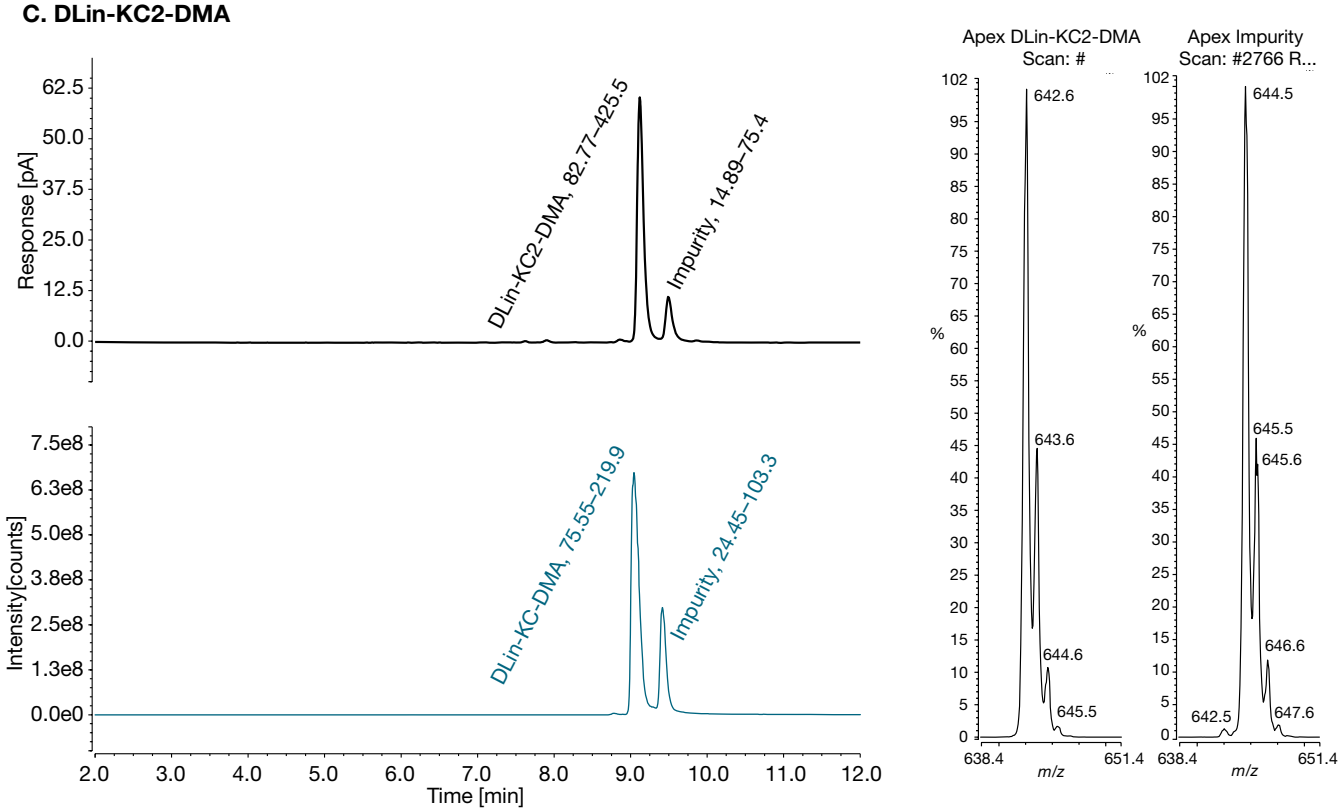


Figure 2C. Example chromatograms and spectra of DLin-KC2-DMA and its impurities. The peak label includes the peak name followed by the relative area percentage and S/N. All concentrations of the lipid standards were around 1 mg/mL.

Table 3. Results of impurity analysis for peak area/height and retention time on the Vanquish CAD HP as average and %RSD (n=6)

Impurity	Peak area average [pA*min]	Peak area %RSD	Peak height average [pA]	Peak height %RSD	Retention time average [min]	Retention time %RSD
R-DOTAP Impurity I	0.74	0.81%	10.66	0.93%	4.163	0.01%
R-DOTAP Impurity II	0.35	0.98%	4.55	1.19%	4.994	0.03%
R-DOTAP Impurity III	0.20	2.06%	1.92	0.72%	6.971	0.03%
DLin-KC2-DMA Impurity	1.05	0.82%	10.26	1.52%	9.119	0.01%
ALC-0315 Impurity	0.17	1.01%	2.84	0.49%	5.174	0.02%

Table 4. Impurity %RSD of peak area/height and retention time on the ISQ EM MS (n=6)

Impurity peaks	*m/z*	Peak area	Peak height	Retention time
R-DOTAP Impurity I	398.5	1.54%	3.00%	0.13%
R-DOTAP Impurity II	438.5	1.70%	4.18%	0.08%
R-DOTAP Impurity III	694.6	1.28%	2.43%	0.05%
DLin-KC2-DMA Impurity	642.6	1.12%	1.62%	0.04%
ALC-0315 Impurity	442.4	2.14%	2.52%	0.02%

Conclusion

The Vanquish Charged Aerosol Detector HP integrates perfectly into the Vanquish Inverse Gradient LC system and offers high sensitivity and excellent repeatability for quantitative cationic lipid impurity analysis, while the ISQ EM MS provides straightforward mass confirmation and high repeatability, with Chromeleon CDS delivering a compliance-ready solution to meet the most stringent regulatory requirements.

References

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