Pharma and biopharma

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

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Abstract

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

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

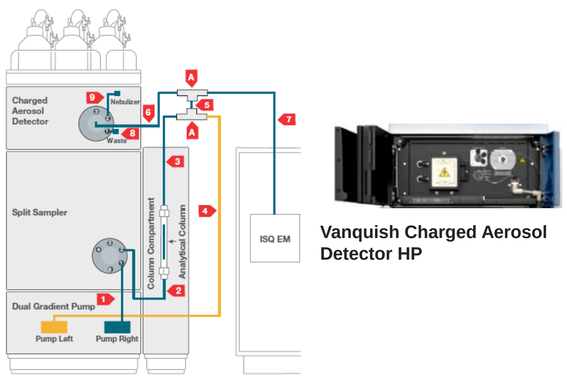
Methods: The workflow uses a Vanquish Flex Inverse Gradient LC system with Vanquish CAD HP and Thermo Scientific™ Hypersil GOLD™ C8 column.

Results: The CAD 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 with high repeatability, all under compliance-ready conditions.

Introduction

Cationic lipid is a critical component in lipid nanoparticle formulations for delivering nucleic acids such as mRNA, siRNA, antisense oligonucleotides etc. [2] Cationic lipids are amphiphilic molecules that possess a hydrophilic region, a hydrophobic region, and a linker structure connecting the two. [1] Charged aerosol detector (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 CAD HP, the same cationic lipids -- R-DOTAP, DLin-KC2-DMA and ALC-0315 -- were measured to this experiment as in Application note 003384 [1]. New diverter valve and software features were showcased, along with method's sensitivity and repeatability.



Vanquish Charged Aerosol Detector HP

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

The new integrated diverter valve in the CAD HP is a 6-port 2-position valve that selects flow between nebulizer and waste. Using the diverter valve to divert everything to waste except the compounds of interest to increase 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 as shown in Figure 1.

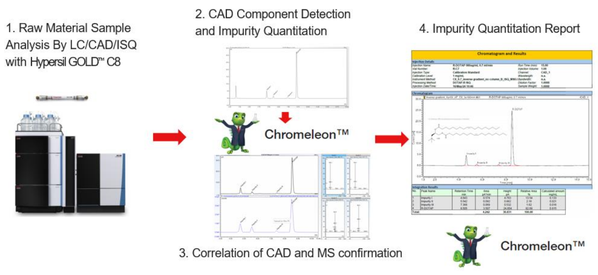


Figure 2. Summary of the method layout

Materials and methods

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 1mg/mL

Test Method(s)

The chromatographic conditions of the LC-CAD-ISQ method are listed in Table1.

Data Analysis

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

Table 1. Chromatographic conditions

Parameter	Value			
Column	Hypersil GOLD HPLC C8 column, 3.0 x 100 mm, 3 µm, P/N 25203-10303			
Solvent A	5 mM ammonium formate in 50% Acetonitrile/50% Water (v/v)			
Solvent B	5mM ammonium formate in 100% Methanol			
Gradient	Analytical		Inverse*	
	Time (min)	%B	Time (min)	%B
	0.0	30	0.829	99
	1.0	50	1.829	79
	6.0	90	6.829	39
	10.0	99	10.829	30
	12.0	99	12.829	30
	12.1	30	12.929	99
	15.0	30	15.0	99
Flow rate	0.7 mL/min			
Column temperature	50 °C with active pre-heater at 50 °C			
Autosampler temperature	8 °C			
Needle wash solution	Solvent B of Mobile phase			
Needle wash mode	Both (before and after)			
Injection volume	1 µL			
CAD settings	Power values: 1.5			
	Evaporation temperature: 35 °C			
	Data collection rate: 10 Hz			
	Filter setting: 5.0 s			
	Diverter valve position: Nebulizer			

* The inverse gradient was automatically calculated using the wizard in the CDS with calculation mode "Keep solvent composition"

Table 2. Instrument and scan settings for the mass spectrometer.

Parameter	Value
Method source type	HESI
Polarity (Spray voltage)	Positive (+3000 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
	Auxiliary gas: 6.5 psig
	Sweep gas: 0.5 psig

Results

High sensitivity and excellent repeatability on the CAD HP

Six consecutive injections were conducted using the method for impurity analysis of cationic lipids as described in application note (AN) 003384 [1].

In the R-DOTAP figure (a), 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 this impurity on CAD HP being 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 in reference [1] the estimated quantitation without reference standard is accurate using the Vanquish Inverse Gradient LC setup. Meanwhile, the extracted MS (ISQ) channel for the same impurity has a S/N ratio of 54, making its LOQ level well above than the LOQ on CAD HP. Depending on the different regulatory impurity thresholds, either CAD HP, ISQ, or both can be chosen for impurity quantification.

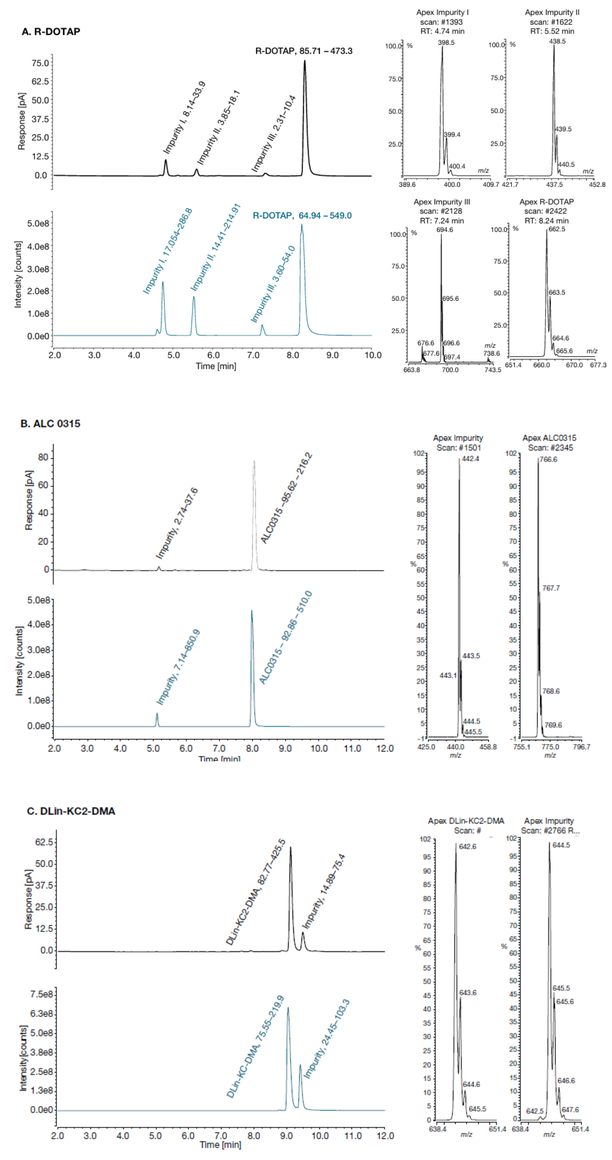


Figure 3. Example chromatograms and spectra of R-DOTAP, ALC0315 and DLin-KC2-DMA and their impurities. The peak label includes peak name followed by the relative area percentage and signal-to- noise ratio. All concentrations of the lipid standards were around 1 mg/mL.

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 (Figure 3). This result (Table 3) demonstrated superior repeatability of retention times and peak areas/heights of the impurity.

Figure 3: Impurity %RSD of peak area and retention time on CAD HP.

Table 3. Impurity %RSD of peak area/height and retention time on CAD HP (n=6).

Impurity	Peak Area average	Peak Area %RSD	Peak Height Average	Peak Height %RSD	Retention time average	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

Mass confirmation with high repeatability

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

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

Impurity peaks	m/z	Peak Area %RSD	Peak Height %RSD	Retention time %RSD
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	2.15	2.52	0.02

Conclusions

The CAD 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 with high repeatability, all under compliance-ready conditions

References

[1] Quantifying impurities in cationic lipids raw materials with the inverse gradient method using LC-CAD-MS - Application note 003384.

[2] Albertsen C. et al, The role of lipid components in lipid nanoparticles for vaccines and gene therapy. Adv Drug Deliv Rev. 2022 Jul 3; 188:114416.

[3] Birdsall R. et al, Monitoring stability indicating impurities and aldehyde content in lipid nanoparticle raw material and formulated drugs. Journal of Chromatography B 1234 (2024) 124005

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