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Research Article

Next Generation Tools in mRNA Purification: The Role of Continuous Raman Spectroscopy Testing with Pretreatment of the Sample

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Abstract

In the biopharmaceutical production field, the purification process is a crucial step in order to obtain Drugs with an impurity profile according to the regulatory agency requirement.

The aim of this work is to verify some relevant and recent literature and after analysis to submit to the researcher new Solutions in order to improve global safety and the toxicological profile: Submit a project related to the continuous testing of the purified materials using Raman spectroscopy – with pre-treatment of the sample: using solvents.

Nanolipis Payload of Biopharmaceutical is not efficiently detected by direct Raman spectroscopy allowed by the regulatory agency for PAT process analytical technology.

Introduction

In various biopharmaceutical productive processes, the final purification is a relevant step and is used in example various chromatographic processes using different systems (resins, columns, monoliths).

Various materials are used for the stationary phase.

Some vials of m RNA vaccine were analyzed by independent researchers to verify the profile of impurity [1].

Many classic and biological drugs use activated carbon for the purification of water needed (pirogen) and in commerce there are producers that use composite materials in their monoliths (carbon-based) [2,3].

In recent times great public debate was involved in finding graphene-like particles in some C19 vials of vaccine as well as in the blood of many vaccinated people [4].

According to the regulatory agency, it is accepted that RAMAN spectroscopy also directs to verification of impurities in the pharmaceutical product.

But this method (the direct one assay), when applied to nano lipids, according to the literature is not the best way to test the payload of the nanoparticle [5,6].

To increase the possibility of finding all impurities it is crucial to pre-treat the sample (nanolipids) with solvent before performing the RAMAN test.

If for injectable water in order to search impurity are performed conductivity test and TOC total organic carbon in the final vials of biopharmaceutical it is necessary to use adequate analytical tests to exclude.

A particle of AC-activated carbon or graphene from filtering or purification steps.

Material and methods

With an observational point of view, some relevant

literature from PUBMED or other biomedical scientific databases is reported and a hypothesis of work is then submitted to increase the global safety related to some impurities for the nanolipids-based drug production.

Results

From literature

Various authors were involved in impurity profile in biopharmaceuticals: P. CAMPRA and its interesting work on vials of vaccine C19 [1], Monoliths used in the purification of biopharmaceuticals [2], ACTIVATED CHARCOAL and its use in filtering [3], findings of suspected graphene-related substantiate on patients blood sample after vaccination [4], direct RAMAN SPECTROSCOPY for PAT and its disadvantages when applied to nano lipids payload [5,6] and since BP PHARMACOPEIA Activated charcoal MONOGRAPHY [7].

"Because various API manufacturing processes used AC products it is necessary to test the final impurity also for graphene: this is due to the different size of the particle of amorphous AC vs crystalline exfoliated graphene. (also for genotoxicity) and the toxicity that can be produced is also below the threshold for impurity.

AC production can imply high temperature with chemical–physical change.

The pharmacopeia monography for AC does not cite the word graphene" [8].

According to R.O. YOUNG 2021

"Steps for analyses were:

1. Dilution in 0.9% sterile physiological saline (0.45 ml + 1.2 ml).
2. Polarity fractionation: 1.2 ml hexane + 120 ul of RD1 sample.
3. Extraction of hydrophilic aqueous phase.
4. UV absorbance and fluorescence spectroscopy scanning.
5. Extraction and quantification of RNA in the sample.
6. Electron and optical microscopy of aqueous phase" [9].

It can be considered a destructive method.

Experimental project hypothesis

In order to increase the power of detecting impurity in nano lipids drugs it is necessary at the end of the production process to test in a continuous way the final product using RAMAN spectroscopy (with pretreatment of the sample).

In this way, only after use of solvent, it is possible to avoid the interference played by the nanolipids.

Discussion

In literature is reported:

The crucial role played by the purification steps

Various chromatographic procedures are used, using resins and monoliths

Some producers use activated carbon composite materials

For the production of water for injectable (PIROGEN) used also charcoal filter systems membrane

The test for PAT (PROCESS ANALYTICAL TECHNOLOGY) analysis in pharmaceutical production use also RAMAN SPECTROSCOPY.

It is allowed by the regulatory agency the direct testing with RS (without pretreatment with solvent of the sample – NOT DESTRUCTIVE METHOD).

This method is not the best to test the payloads for nanolipid drugs. According to Vanden-Hehir S, et al. "A major advantage of Raman is that it allows direct imaging of the nanocarriers, and not the payload encapsulated within them."

Independent researchers find graphene-like particles in some vials of some C19 vaccine [1].

Another researcher found graphene particles in the blood of the vaccinated [4].

From the membrane activated carbon can be released impurity.

One method to produce water for injection (pirogen removal) is using activated carbon.

(The same to remove pyrogen from injectable).

Activated charcoal is produced also using high temperatures and it can exfoliate graphite and graphene [8] even if under the threshold requirement of the regulatory agency.

In the monography of activated carbon of various pharmacopeia, the term graphene is not reported.

Conclusion

For all reported in the discussion it is the opinion of the authors that to increase the level of safety IN m RNA PRODUCTION and purifications it is necessary to test the final product in biopharmaceutical production in a continuous way using a RAMAN SPECTROSCOPY with pretreatement of the sample with solvent. This is to avoid interference played by the nanolipids.

Also for the classic chemical drugs and their productive process, it is of interest to verify the absence of impurity from

activated carbon membrane used related to the level today admitted (thresholds) by pharmacopeia.

What are the toxicological effects played by some impurities if present in drugs' final product also under the regulatory thresholds?

The m RNA purification steps can be improved using new technology, classic chemical analytical tests whit pre pre-treatment of the samples, and performed in a continuous way.

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