The Lipids Purification for LNPs Formulations, Which Reversed Phase Column is the Best- C18, C8, or C4?

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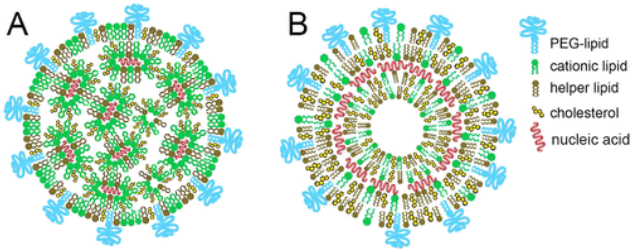

The huge success of lipid nanoparticles (LNPs) encapsulated mRNA vaccines against COVID-19 has not only demonstrated a new method for vaccination, but also provides a proven method for the delivery of mRNA for gene therapeutic. For over three decades, researchers have been trying to overcome major challenges such as intracellular delivery, stability for utilizing RNAs in a therapeutic context, lipid nanoparticles (LNPs) are now solving those problems. With their ability to encapsulate and deliver therapeutics to specific locations within the body and to release their contents at a desired time, furthermore, LNPs provide a valuable platform for treatment of a variety of diseases.

Cationic LNPs[1] with anionic nucleic acids, are the most widely used nonviral delivery system for nucleic acid drugs. They consist of four different types of lipids: PEG-lipids, cationic lipids, helper lipids and cholesterol. For their development, a good purification method for various lipids is crucial.

Here we present a case study of the reversed phase flash chromatography purification of an ionizable lipids, nonanoic acid, $CH_3(CH_2)_7COOH$ which are widely used as the building block for more complex lipids. In some of the reversed phase columns (C4, C8, and C18), the silica is modified with hydrocarbon chains, which interact with the hydrocarbon chains on the lipids. Having the appropriate interaction is the key to good retention and separation. This nonanoic acid sample contains a minor impurity (< 5%), which is important to be removed. The solvent system is water/methanol.

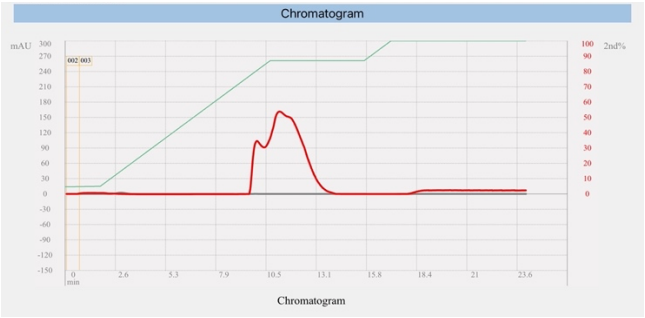

Figure 1. Reversed-phase flash chromatography purification of nonanoic acid (40 mg) by C18 (SW-8201-012-IR, Irregular C18, 40~63um, 60A) with UV (205 nm).

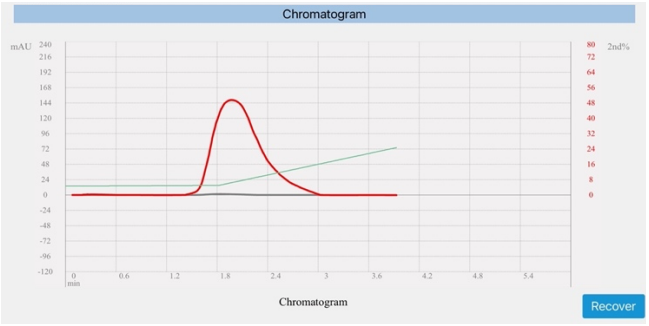

Figure 2. Reversed-phase flash chromatography purification of nonanoic acid (40 mg) by C8 (SW-5822-012-SP, Spherical C8, 20-45um 100A) with UV (205 nm). The lipid is poorly retained, the minor impurity can't be isolated.

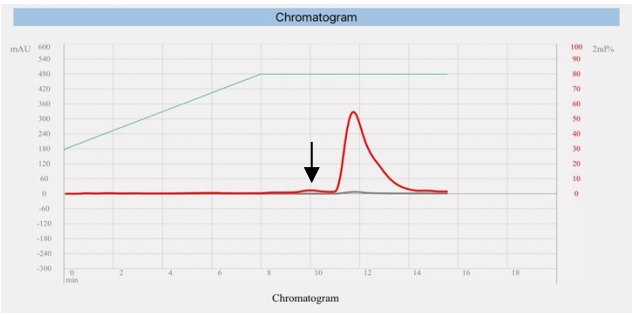

Figure 3. Reversed-phase flash chromatography purification of nonanoic acid (40 mg) by C4 (SW-5422-012-SP, Spherical C4, 20-45um 100A) with UV (205 nm). The lipid is well retained, and the minor impurity is nicely isolated.

Only with C4 modified reversed phase column, the minor impurity is isolated from the main peak with baseline separation and the retention is very

1. [1]*Tenchov, Robert Bird, Allison E. Curtze, and Qiongqiong Zhou* ACS Nano ***2021*** 15 *(11), 16982-17015*

reasonable. With C8 column, the sample eluted at 5% methanol, the sample retention is very poor and the impurity can't be resolved. With C18 column, the complex interaction between the long hydrocarbon chains from the column and the lipids are shown as the non-Gaussian separation diagram with no separation of the impurity. It is important to know that these reversed phase columns are all reusable for minimum 20 times while stored in 80% methanol. This method is scalable up to 10kg media with SepaBean machine L for the purification of 200 g per run. For more info please consult our website at www.santaisci.com.

Table 22: High-efficiency spherical C4, 20-45 µm, 100 Å
(carbon content 5.8%, end-capping, surface area 320 m^2/g, loading capacity 0.1–2%)

Item Number	Column Size	Sample Size	Flow Rate (mL/min)	Cartridge Length(cm)	Cartridge ID (mm)	Max. Pressure (psi/bar)	Quantity per Box
SW-5422-004-SP	5.4 g	5.4 mg–108 mg	5–15	113.8	12.4	400/27.5	2
SW-5422-012-SP	20 g	20 mg–0.40 g	10–25	134.8	21.4	400/27.5	1
SW-5422-025-SP	33 g	33 mg–0.66 g	10–25	184.0	21.4	400/27.5	1
SW-5422-040-SP	48 g	48 mg–0.96 g	15–30	184.4	26.7	400/27.5	1
SW-5422-080-SP	105 g	105 mg–2.1 g	20–50	257.4	31.2	350/24.0	1
SW-5422-120-SP	155 g	155 mg–3.1 g	30–60	261.5	38.6	300/20.7	1
SW-5422-220-SP	300 g	300 mg–6.0 g	40–80	223.5	61.4	300/20.7	1
SW-5422-330-SP	420 g	420 mg–8.4 g	40–80	280.2	61.4	250/17.2	1

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