Elemental impurities in process water for liposome and LNP manufacturing: rationale for element-specific control

In liposome and LNP manufacturing, water is the input used in the largest quantity, and it is used in nearly every process step. Several trace metals that may be present in this water are relevant beyond toxicology, as they can catalyse the degradation of lipids and nucleic acids. This paper summarises which metals are relevant, why standard WFI release testing does not capture individual elemental impurities, and what follows for the specification of process water.

By Sarah Gierl, Head of R&D, PAN-Celltech · TP-09 · Rev 1.0 · 22 Oct 2026 · elemental impurities in process water for liposome and LNP manufacturing · Download the PDF

Key takeaways

- In liposomes and LNP, trace metals are relevant to product stability as well as to patient safety, as several of them act as **catalysts** of degradation reactions.
- **Iron and copper** promote lipid oxidation[1,2], and reactive products of oxidised lipids can form adducts with the nucleic-acid payload[3,4]. **Zinc and lead** can cleave RNA directly[2,5].
- Water is the largest input by mass. In our assessment, metals that bind to the particles are not reliably removed by diafiltration.
- **Standard WFI is not released on individual elemental impurities**, only on summary parameters such as conductivity and TOC. In addition, iron, manganese and zinc are not part of the ICH Q3D panel.
- The effect of chelators such as EDTA differs between systems[1,4,6,7]; chelators do not replace control of the inputs.

- **WFI Beyond** is released per batch on the 24 ICH Q3D elements (Certificate of Analysis) plus iron, manganese and zinc (CoA addendum) [8].

In brief. The published studies establish the mechanisms by which trace metals can damage lipid-based products and identify the relevant elements. As the critical level is product-specific, element-specific data on the process water are a necessary part of the product risk assessment.

1. Elemental impurities in lipid-based products: toxicology and catalysis

ICH Q3D assesses elemental impurities with respect to patient safety. It defines permitted daily exposures and sums the contributions of all sources; this framework applies to liposome and LNP products as to other injectables.

ICH Q3D does not address degradation reactions within the product. Liposomes and LNP contain three components that are susceptible to metal-catalysed degradation:

- unsaturated lipids, which are susceptible to oxidation;
- ionizable or cationic lipids, which can form reactive degradation products such as N-oxides and aldehydes;
- the payload itself, whether mRNA, siRNA or a small molecule, which can be modified or cleaved.

Redox-active metals such as iron and copper act catalytically: in a redox cycle, for example between Fe^{2+} and Fe^{3+}, the metal ion is regenerated and can initiate further reactions[7]. Low concentrations can therefore contribute to cumulative degradation and cannot be dismissed a priori for products with a shelf life of several months.

2. Process water as the largest input by mass

Water is the main component of liposome and LNP dispersions, and the process consumes considerably more water than the final product contains: for the aqueous phase and buffers, for dilution after mixing, typically for several

diavolumes in tangential flow filtration, and for rinsing. A low metal concentration in the water can therefore contribute a cumulative load over the process.

Diafiltration removes free low-molecular-weight solutes. In our assessment, this includes free metal ions, which permeate the membrane, but not reliably ions bound to lipid headgroups or to the RNA backbone, which can be retained with the particles.

Other inputs contribute as well. The authors of a study on lipid/DNA complexes report that excipients such as sugars can contain metals, and that even very low levels may cause damage during long storage[7]. A recent review of mRNA-LNP degradation also names trace metals among the manufacturing variables that may significantly influence product quality[2]. Control at the source therefore begins with the input that is largest by mass.

3. Degradation pathways and published evidence

3.1 Iron and copper oxidise lipids

Copper and iron both caused lipid oxidation in phosphatidylcholine liposomes[1]. In a study on lipid/DNA complexes, iron(II) and copper(II) were added before freeze-drying. Both increased the formation of reactive oxygen species and reduced DNA integrity and biological activity, and iron(II) had the strongest effect[7]. The authors suggest that radicals from oxidising lipids may damage the DNA as well[7]. For LNP, residual iron and copper ions are described as triggers of lipid oxidation[2].

Mechanistically, reduced iron and copper ions (Fe^{2+}, Cu^{+}) generate reactive oxygen species, including hydroxyl radicals, in Fenton-type reactions with peroxides[7]. In unsaturated lipid chains, these radicals initiate chain reactions that yield lipid hydroperoxides and, as secondary products, reactive aldehydes such as malondialdehyde and 4-hydroxynonenal[2]. The oxidised species alter membrane packing and increase permeability[2].

3.2 Oxidised lipids can damage the payload

Reactive lipid degradation products can also react with the payload. In mRNA-LNP, reactive impurities derived from the ionizable lipid formed covalent adducts with the mRNA, which could then no longer be translated[3]. The underlying pathway starts with oxidation of the tertiary amine of the ionizable lipid to the N-

oxide; its degradation releases reactive aldehydes, which modify the mRNA predominantly at cytidine residues[2,3]. In siRNA-LNP, oxidation of the ionizable lipid generated a reactive degradation product that reacted with the siRNA; a histidine buffer reduced the oxidation[4].

Neither study identifies metals as the cause. In our assessment, metal-catalysed oxidation is a plausible route to comparable reactive products; metal control is therefore relevant for the payload as well as for the lipids.

3.3 Zinc and lead cleave RNA

Metal ions can also promote cleavage of the RNA backbone, independently of lipid oxidation. Non-enzymatic cleavage proceeds by intramolecular transesterification: the deprotonated 2'-hydroxyl group attacks the adjacent phosphorus, the strand is cleaved and a 2',3'-cyclic phosphate is formed; divalent metal ions accelerate this reaction[2]. In a kinetic study with short model oligonucleotides, zinc and lead ions cleaved the phosphodiester backbone, preferentially in single-stranded regions[5]. Magnesium, tested at 30 mM and pH 7.4, caused no cleavage within five days at 50 °C[5]; at higher pH, however, magnesium also accelerates RNA hydrolysis[2]. Manganese and lead are used as RNA-cleaving reagents in structural studies, which illustrates their capacity to cleave RNA[5]. Each strand break reduces the fraction of intact, translatable mRNA.

The mechanisms described in this section follow general chemistry and have been described in liposomes, lipid/DNA complexes, oligonucleotides and LNP[1,5,7]; a recent review of mRNA-LNP degradation lists both pathways[2]. The relevant elements are therefore well defined. The concentration at which a given product is affected depends, as for any stability-relevant impurity, on its formulation, payload, storage conditions and shelf life.

4. What standard WFI testing captures and what WFI Beyond adds

Standard Water for Injection is released on compendial parameters such as conductivity, total organic carbon (TOC) and endotoxin. These are summary or non-elemental parameters; individual elemental impurities are not part of this routine release. A low conductivity therefore does not show whether catalytic metals such as iron or copper are present at trace level.

WFI Beyond is additionally released element by element, on two levels:

- **Certificate of Analysis:** the 24 elements of the ICH Q3D panel, each with its ICH Q3D limit and a beyond-compendial limit at half of it.
- **CoA addendum:** iron, manganese and zinc, which are not covered by ICH Q3D, against in-house reference limits.

Both levels are needed for lipid-based products. ICH Q3D selects its elements on toxicological grounds, and of the catalytic metals discussed here only copper and lead belong to the panel. **Iron, manganese and zinc are captured only by the additional testing on the addendum.** The table summarises the relevant elements, their effects and the corresponding WFI Beyond data.

Element	Effect on lipid particles (evidence)	WFI Beyond: limit and release result
Iron (Fe)	Catalyses lipid oxidation and radical formation; ferrous iron the most damaging in lipid/DNA complexes[1,7]	Addendum: reference limit 25,000 µg/L (in-house; not covered by ICH Q3D). Result < 5 µg/L (LOQ)
Copper (Cu)	Catalyses lipid oxidation and radical formation[1,7]	CoA: ≤ 15,000 µg/L beyond compendial (ICH Q3D ≤ 30,000 µg/L). Result < 6 µg/L (LOQ)
Zinc (Zn)	Promotes cleavage of the RNA backbone[5]	Addendum: reference limit 25,000 µg/L (in-house; not covered by ICH Q3D). Result < 5 µg/L (LOQ)
Lead (Pb)	Promotes cleavage of the RNA backbone[5]; ICH Q3D Class 1 toxicant	CoA: ≤ 250 µg/L beyond compendial (ICH Q3D ≤ 500 µg/L). Result < 0.10 µg/L (LOQ)
Manganese (Mn)	Promotes RNA cleavage (used for this in structure probing)[5]	Addendum: reference limit 25,000 µg/L (in-house; not covered by ICH Q3D). Result < 5 µg/L (LOQ)
Nickel, chromium, molybdenum	Markers of stainless-steel contact; specific evidence for lipid particles is not covered here	CoA, beyond compendial: Ni ≤ 1,000, Cr ≤ 55,000, Mo ≤ 75,000 µg/L. Results Ni < 0.40, Cr < 22, Mo < 30 µg/L (LOQ)
Magnesium (Mg)	Practically inactive in RNA cleavage at neutral pH[5]; accelerates hydrolysis at elevated pH[2]; cofactor of in-vitro transcription	A process ion to manage, not a contaminant

Limits as defined on the WFI Beyond Certificate of Analysis (24 ICH Q3D elements: ICH Q3D(R2) limit and the beyond-compendial limit at half of it) and on the CoA addendum (elements in addition to the ICH Q3D panel). Results are typical release results by ICP-MS; LOQ = limit of quantification of the accredited laboratory[8]. Stability data are reported separately[9,10].

5. Chelators: effect depends on metal and formulation

Chelators are frequently considered as a formulation measure. The published results, however, are system-dependent:

- In liposomes, EDTA and NTA reduced copper-induced oxidation but increased iron-induced oxidation[1].
- In another liposome system, EDTA strongly reduced iron-induced oxidation, while NTA and ADP increased it in the presence of iron(II)[6].
- In lipid/DNA complexes during freeze-drying, EDTA increased radical formation, although DNA integrity was maintained[7].
- In siRNA-LNP, EDTA and citrate were among the most stabilising additives[4].

The effect depends on the metal, the chelator and the formulation. Chelators therefore have to be evaluated for each product; they do not replace control of metals in the raw materials and the water.

6. Points to consider in specifications

The following points should be considered when specifying the inputs to a liposome or LNP process:

- **Process water:** element-specific release data per batch with defined limits, covering the ICH Q3D panel plus iron, manganese and zinc, together with stability data. Conductivity and TOC alone are not sufficient.
- **Excipients:** iron and copper data for sugars, salts and buffer substances, and for RNA products also zinc and manganese.
- **Equipment:** stainless-steel contact and single-use components as part of the leachables assessment.
- **Formulation:** chelators, antioxidants and buffer choice, for example histidine, tested in the actual formulation[1,4,6,7].
- **Analytics:** methods that detect lipid oxidation products and lipid-payload adducts, alongside payload integrity[3,4].

7. Data available for WFI Beyond

WFI Beyond is Water for Injection with elemental impurities controlled beyond compendial requirements. Each batch is released by ICP-MS in accordance with Ph. Eur. 2.4.20 and USP <233>, performed by an external accredited laboratory. The Certificate of Analysis covers the 24 ICH Q3D elements, each with its ICH Q3D limit and a beyond-compendial limit at half of it. Iron, manganese and zinc, which are not covered by ICH Q3D, are evaluated on the CoA addendum against in-house reference limits. All four catalytic metals discussed here are therefore part of the release data[8]; stability data are reported separately[9,10].

The ICH Q3D limits on the Certificate of Analysis are derived from the parenteral permitted daily exposures according to ICH Q3D(R2), Section 7, Option 1, assuming a daily intake of 10 g. For aqueous solutions with a density of 1.00 g/mL, 1 µg/g corresponds to 1 mg/L. The assumed daily intake has to be verified by the manufacturer of the finished medicinal product against its actual maximum daily intake[8].

In the PAN liposome and LNP toolkit, WFI Beyond is used as process water in every step, and aqueous buffers can be produced on a WFI Beyond base on request. The toolkit also includes EDTA, histidine and citrate as GMP-grade solutions.

8. Summary

In lipid-based products, the relevance of trace metals extends beyond toxicology. Iron and copper catalyse lipid oxidation, which can affect the payload, and zinc and lead can cleave RNA. Process water is the largest input by mass, standard WFI is not released on individual elemental impurities, the ICH Q3D panel does not include iron, manganese and zinc, and the effect of chelators is system-dependent. Element-specific control of process water is therefore a relevant control point and a data basis for the product-specific risk assessment.

Frequently asked questions

Why should elemental impurities in process water be measured element by element?

Standard WFI is released on summary parameters such as conductivity and TOC, which do not resolve individual metals, and the ICH Q3D panel does not include iron, manganese or zinc. In liposomes and LNP, these metals can catalyse lipid oxidation or RNA cleavage, and process water is the largest input by mass. Element-specific data on the water therefore provide a basis for the product risk assessment.

Does a low conductivity indicate low trace-metal levels?

No. Conductivity is a summary parameter for the total ionic content of the water. It does not show whether catalytic metals such as iron or copper are present at trace level. For this, element-specific testing is required.

Are elemental impurities a safety or a quality topic for liposomes and LNP?

Both: ICH Q3D covers patient safety, and in lipid-based products some metals also act as catalysts of lipid oxidation or RNA cleavage, which affects product quality and stability.

Which elements are most relevant?

The cited studies point to two groups of elements. Iron and copper catalyse lipid oxidation and the formation of reactive oxygen species; in lipid/DNA complexes, iron(II) had the strongest effect. Zinc and lead, and also manganese, promote cleavage of the RNA backbone.

Is testing according to ICH Q3D sufficient?

For liposome and LNP applications, it is not sufficient on its own. ICH Q3D covers 24 elements selected on toxicological grounds. Of the catalytic metals discussed here, only copper and lead are included, so iron, manganese and zinc have to be tested in addition.

Is magnesium relevant?

Not as a cleavage catalyst at neutral pH: at 30 mM and pH 7.4, magnesium caused no RNA cleavage within five days at 50 °C. At higher pH, however, magnesium accelerates RNA hydrolysis. As magnesium is also the cofactor of in-vitro transcription, pH control is the relevant measure.

Does the addition of EDTA prevent metal-catalysed degradation?

EDTA does not reliably prevent it. Depending on the system, EDTA reduced or increased metal-induced oxidation, although in one siRNA-LNP study it was among the most stabilising additives. Its effect has to be tested in each formulation.

Does diafiltration remove trace metals?

Diafiltration does not reliably remove them. In our assessment, free ions pass through the membrane, but metals bound to lipids or to the RNA backbone can stay with the particles. Control at the source, in particular of the process water, is more robust.

What data are available for WFI Beyond?

Every batch is released by ICP-MS in accordance with Ph. Eur. 2.4.20 and USP <233>. The Certificate of Analysis covers the 24 ICH Q3D elements with ICH Q3D and beyond-compendial limits, and the CoA addendum covers iron, manganese and zinc against an in-house reference limit of 25,000 µg/L. In release testing, iron, copper, zinc, manganese and lead are typically below the limit of quantification. Stability data are reported separately.

References

1. Yoshida Y, Furuta S, Niki E. Effects of metal chelating agents on the oxidation of lipids induced by copper and iron. Biochim Biophys Acta. 1993;1210:81-88. https://www.sciencedirect.com/science/article/abs/pii/000527609390052B
2. Khan MFH, Islam T, Mishra A, Kamen AA. Possible mechanisms of mRNA-LNP degradation: a comprehensive review. Vaccines. 2026;14(7):620. https://doi.org/10.3390/vaccines14070620
3. Packer M, Gyawali D, Yerabolu R, Schariter J, White P. A novel mechanism for the loss of mRNA activity in lipid nanoparticle delivery systems. Nat Commun. 2021;12:6777. https://doi.org/10.1038/s41467-021-26926-0

4. Estabrook DA, Huang L, et al. Buffer optimization of siRNA-lipid nanoparticles mitigates lipid oxidation and RNA-lipid adduct formation. Nat Commun. 2025;16:8380. https://doi.org/10.1038/s41467-025-63651-4
5. Zagórowska I, Kuusela S, Lönnberg H. Metal ion-dependent hydrolysis of RNA phosphodiester bonds within hairpin loops. Nucleic Acids Res. 1998;26(14):3392-3396. https://doi.org/10.1093/nar/26.14.3392
6. Arora A, Strasburg GM. Development and validation of fluorescence spectroscopic assays to evaluate antioxidant efficacy - application to metal chelators. J Am Oil Chem Soc. 1997;74(9):1031-1040.
7. Molina MdC, Anchordoquy TJ. Metal contaminants promote degradation of lipid/DNA complexes during lyophilization. Biochim Biophys Acta. 2007;1768(3):669-677. https://pmc.ncbi.nlm.nih.gov/articles/PMC1851895
8. PAN-Celltech. WFI Beyond Certificate of Analysis and CoA addendum: release testing by ICP-MS to Ph. Eur. 2.4.20 / USP <233>, external accredited laboratory. Data on file.
9. PAN-Celltech. WFI Beyond: metal control that lasts - every ICH Q3D element still below limit at the T18 stability point (TP-08). Technical paper, Rev 1.0. https://pan-biotech.com/wfi-beyond-elemental-impurity-stability/
10. PAN-Celltech. WFI Beyond GMP stability reports, elemental impurities by ICP-MS to Ph. Eur. 2.4.20 / USP <233>, ISO/IEC 17025-accredited laboratory. Data on file.

Related reading

- WFI Beyond - Elemental Stability (TP-08) - the 24-element and copper/iron data behind this paper.
- One Foundation for the Whole Liposome Process (In-Depth A06) - the liposome and LNP process, step by step.
- Liposomes & LNP - the PAN toolkit for every step.
- The WFI Beyond Standard (In-Depth D02) - the water behind the beyond-compendial standard.

Data and documentation

The WFI Beyond elemental-impurity data, a representative Certificate of Analysis and information on GMP buffers on a WFI Beyond base are available on request.

info@pan-celltech.com