



The Inorganic impurity for injectable WATER ,chemical DRUGS and biopharmaceuticals : Conductivity TOC and Activated charcoal - graphene testing in Quality control- Hypotesys of work

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Abstract: This work is involved inorganic impurity profile in drugs and in biopharmaceuticals : according FDA and EMA for chemical Drugs are Adopted the thresholds, below this value they not require identification as mandatory. In order To eliminate pirogen In the production of WIFI water (for injectable) are used also activate carbon filter and FDA consider this product as a possible source of inorganic impurity. But for Injectable water production to test the quality is required Conductivity measure and Toc total organic carbon assay. In this way WIFI water can be considerdc safe because with this analisys it is possible to exclude both inorganic and organic impurity. In biopharmaceutical purification steps are used also composite material filter (some producer use activated charcoal) and this materials can in teory exfoliate graphite and graphene in mechanical way. (Graphene is a good conductor of eletrricity and it is classify as inorganic.) In the final vials of biopharmaceuticals is not requested conductivity test. For chemical drugs there are regulatory requirement for global impurity : if the test show results below the fixed thresholds no more action is required. (no needed identification) , genotoxicity and cancerogenicity evalutation are also performed. For biotech product there are not fixed limits for all inorganic molecule (only suggested), instead fixed for elemental impurities and residual solvents : regulatory rules report a responsibility of the producer for actions. There are guideline ICH for preclinical study of mutagenicity and cancerogenicity.

Aim of this work is to analyze the conductivity and TOC testing role for WIFI water production and its meaning in PAT and to discuss about the need to verify also below thresholds adopted or suggested for molecule like graphene for the drugs substantie produced. In this study are reported various commercial products , but it is not intent of the authors to put in relationship the profile of impurity of the final product using this with any toxicological aspects : this are only reported for a pure Material science purpose.

Keywords: Inorganic Impurity, regulatory, purification, activated charcoal, graphite, graphene, cartridge, membrane, monoliths, composite materials, TOC,conductivity, WIFI water, biopharmaceuticals, biological drugs,genotoxicity,mutagenesis, RAMAN spettroscopy, PAT, brief, medium long term toxicity profile, toxicology, material science.

INTRODUCTION

This work start observing the chemico -physical properties of some carbon allotropes and various product characteristics under a material science approach:

<https://www.intertek.com/pharmaceutical/biopharmaceuticals/process-residuals/>

Process Related Impurities and Residuals Analysis

“Effective removal of process-related impurities or process residuals is important to pharmaceutical and biopharmaceutical development. Process impurities are related to the manufacturing process and may include the cell substrates (host cell proteins, host cell DNA), cell culture (inducers,

antibiotics, or media components), or **chromatographic media used in purification**, solvents and buffer components. Process-related impurities analysis to support bioprocess validation in line with **ICH Q6B**, through mass spectrometry, spectroscopy and chromatography assays” Great public debate was involved in graphene like particles impurity in some vials of C19 vaccine by independent researcher so it is interesting to observe some related properties:

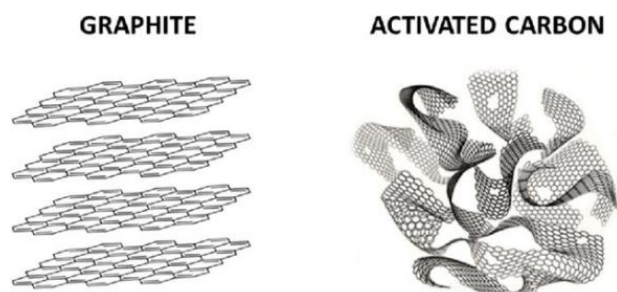


Fig n 1 Illustrative representation of 3-dimensional crystal lattice of graphite and amorphous activated carbon. From DOI: 10.1016/j.rser.2021.111783 From <https://blog.metcar.com/what-is-graphitization>

“**Graphitization is the process of heating amorphous carbon for a prolonged period of time**, rearranging the atomic structure to achieve an ordered crystalline structure that is typical of solids. During graphitization, carbon atoms are rearranged to fill atom vacancies and improve atom layout.” <https://www.thomasnet.com/articles/chemicals/producing-activated-carbon/>

“The production process of activated carbon AC, or the activation of carbon, exists in 2 forms. A carbonaceous source, which can exist as coal, peat, or any organic carbonaceous material is carbonized, which means the pure carbon is extracted by a heating method known as pyrolysis. Once the material is carbonized, it needs to be oxidized, or treated with oxygen, either by exposure to CO₂ or steam, or by an acid-base chemical treatment.

Carbonization

Carbonization is the process of taking a carbon-rich piece of material and converting it to pure carbon through heating. This heating process, called pyrolysis, comes from an ancient technique for making charcoal. Very dense carbonaceous material is used in the beginning, because the end result needs to be extra-porous for activated carbon AC purposes. Carbon-rich material is placed in a small (relative to the amount of material) **furnace and cooked at extreme temperatures topping 2000 degrees Celsius**. What remains is usually 20-30 % of the beginning weight, and consists of mostly carbon and a small percentage of inorganic ash,”

Carbon Volume 161, May 2020,

Assessing the structural properties of graphitic and non-graphitic carbons by Raman spectroscopy Dominique B. Schuepfer , F. Badaczewski , Juan Manuel Guerra-Castro, Detlev M. Hofmann,

Christian Heiliger, Bernd Smarsly , Peter J. Klar <https://doi.org/10.1016/j.carbon.2019.12.094>

“Experimental details and theoretical modelling 5 series of samples spanning the transformation chain of the carbonization process from a molecular precursor to graphitic carbon have been studied. The series were based on 5 different precursors, 3 pitches and 2 resins.

All precursor substances were heat-treated up to 3000 +C in Ar (argon) atmosphere, which results in a carbonization process.

Typical stages which occur during the heat-treatment causing atomic rearrangements are the following: First, **amorphous carbon exhibiting all 3 kinds of carbon bonds (sp¹, sp² and sp³)** without any stacking is formed at temp. above 700 +C. The amorphous carbon AC of mixed bonding turns into purely sp²-bonded amorphous carbon at round about 1200+C on further heating. Non-graphitic carbon is formed in the temperature range from about 1400+C to 1700+C. **Above 1700+C the crystallites transform into graphene sheets, which arrange in an ordered stacks.”**

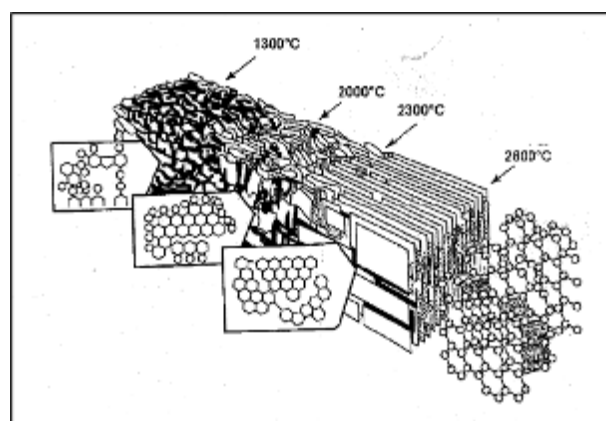


Fig n 2 Evolution of graphitization process, at 1300°C – 2000°C: Crystal structure begins to grow. This indicates movement and rearrangement of atoms. The interlaying spacing of atoms begin to rearrange and shrink to resemble that of graphite as depicted in Figure

The process of exfoliation of activate carbon or graphite is of great interest: According the work : Structure of Carbon Materials Explored by Local Transmission Electron Microscopy and Global Powder Diffraction Probes by Karolina Jurkiewicz, M.Pawlyta ,Andrzej Burian <https://doi.org/10.3390/c4040068> : Dec 2018 “In 1934 Warren gave a deeper insight into the structure of carbon black and based on the Fourier integral analysis of the XRD patterns proposed that carbon black is not truly crystalline or an **amorphous form of carbon but it may be a heterogeneous mixture of domains which range from a single graphite layer (named graphene) up to graphite crystals several layers thick**”

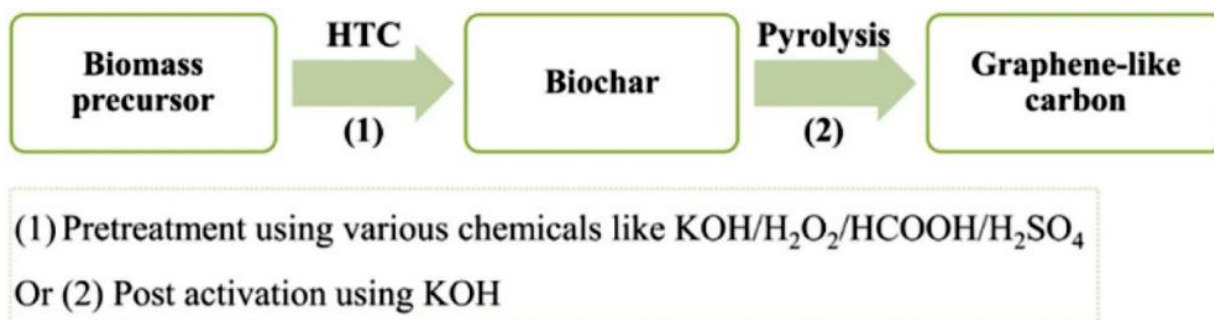


Fig n 3 from <https://doi.org/10.1007/s12649-021-01577-w>

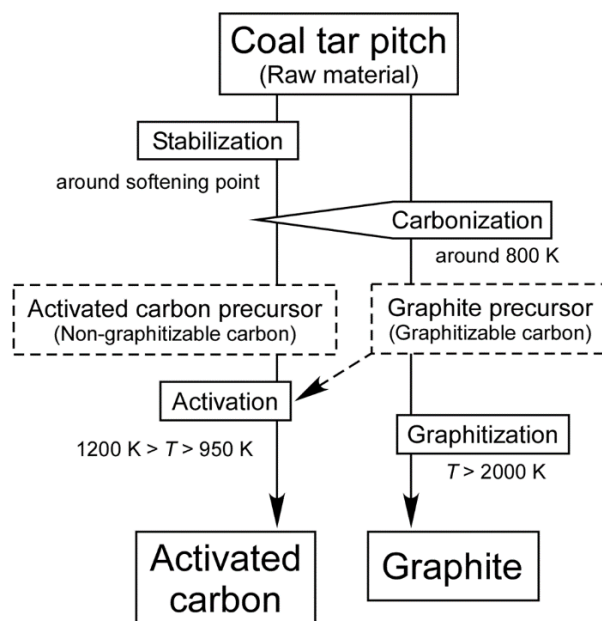


Fig n 4 General preparation method of activated carbon AC and graphite from coal tar pitch.From <https://doi.org/10.1038/s41598-019-43818-y>

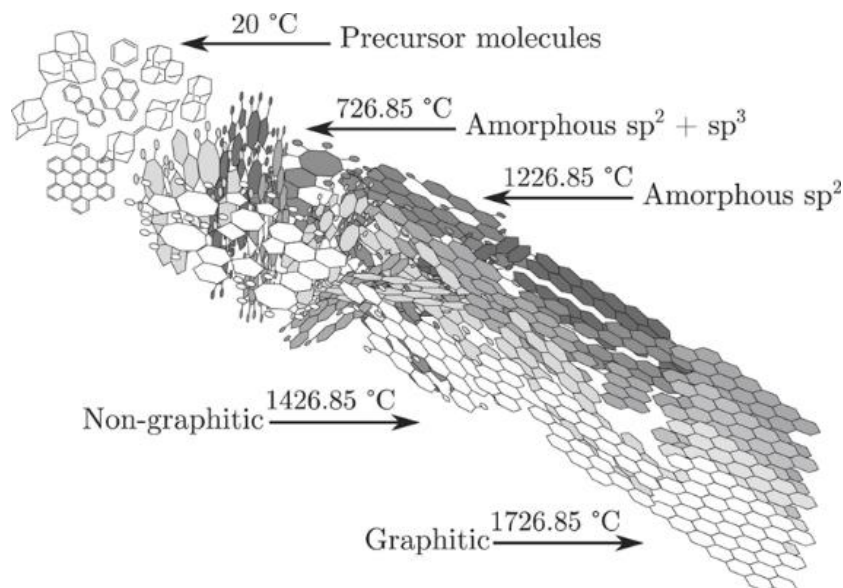


Fig n 5 During heat-treatment the structural units increase in size and crystallinity and temperatures above 1700 °C yield high stacking order .From <https://doi.org/10.1016/j.carbon.2019.12.094>

4.6. Mechanical exfoliation of graphite

Geim and Novoselov [7] prepared a single graphene sheet by peeling off a sheet of graphite using Scotch tape (see Figure 8) [82]. This method involves repeatedly peeling highly oriented pyrolytic graphite (HOPG) using scotch tape. The process has been optimized to produce single-layer graphene (SLG) with high structural quality and more than $100 \mu\text{m}^2$ in size [83]. This method is called the Scotch tape approach, which is not suitable for large production of graphene.

4.7. Liquid-phase exfoliation (LPE)

Layered materials such as graphite consist of two-dimensional platelets weakly stacked to form three-dimensional structures. In graphite, these layers form strong chemical bonds in-plane but display weak out-of-plane bonding. This

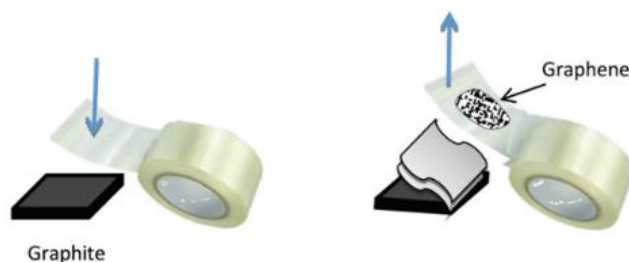


Figure 8. Scotch tape method for graphite exfoliation [82].

Fig . n . 6 from Turkish Journal of Chemistry Volume 45 Number 3 Article 11-1-2021 Graphene preparation and graphite exfoliation AHMED MOOSA MAYYADAH ABED

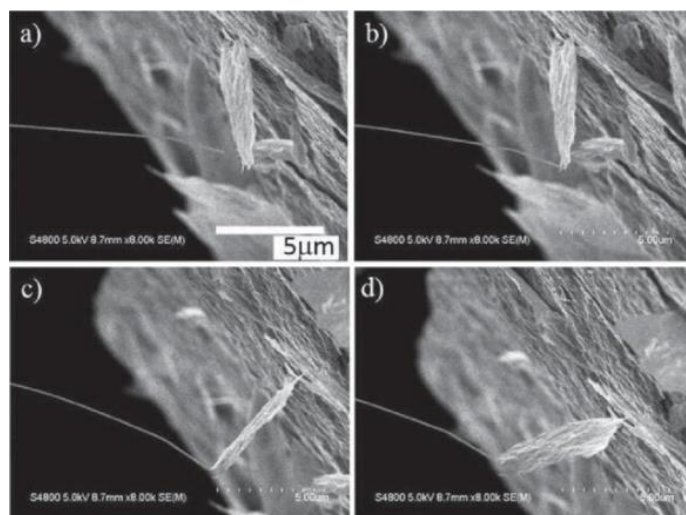


Fig n 7 EM images of the graphene flake exfoliation from the pyrolytic graphite surface: (a) Ge nanowire near the graphene flake delaminated from the graphite surface; (b) Ge nanowire in contact with the graphene flake; (c)-(d) withdrawing of the Ge nanowire together with graphene flake. From DOI: 10.1166/sam.2014.2139

From <https://www.pall.com/en/food-beverage/spirits/activated-carbon-treatment.html>

“Activated carbon AC mainly consists of elementary carbon in a graphite like structure. It differs from graphite by having a random imperfect structure which is highly porous over a broad range of pore sizes”

<https://www.fishersci.se/shop/products/carbon-activated-norit-row-0-8mm-pellets-steam-activated-thermo-scientific/11309115>

“**Synonym:graphite, activated charcoal, norit, mineral, carbon-12, carbono, graphene,** acticarbon, anthrasorb, carbo sieve. Carbon, activated, Norit ROW 0.8mm pellets, steam activated is suitable for decolorization, deodorization and purification applications” 3M™ Zeta Plus™ Activated Carbon Series Filter Cartridge “Our Zeta Plus™ Activated Carbon AC Series Filter cartridges are specifically designed for use in the manufacture of biopharmaceutical, vaccine, blood fractionation and small molecule API drug substances.” Impurities in biologic drug

substances can be classified into : Product-Related Impurities: variants, aggregates, and degradation products of the desired therapeutic protein.

Process-Related Impurities: That arise from the manufacturing process and include host cell proteins, DNA, endotoxins, and residual solvents.

A well-designed manufacturing process is vital in minimizing this impurities. Key strategies can include: Selection of Appropriate Host Systems: Choosing the right host cell line and expression system can minimize some impurities. Optimization of the Upstream Processes: Control of media components, pH, temperature, and culture conditions can reduce the unwanted variations.

Downstream Process Development: Employing various purification techniques, like as chromatography, can further reduce impurities.

Analytical methods to detect and quantify impurities Method Development: Employing techniques like mass spectrometry MS, liquid chromatography LC, and capillary electrophoresis to identify impurities.

Rudolf W. Kessler, W. Kessler, in Comprehensive Chemometrics , 2020 Definition of inline, online, atline and offline measurements “PAT is defined by FDA as a system for designing, analyzing, and controlling manufacturing through timely measurements (during processing) of critical quality and performance attributes of raw and in-process materials and processes with the goal of ensuring the final product quality. In the FDA’s PAT definition, “analyzing” equates to in situ analytical tools and it includes many measurement and instrument types like a pH probe, optical spectroscopy, mass spectrometry MS and chromatography.”

Guidance for Industry

PAT — A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance Office of Training and Communication Division of Drug Information, HFD-240 Center for Drug Evaluation and Research FDA <http://www.fda.gov/cder/guidance/index.htm> Sep 2004

Pharmaceutical CGMPs

“The Agency considers PAT to be a system for designing, analyzing, and controlling manufacturing through timely measurements (during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality. These measurements can be:

at-line: Measurement where the sample is removed, isolated from, and then analyzed in close proximity to the process stream.

on-line: Measurement where the sample is diverted from the manufacturing process, and may be returned to the process stream.

in-line: Measurement where the sample is not removed from the process stream , can be invasive or **noninvasive**.”

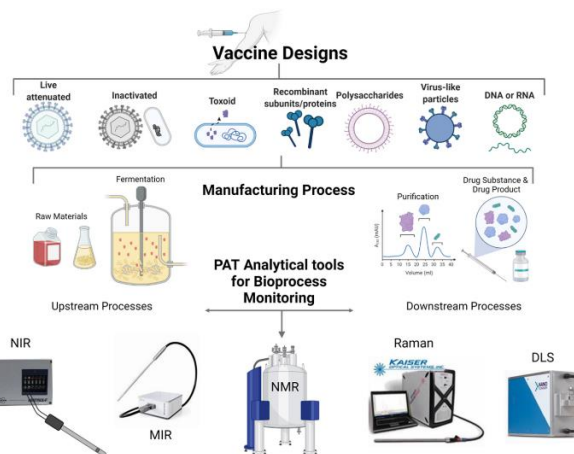
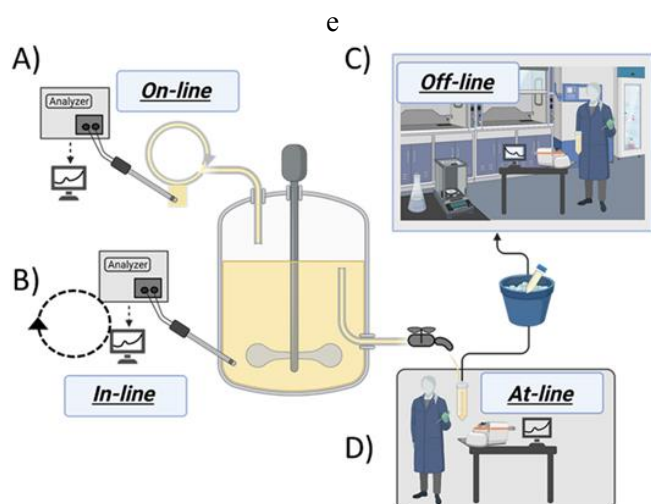


Fig n 8 (PAT) tools for Bioprocess monitoring throughout the Manufacturing Lifecycle. Schematic for the use of analytical tools (Near-infrared spectroscopy (NIR), Mid-infrared spectroscopy (MIR), Nuclear Magnetic Resonance (NMR), Raman Spectroscopy, and Differential Light Scattering (DLS)) for monitoring different types of biological- biotechnological products throughout upstream and downstream production processes. From : <https://doi.org/10.1016/j.jpba.2021.114379>

G. Gerzon , Yi Sheng , M. Kirkitadze. Process Analytical Technologies – Advances in bioprocess integration and future perspectives. Journal of Pharmaceutical and Biomedical Analysis, Volume 207, 5 January 2022. <https://doi.org/10.1016/j.jpba.2021.114379>

“analytical PAT tools including NIR, IR, NMR, Raman, DLS, and particle count and imaging probes during the various phases of manufacturing lifecycle.” Application of PAT-Based Feedback Control Approaches in Pharmaceutical Crystallization Ye Gao ,Teng Zhang ,Yiming Ma ,F. Xue ,Z. Gao ,Baohong Hou ,Junbo Gong Crystals , <https://doi.org/10.3390/cryst11030221> 24 February 2021

“For the pharmaceutical production process PPP, the purity of the product is a crucial quality indicator. Impurities in the drug will reduce the efficacy and even have physiological toxicity.”

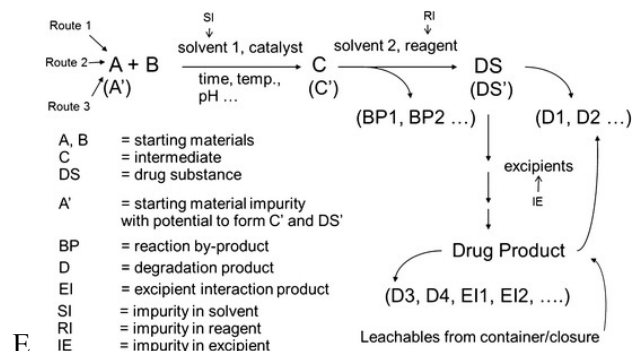


Fig. n 9 The potential sources of drug impurities during development for both drug substances and drug products From doi: 10.1208/s12249-013-0061-z

Into the group of biopharmaceuticals the Quality of vaccines is controlled at multiple levels:

1) Before approval > in depth review of the registration file (MA MARKETING AUTHORIZATION) by medicines agencies (FDA -

EMA). The MA contains all information on production process, materials and testing.

2) Quality control testing: every single vaccine batch has to be tested by the manufacturer according to predefined specifications approved by the authorities.

3) GMP inspections: before and after the approval (every 2-3 years): verification of compliance with legislation and the registration file.

4) Pharmacovigilance PV: post-approval clinical monitoring.

5) Additional QC testing performed by independent laboratory (OMCL) Quality review of MA file

All the materials used for production need to be described in detail. Any biological materials used in the process need to be screened for the possible contaminants (bacteria, viruses). Process, process controls, QC tests need to be well described. Containers and process equipment has to be evaluated for the possible leaching of elements into the vaccine.

Impurity removal capacity of process needs to be demonstrated.

Assessment has to be made confirming the safety of the final product.

Any residuals need to be below the acceptable safety limits. All this info has to be included in the MA file, which has to be reviewed and approved by the authorities (FAMHP / EMA/FDA)

Benedette Cuffari, D. Ellis, The Power of Process Analytical Technology (PAT) in Pharmaceutical Manufacturing "PAT is a system that allows for the design, analysis, and control of manufacturing through real time quality control and performance attribute measurements.

Spectroscopic techniques such as infrared (IR), Raman, and UV-Vis spectroscopy, chromatographic techniques, as well as particle size and mass measurement tools, can be incorporated in situ or through continuous flow systems to be considered PAT.

In example, combining Raman and IR spectroscopy with advanced data analysis strategies such as machine learning and chemometric tools allows for extracting crucial information embedded within the data. Comparatively, chromatographic techniques such as gas and liquid chromatography LC, which are the most common methods for PAT, separates samples into their gas and liquid mobile phases, respectively. When combined with other separation analytical techniques, these tools provide relevant information for evaluating pharmaceutical drugs to improve the manufacturing process ultimately"

The Power of Process Analytical Technology (PAT) in Pharmaceutical Manufacturing, Benedette Cuffari, M.Sc.

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ACCORDING FDA FDA Guidance for Industry: "Q11 Development and Manufacture of Drug Substances (Chemical

Entities and Biotechnological/Biological Entities)." Impurity: See ICH Q3A, ICH Q6A, and ICH Q6B. ICH Q 6 B

Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products SEP 1999 "Process-Related Impurities:

Impurities that are derived from the manufacturing process. They may be derived from cell substrates (host cell proteins, host cell DNA), cell culture (inducers, antibiotics, media components), or **downstream processing, processing reagents or column leachables). Process-related impurities** in the drug substance may include cell culture media, host cell proteins, DNA, monoclonal antibodies or **chromatographic media used in purification, solvents and buffer components. These impurities should be minimised by the use of appropriate well-controlled manufacturing processes**

Product-Related Impurities: Molecular variants of the desired product (precursors, certain degradation products arising during manufacture and/or storage) which do not have properties comparable to those of the desired product with respect to activity, efficacy, safety. Downstream-derived impurities include, but are not limited to, enzymes, chemical and biochemical processing reagents (cyanogen bromide, guanidine, oxidising and reducing agents), inorganic salts (heavy metals, arsenic, non metallic ion), solvents, carriers, ligands (mabs), other leachables. Scenario hypotesys : Residual Solvents in the Downstream Processing Problem: A company identifies residual solvents in the purification stage, which can affect the final product's quality.

Solution:

Optimize the purification Steps: Adjust chromatography conditions to enhance removal of the solvents. Implement Rigorous Testing: Use validated analytical methods to detect the residual solvents early in the process. Review Supplier Quality: Ensure that the raw materials, including solvents, meet the quality standard."

After this list of impurities it is interesting to observe that: In various biopharmaceutical or classic drug production are used also materials for purification based on activated charcoal (filtering cartridge, carbon based composite materials: monoliths):In ex. the purification steps in production of mRNA vaccine. Various methods are used to produce WFI water and to avoid pyrogen and between this methods some use activated carbon AC (cartridge, membrane). During last Pandemia C19 Great public debate raised after findings of some independent reseacher of impurity of graphene in some vials of C19 Vaccine as well as in blood of many patients after vaccination.

Activated charcola is produced using chemico physical procedure (really high temperature) and it is showed by literature as it can exfoliate graphite and also graphene in mechanical way. But if for the production of WFI water for injectable is required by regulatory agency testing of

Conductivity and TOC analisys (TOTAL organic carbon) what happen for finished vaccine ? for finished drugs products are fixed thresholds for global impurity and below it is not required to identify the molecule involved. Between the inorganic impurity FDA and EMA report also activated charcoal from purification step in drug production. Because form activated charcoal due by its production technique can exfoliate graphite or graphene it is

relevant to test the final product verifying the global absence of this impurities even if under the required thresholds. The EMA's scientific guidelines on impurities in drug products and drug substances help medicine developers prepare marketing authorisation applications for human medicines.

Guidelines: Control of impurities of pharmacopoeial substances

ICH Q3A (R2) Impurities in new drug substances, ICH Q3B (R2) Impurities in new drug products

ICH Q3C (R6) Residual solvents, ICH Q3D Elemental impurities

ICH M7 Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk - Scientific guideline

Impurities	Drug substances	Drug products	Biological products
Organic impurities: Process-related	ICH Q3A, FDA 2009, USP <1086>	USP <1086>	WHO 2014 (Series No. 987)
Organic impurities: Drug-related products		ICH Q3B, FDA 2010	
Residual solvents	ICH Q3C, USP <467>		ICH Q3C*
Inorganic & elemental	ICH Q3D, USP <232>, <233>, <1086>, EMA 2007, 2008, 2017		
Genotoxic	FDA 2018		
	FDA 2008		
	ICH M7		
	EMA 2006		

Table 1 Comparison of the application scopes of regulatory guidelines/guidance for the management of impurities in pharmaceutical products
From Determination of Impurities in Pharmaceuticals: Why and How?

WRITTEN BY Kung-Tien Liu and C.-Hsin Chen 31 Jan 2019
DOI: 10.5772/intechopen.83849 WHO 987: "The **control of relevant process-related impurities should be included** in the plan for quality control. Control of process-related impurities (protein A, host-cell protein, DNA, and other potential culture or purification residues) is typically part of the drug substance specification, as appropriate" ICH Q3D: "Special considerations for biotechnologically-derived products For biotechnology-derived products, the risks of elemental impurities being present at levels that raise safety concerns at the drug substance stage are considered low. This is largely because: a) elements are not typically used as catalysts or reagents in the manufacturing of biotech products; b) elements are added at trace levels in media feeds during cell culture processes, without accumulation and with significant dilution/removal during further processing; c) typical purification schemes in biotech manufacturing such as extraction, chromatography steps and dialysis or Ultrafiltration-Diafiltration (UF/DF) have the capacity to clear elements introduced in cell culture/fermentation steps or from contact with manufacturing equipment to negligible levels Specific controls on elemental impurities up to the biotech drug substance are generally not needed. In cases where the biotechnology-derived drug substance contains synthetic structures (such as antibody-drug conjugates), appropriate controls on the small molecule component for elemental impurities should be evaluated. Potential elemental impurity sources included in drug product manufacturing (excipients) and other environmental sources should be considered for biotechnologically-derived drug products. The **contribution of these sources to the finished product should be assessed** because they are typically introduced in the drug product manufacture at a step in the process where subsequent elemental impurity EI removal is not generally performed. Risk factors that should be considered in this assessment should include the type of excipients used, the processing conditions and their susceptibility to contamination by environmental factors (controlled areas)" In ICH

Q3A 3.2 Inorganic Impurities Inorganic impurities are normally detected and quantified using pharmacopoeial or other appropriate procedures. Carry-over of catalysts to the new drug substance should be evaluated during development.

The need for inclusion or exclusion of inorganic impurities in the new drug substance specification should be discussed. Acceptance criteria should be based on pharmacopoeial standards or known safety data.

ANALYTICAL PROCEDURES

The registration application should include documented evidence that the analytical procedures are validated and suitable for the detection and quantification of impurities (see ICH Q2A and Q2B Guidelines for Analytical Validation). Technical factors (manufacturing capability and control methodology) can be considered as part of the justification for selection of alternative thresholds based on manufacturing experience with the proposed commercial process. The use of 2 decimal places for thresholds does not necessarily reflect the precision of the analytical procedure used for routine quality control purposes. The use of lower precision techniques (thin-layer chromatography TLC) can be acceptable where justified and appropriately validated. Differences in the analytical procedures used during development and those proposed for the commercial product should be discussed in the registration application.

The quantitation limit for the analytical procedure should be not more than ($\leq$) the reporting threshold." Related WATER for injectable : regulatory pharmacopea tests required: Acidity – alkalinity, conductivity (1,1 uS/cm, Oxidizable substance, color, residual after evaporation Particulate contamination, sterility, bacterial endotoxins. And about PIROGEN removal process from water : between the METHODS in use: sterilization (dry heat 250 centigrades for 30 minutes) acid or alkaline treatment, Activated charcoal absorption, distillation, reverse

osmosys

ultrafiltration.

https://pharmaceuticalmanufacturer.media/pharmaceutical-industry-insights/top-tweets-from-md-m-east_1/

“It is within these demanding circumstances that total organic carbon (TOC) analysis has emerged as a quality assurance gold standard, with regulators such as the US FDA and the UK’s Medicines and Healthcare products Regulatory Agency (MHRA) adopting it as a best practice method for evaluating organic contaminants throughout the pharmaceutical industry.

Proven benefits of TOC analysis

TOC analysis has 2 primary applications in pharmaceutical production: for identifying and facilitating the removal of carbon impurities found in the products themselves, and for verifying the effectiveness of the cleaning work carried out between production batches. The instruments operate by releasing the carbon contained within the sample in question by oxidising it, either by breaking it down through the use of UV/persulfate decomposition, or by placing it inside a high-temperature combustion furnace. This converts the carbon into carbon dioxide gas, which can then be identified using an IR detector.

Pharma manufacturers usually find that the UV/persulfate digestion method delivers the greatest benefit - drug production usually deals with relatively pure samples, without many instances of the kind of large solid mass that environmental scientists rely on furnace combustion-based TOC analysers to break down. The digestion-based approach proves to be cheaper and more efficient, as well as offering lower detection limits due to the fact that larger sample sizes can be analysed. The use of TOC analysis in cleaning validation has been shown to deliver particularly considerable value for the drug makers, allowing manufacturers to ensure that any containers or vessels used in the production have been decontaminated thoroughly before the next batch is produced.

This can be done by testing the final rinse solution of washing water for TOCs, or by analysing a swab sample. This method offers various benefits - it has been shown to prevent microbial build-up on equipment and stops the transfer of bio-burdens between products, enhancing lot integrity in a way that is quicker and more precise than other methods; Because it is not ingredient-specific, it is ideal for situations where more than 1 type of impurity might be expected” TOC is an aspecific test : in Toc analysys the Total organic carbon is measured : total carbon = ORGANIC CARBON + INORGANIC So $TOC = TOTAL\ CARBON - INORGANIC = TC - IC$
https://ispe.org/sites/default/files/attachments/public/Jan-Feb-2009_v1_compressed.pdf

“TOC concentration is indirectly obtained by calculating the difference between 2 directly measured parameters; Total Carbon is determined by oxidizing organic carbon containing compounds to CO₂ and quantifying both the inorganic carbon already present in the sample along with the evolved CO₂. In the case of a membrane conductometric analyzer , Inorganic Carbon in analyzed samples results from dissolved CO₂ species (HCO₃⁻, CO₃²⁻), and may bemeasured directly without oxidation of the sample”

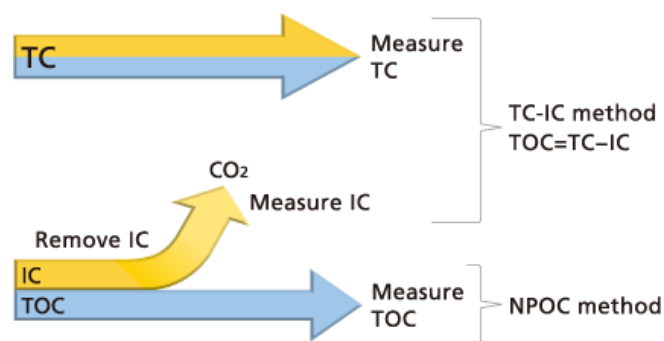


Fig n 10 from <https://www.shimadzu.ro/what-is-toc> 2 main TOC determination methods are used:

Difference method: TOC is determined by subtracting results for TC and IC ($TOC = TC - IC$).

Direct method: TOC is determined by measuring NPOC, in other words TC after IC removal ($TOC = NPOC$).
<https://www.shimadzu.ro/what-is-toc>

“TOC Oxidation methods

TOC analyzers are, in general terms, CO₂ gas analyzers with an upstream oxidation stage and sample preparation system. Regardless of which TOC determination method is used, TOC is measured by the oxidation of organic carbon and subsequent quantification of the resulting CO₂ using an infrared detector. There are different oxidation methods for conversion to CO₂, of which 2 have become established; combustion oxidation and wet oxidation.

Combustion oxidation method

The sample is injected into a high-temperature (650 to 1,200 °C) combustion furnace to incinerate all organic carbon in the sample and measure it as fully oxidized co₂. Due to the simplicity of using heat/combustion as the principle for oxidation, the method requires no reagents for pretreatment or posttreatment processes. 1 of the main features of this method is its ability to efficiently oxidize organic carbon matter that is otherwise resistant to decomposition, such as particulate matter or macromolecular organic substances. In the past, high temperatures (1000°C and more) were required because the first TOC instruments used peak height for integration. The conversion to CO₂ had to be extremely fast so that the signal was recorded as sharp as possible to achieve the best possible resolution.

Very high combustion temperatures lead to the formation of salt melts in the analyzer, which in turn cause increased maintenance due to the deactivation of catalyst, corrosion of the combustion tube and the detector cell. Salt interference on the detector cell from the molten salt products can affect the quality and accuracy of the data. Maintenance time is extended due to the longer cooling and reheating time required because of the higher combustion temperature.

Shimadzu has developed the high-temperature catalytic oxidation at 680°C. While the platinum catalyst ensures complete conversion of all carbon components, the combustion temperature is below the melting points of common salts.

Problems caused by salt are minimized while excellent recovery rates are achieved for all organic components. TOC combustion

oxidation can easily be expanded to include the determination of an additional sum parameter for nitrogen, Total bound Nitrogen .

Wet Oxidation method

With this method, an oxidizing agent is added to samples to chemically decompose carbon in organic matter for measurement as carbon dioxide. Though heat (up to 100 °C) or ultraviolet UV irradiation can be applied to promote the oxidation reaction, the ability of the chemical reaction to oxidatively decompose matter is weaker than combustive oxidation, which tends to result in lower carbon recovery rates from suspended or other particulate organic matter, or persistent substances. It does allow the injection of comparably larger amounts of sample to reach lower limits of detection. Due to its superior oxidative reaction, the combustion oxidation method is commonly used to measure TOC levels in environmental water, factory effluent, and similar samples, where water samples often contain large amounts of insoluble organic carbon."

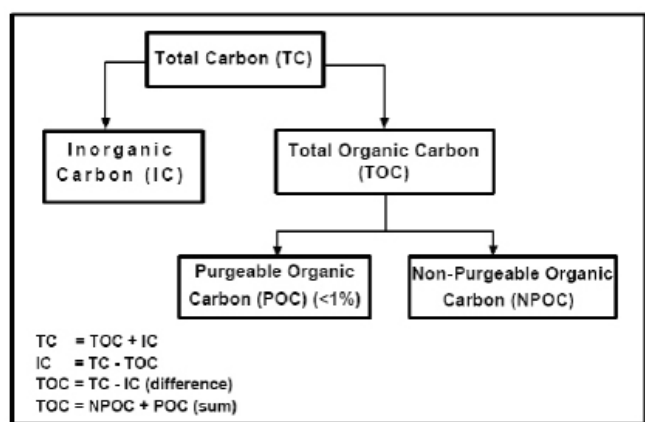


Fig . n 11 from Understanding Total Organic Carbon (TOC) and Why it should be Measured

Posted by Betsey Seibel on Mon, Jul 21, 2014 The Role of Total Organic Carbon (TOC) Validation Techniques in the Pharmaceutical Industry Tyler Trent on Wed, May 18, 2022

"TOC analysis is an indirect means to ensure cleanliness of the water used in manufacture of pharmaceutical products and the manufacturing equipment that produces them. TOC is a useful "catch all" cleaning validation procedure for major manufacturing contaminants because the presence of carbon is indicative of a variety of cross-contamination including the cleaning agents, foreign materials (paint, hair, building materials) and bacteria. https://www.larllc.com/app_pharmaceutical.html

Application: TOC in Pharmaceutical Waters The US Pharmacopeia specifies Total Organic Carbon (TOC) for the detection of organic contaminants in Purified Water (PW) and Water For Injection (WFI).

"What is TOC?"

Since there are millions of organic compounds, organic contaminants are detected as an aggregate rather than analyzing for specific analytes, and since all organic compounds contain carbon, measuring carbon content provides a means of detecting organic compounds. A few carbon compounds are not organic. To achieve a true TOC measurement, these inorganic compounds can be removed through acidification before measuring the sample."

Total Organic Carbon (TOC)

The TOC method can employ 1 of 2 different oxidation techniques:

Chemical Oxidation (UV Persulfate [UVP] Systems)

Catalyst Combustion Oxidation

Chemical oxidation transfers a sample aliquot to a UV reactor where oxidation is achieved through the combination of a chemical oxidizer, (usually sodium persulfate) and UV light. The oxidized carbon in the sample is converted to carbon dioxide (CO₂) and swept through a detector that uses traditional non-dispersive infrared (NDIR) detection. The second option, catalytic combustion oxidation, uses a catalyst to assist in the combustion of organic carbon into CO₂. In these systems, the furnace-enclosed catalyst tube with sample is heated to 680 °C -1000 °C to oxidize carbon to CO₂, through a combination of temperature, oxygen-rich environment (generally ultra-zero air or oxygen carrier gas) and catalyst. The CO₂ is then swept to the NDIR detector.

Because the catalyst used in catalytic combustion systems can have carbon artifacts that create carbon background, UVP systems are recommended for the low-level analysis needed for PW and WFI applications. The only major interferences to the NDIR detection used in both systems are halogens and sulfur. To mitigate their effects, most TOC systems include a halogen scrubber and offer an optional sulfur scrubber."

<https://www.usp.org/frequently-asked-questions/water-pharmaceutical-and-analytical-purposes>

"What is the total organic carbon (TOC) limit for Purified Water and Water for Injection? :There is a "target limit response" of 500 µg of Carbon/L."

American Journal of Analytical Chemistry > Vol.9 No.4, April 2018. Impurity Profiling of Solid Oral Drug Products to Sail through GDUFA-II Requirements Raghuram Pannala Quality Assurance and Regulatory Affairs, ScieGen Pharmaceuticals, Inc., Hauppauge, NY, USA. DOI: 10.4236/ajac.2018.94016

"Impurities in Drug substances and Drug product can be classified as follows as per the current guidance under GDUFA-II requirements

- 1) Organic Impurities i) Process ii) Degradation iii) Chiral Impurities
- 2) Genotoxic Impurities (discussed as separate topic)
- 3) Inorganic Impurities (Elemental impurities erstwhile Heavy metals)
- 4) Residual Solvents
- 5) Polymorphic Impurities

And About the Inorganic Impurities :Inorganic impurities can result from the manufacturing process. They are normally known and identified and include Reagents, Ligands, and Catalysts, Heavy metals or Other residual materials, Inorganic salts, Other materials (filter aids, charcoal) . Heavy metals test by color comparison is a part of USP from 1904, due to numerous events on health of patient population the controls on metallic impurities is effective and in full control from Jan. 1st 2018 across all the major regulatory authorities."

Center for biologics Evaluation and Research (CBER) June 2008. ICH Revision 2 Guidance for Industry Q3A Impurities in New Drug Substances U.S. Department of Health and Human Services FDA Center for Drug Evaluation and Research (CDER) "Inorganic impurities are normally detected and quantified using pharmacopoeial or other appropriate procedures. Carry-over of catalysts to a new drug substance should be evaluated during development. The need for inclusion or exclusion of inorganic impurities in a new drug substance specification should be discussed. Acceptance criteria should be based on pharmacopoeial standards or known safety data." Total Organic Carbon (TOC): Back to Basics. Monday, October 26, 2020 Michelle Neumeyer

"USP <643> Total Organic Carbon and USP <645> Conductivity are the applicable United States compendia. Is Measuring TOC Suitable for All Compounds, APIs, Excipients, and Detergents?"

Total organic carbon testing is a non-specific test method. Rather than detecting a single analyte of interest, TOC analysis gives a comprehensive understanding of purity and cleanliness.

A wide variety of compounds can be detected with satisfactory recovery. While many different compounds can be recovered from

detergents and degradants to APIs and excipients, it is necessary to demonstrate this with robust recovery studies during method development and validation. Water soluble compounds can be analyzed using TOC analysis with little to no method variation. Compounds that do not readily solubilize in water can still be detected using small adjustments such as agitation, pH, or temperature.

The SUEZ applications team can help with these types of development and validation activities. **In cleaning validation, TOC, inorganic carbon, and conductivity viewed together can provide an all-inclusive understanding of cleanliness.** Rather than looking for a single API, using TOC and conductivity data together encompasses APIs, detergents, degradants, and excipients that would otherwise go undetected. Whether using TOC and conductivity for pharmaceutical grade water monitoring or for cleaning verification and validation of equipment, choosing technology and consumables that are fit for purpose gives manufacturers confidence in the accuracy and reliability of data."

https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-3-r2-impurities-new-drug-substances-step-5_en.pdf

Unidentified impurities

Attachment 1: Thresholds

Maximum Daily Dose ¹	Reporting Threshold ^{2,3}	Identification Threshold ³	Qualification Threshold ³
≤ 2g/day	0.05%	0.10% or 1.0 mg per day intake (whichever is lower)	0.15% or 1.0 mg per day intake (whichever is lower)
> 2g/day	0.03%	0.05%	0.05%

Fig n 12 not applied for biotechnological

L. GALATI 2019. **"Special Considerations for Biotechnologically Derived Products:** For biotechnology-derived products, the risks of elemental impurities being present at levels that raise safety concerns at the drug substance stage are considered low.

a) elements are not typically used as catalysts or reagents in the manufacturing of biotech products;

b) elements are added at trace levels in media feeds during cell culture processes, without accumulation and with significant dilution/removal during further processing;

c) typical purification schemes used in biotech manufacturing such as extraction, chromatography steps and dialysis or Ultrafiltration-Diafiltration (UF/DF) have the capacity to clear elements introduced in cell culture/fermentation steps or from contact with manufacturing equipment to negligible levels.

As such, specific controls on elemental impurities up to the biotech drug substance are generally not needed. In cases where the biotechnology-derived drug substance contains synthetic structures

(such as antibody-drug conjugates), appropriate controls on the small molecule component for elemental impurities should be evaluated.

Potential elemental impurity sources included in drug product manufacturing (excipients) and other environmental sources should be considered for biotechnologically-derived drug products. The contribution of these sources to the finished product should be assessed because they are typically introduced in the drug product manufacture at a step in the process where subsequent elemental impurity removal is not generally performed."

See ICH Q6B Specifications: test procedures and acceptance criteria for biotechnological/biological products - Scientific guideline

"The principles adopted and explained in this document apply to proteins and polypeptides,

their derivatives, and products of which they are components (conjugates). These proteins and polypeptides are produced from recombinant or non-recombinant cell-culture expression

systems and can be highly purified and characterised using an appropriate set of analytical procedures.

The principles outlined in this document may also apply to other product types such as proteins and polypeptides isolated from tissues and body fluids. To determine applicability, manufacturers should consult with the appropriate regulatory authorities. This document does not cover antibiotics, synthetic peptides and polypeptides, heparins, vitamins, cell metabolites, DNA products, allergenic extracts, conventional vaccines, cells, whole blood, and cellular blood components. A separate ICH Guideline, "Specifications: Test Procedures and Acceptance Criteria for New Drugs Substances and New Drug Products: Chemical Substances" addresses specifications, and other criteria for chemical substances. This document does not recommend specific test procedures or specific acceptance criteria nor does it apply to the regulation of preclinical and/or clinical research material.

Characterisation of a biotechnological or biological product (which includes the determination of physicochemical properties, biological activity, immunochemical properties, purity and impurities) by appropriate techniques is necessary to allow relevant specifications to be established. **Acceptance criteria should be established and justified based on data obtained from lots used in preclinical and/or clinical studies, data from lots used for demonstration of manufacturing consistency and data from stability studies, and relevant development data.**

Impurities

To evaluating the purity of the drug substance and drug product, which may be composed of the desired product and multiple product-related substances, the manufacturer should also assess impurities, which may be present. Impurities may be either process or product-related. They can be of known structure, partially characterised, or unidentified. When adequate quantities of impurities can be generated, these materials should be characterised to the extent possible and, where possible, their biological activities should be evaluated. Process-related impurities encompass those that are derived from the manufacturing process, cell substrates (host cell proteins, host cell DNA), cell culture (inducers, antibiotics, or media components), or downstream processing . Product-related impurities (precursors, certain degradation products) are molecular variants arising during manufacture and/or storage, which do not have properties comparable to those of the desired product with respect to activity, efficacy, and safety.

The acceptance criteria for impurities should be based on data obtained from lots used in preclinical and clinical studies and manufacturing consistency lots. Individual and/or collective acceptance criteria for impurities (product-related and process-related) should be set, as appropriate. Under certain circumstances, acceptance criteria for selected impurities may not be necessary. For the impurities, the choice and optimisation of analytical procedures should focus on the separation of the desired product and product-related substances from impurities. Individual and/or collective acceptance criteria for impurities should be set, as appropriate. Under certain circumstances, acceptance criteria for selected impurities may not be required . Purity and impurities Impurities may be generated or increased during manufacture

and/or storage of the drug product. These may be either the same as those occurring in the drug substance itself, process-related, or degradation products which form specifically in the drug product during formulation or during storage. If impurities are qualitatively and quantitatively (relative amounts and/or concentrations) the same as in the drug substance, testing is not necessary. If impurities are known to be introduced or formed during the production and/or storage of the drug product, the levels of these impurities should be determined and acceptance criteria established.

Acceptance criteria and analytical procedures should be developed and justified, based upon previous experience with the drug product, to measure changes in the drug substance during the manufacture and/or storage of the drug product. For certain impurities, testing of either the drug substance or the drug product may not be necessary and may not need to be included in the specifications if efficient control or removal to acceptable levels is demonstrated by suitable studies.

In-process acceptance criteria and action limits

In-process tests are performed at critical decision making steps and at other steps where data serve to confirm consistency of the process during the production of either the drug substance or the drug product. The results of in-process testing may be recorded as action limits or reported as acceptance criteria. Performing such testing may eliminate the need for testing of the drug substance or drug product . In-process testing for adventitious agents at the end of cell culture is an example of testing for which acceptance criteria should be established.

The use of internal action limits by the manufacturer to assess the consistency of the process at less critical steps is also important. Data obtained during development and validation runs should provide the basis for provisional action limits to be set for the manufacturing process.

These limits, which are the responsibility of the manufacturer, may be used to initiate investigation or further action. They should be further refined as additional manufacturing experience and data are obtained after product approval."

ICH Topic Q 6 A

Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances 2000

"This guideline may be applicable to synthetic and semi-synthetic antibiotics and synthetic peptides of low molecular weight; But it is not sufficient to adequately describe specifications of higher molecular weight peptides and polypeptides, and biotechnological/biological products.

The ICH Guideline Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products addresses guideline specifications, tests and procedures for biotechnological/biological products. Radiopharmaceuticals, products of fermentation, oligonucleotides, herbal products and crude products of animal or plant origin are similarly not covered. d) Impurities: Organic and inorganic impurities and residual solvents are included in this category.

Refer to the ICH Guidelines Impurities in New Drug Substances and Residual Solvents in Pharmaceuticals for detailed information.

Limited data available at filing

It is recognized that only a limited amount of data may be available at the time of filing, which can influence the process of setting acceptance criteria. As a result it may be necessary to propose revised acceptance criteria as additional experience is gained with the manufacture of a particular drug substance or drug product (**acceptance limits for a specific impurity**). **The basis for the acceptance criteria at the time of filing should necessarily focus on safety and efficacy. When only limited data are available, the initially approved tests and acceptance criteria should be reviewed as more information is collected, with a view towards possible modification.**

This could involve loosening, as well as tightening, acceptance criteria as appropriate Decision tree #1 addresses the extrapolation of meaningful limits on impurities from the body of data generated during development. At the time of filing it is unlikely that sufficient data will be available to assess process consistency. Therefore it is considered inappropriate to establish acceptance criteria which tightly encompass the batch data at the time of filing. f) Inorganic impurities: The need for inclusion of tests and acceptance criteria for inorganic impurities (catalysts) should be studied during development and based on knowledge of the manufacturing process. Procedures and acceptance criteria for sulfated ash / residue on ignition should follow pharmacopoeial precedents; other inorganic impurities may be determined by other appropriate procedures, atomic absorption spectroscopy.”

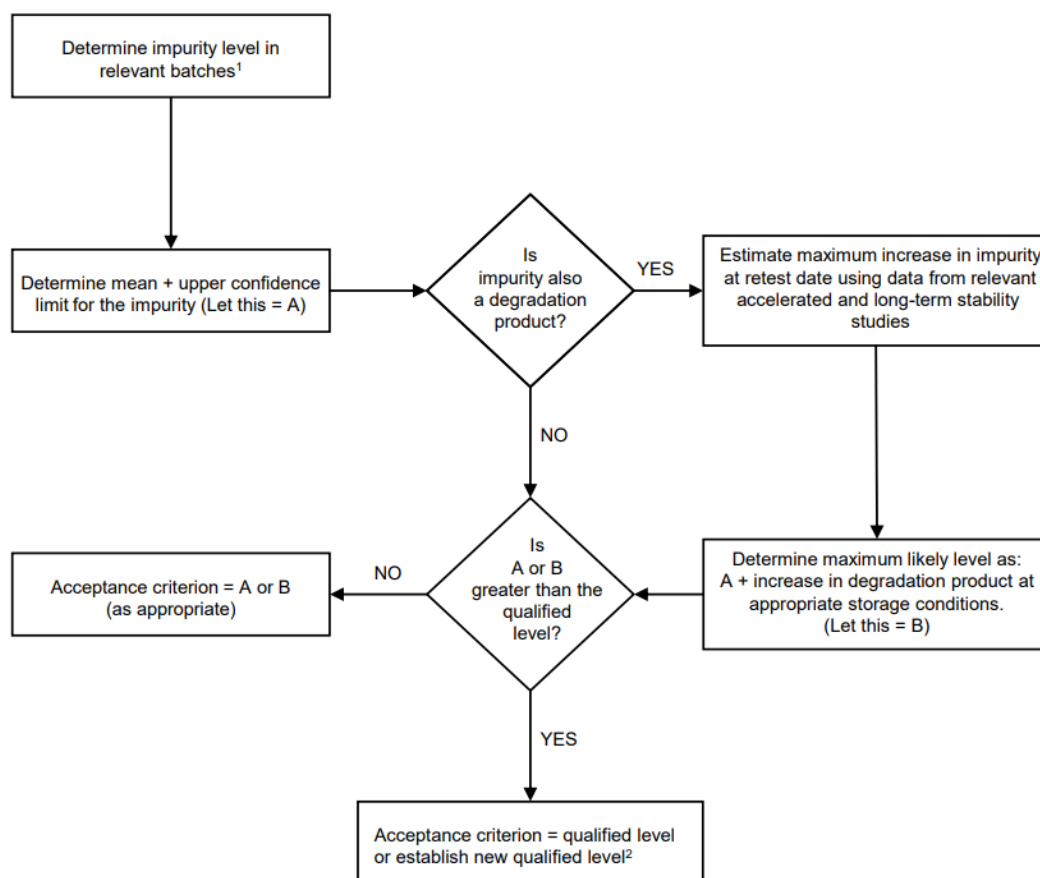


Fig n 13 decision three impurity ICH Q6A

Contains Nonbinding Recommendations

Table 5b. Example of a Possible Control Strategy Summary – Chemical Entity.

Drug Substance CQA (3.2.S.2.6) / Limit in Drug Substance ¹	Type of Control →	In process Controls (including In-process testing and process parameters)	Controls on material attributes (raw materials/starting materials /intermediates)	Impact of Manufacturing Process Design	Is CQA tested on drug substance/ included in Drug Substance specification (3.2.S.4.1)
Organic Purity					
- Impurity X NMT* 0.15%		Design space of the reflux unit operation composed of a combination of percentage of water in Intermediate E and the reflux time in step 5 that delivers Intermediate F with Hydrolysis Impurity ≤0.30% (3.2.S.2.2)			Yes/Yes
- Impurity Y NMT 0.20%		Process parameters step 4 (3.2.S.2.2) p(H ₂) ≥2 barg T <50°C In-process test step 4 (3.2.S.2.4) Impurity Y ≤0.50%			Yes/Yes
- Any individual unspecified impurity NMT 0.10%			Spec for starting material D (3.2.S.2.3)		Yes/Yes
- Total impurities NMT 0.50%					Yes/Yes
Enantiomeric purity					
- S-enantiomer NMT 0.50%			Spec for starting material D (3.2.S.2.3) - S-enantiomer ≤0.50%	Stereocenter is shown not to racemize (3.2.S.2.6)	No/No
Residual Solvent					
- Ethanol NMT 5000 ppm		In-process test during drying after final purification step (3.2.S.2.4) LOD ≤0.40 %		In-process results correlated to test results on drug substance (3.2.S.2.6)	No/Yes
- Toluene NMT 890 ppm		In-process test step 4 (3.2.S.2.4) ≤2000 ppm by G.C		Process steps after step 4 are shown to purge toluene to levels significantly below (less than 10%) that indicated in ICH Q3C (3.2.S.2.6)	No/No

* NMT: not more than

¹This approach could be acceptable as part of a control strategy when justified by submission of relevant process data that confirm the adequacy of the process design and control. The manufacturing process should be periodically evaluated under the firm's quality system to verify removal of the solvent.

Table 2 Any individual specific impurity NMT no more then 0,10% , TOTAL IMPURITIES NMT 0,5% EXAMPLE OF POSSIBLE CONTROL STRATEGY Guidance for Industry Q11 Development and Manufacture of Drug Substances FDA GOV

<https://www.tga.gov.au/sites/default/files/presentation-detailed-look-impurities-medicines.pdf>

“Drug substances Inorganic impurities

- Moisture ($\leq 0.5\%$)
- Sulphated ash / Residue on ignition ($\leq 0.1\%$)
- Heavy metals (≤ 20 ppm)
- Other metals (catalyst residues)”

Guideline on the limits of genotoxic impurities (EMA/CHMP/QWP/251344/2006)

Genotoxicity:- positive findings in established in vitro (Ames test, chromosomal aberration test) or in vivo genotoxicity tests with the main focus on DNA reactive substances that have a potential for direct DNA damage.’ -encompasses mutagenicity through DNA reactivity, DNA damage, and chromosomal damage, both structural chromosome breakage and aneuploidy

- based on evidence for ‘threshold related mechanism’ but if a substance acts directly with DNA then there is no ‘safe exposure level’ (no threshold mechanism)

– Type 1. With sufficient evidence, calculate PDE as per ICH Q3C for Class 2 solvents

– Type 2. Without sufficient evidence, remove impurity or control levels to ‘as low as reasonably practicable’, i.e. apply ‘threshold of toxicological concern’ (TTC). TTC set at 1.5 $\mu\text{g/day}$ -high potency genotoxic carcinogens ‘aflatoxin-like-’, N-nitroso-, and azoxy-compounds are excluded from the TTC approach”

From

<https://www.yokogawa.com/us/library/resources/application-notes/pharmaceutical-waters/>

“USP 23 and 24

Before 1996, the quality of the pharmaceutical waters was determined by several off-line, laboratory tests that are now considered to be antiquated. The USP monograph 23 (and currently 24) replaced these tests with an on-line conductivity measurement as the initial marker. While PW only needs to meet a TOC limit, the WFI has to meet bacterial tests in addition to the TOC and conductivity limits. This application note focuses on the USP requirements for conductivity only. This change to an on-line conductivity measurement was done to: Improve the reliability of the testing by using modern instrumentation Provide immediate alarms and options for quality control Eliminate sample collection and handling errors Reduce the cost of testing.”

Pharmaceutical Water System Investigations

Sponsored Content by Beckman Coulter Life Sciences - Particle Counting and Characterization May 14 2020. “FDA water quality expectations The employment of the terms Water For Injection (WFI) and Purified Water (PW) implies that these waters adhere to the quality expectations defined by the pharmacopoeias. There are 4 vital quality attributes that define PW and WFI: Endotoxins – Pyrogenic substance found in the cell wall of gram-negative bacteria that can cause a fever if injected into patients

Conductivity – Inorganic contaminants which could cause illness in patients and are not meant to be present

Microbial colony forming units (CFU) – Potentially harmful bacteria which could cause infection and illness in patients. Total

organic carbon (TOC) – Organic material which could encourage microbial growth in water systems At present, only TOC and conductivity can be measured online in real time. The CFU and endotoxin tests are both laboratory-based tests.”

“While the presence of many inorganic impurities at low concentrations have few toxicological consequences, significant variation in the impurity levels from batch-to-batch can indicate that the manufacturing process of the drug product is not adequately controlled.

In most cases, these impurities should be removed or at least minimized in the final product. Therefore, the identification, quantification, and control of impurities are important during drug development in the pharmaceutical industry. Ion chromatography (IC) with suppressed conductivity detection is a well-established technique for the determination of inorganic and organic ions in pharmaceuticals. For the determination of anions, a hydroxide eluent is commonly used. Hydroxide is suppressed to water, which provides exceptionally low background conductivity and baseline noise and, therefore, very low detection limits. In Application Note 190 (AN190), we demonstrated the determination of sulfate counter ion and anionic impurities in several watersoluble aminoglycoside antibiotics.

Most of the samples described in AN190 could be analyzed by direct injection after dilution with deionized water. In this application note (AN), we demonstrate the development of an IC method for the determination of anionic impurities in a proprietary water-insoluble pharmaceutical. A 2-mm Thermo Scientific™ Dionex™ IonPac™ AS15 Column with an electrolytically

APPLICATION NOTE 220

generated potassium hydroxide eluent was used for the determination of sub-mg/L concentrations of inorganic anion impurities in a proprietary pharmaceutical dissolved in 100% MeOH. A 100 μL sample was concentrated on a Thermo Scientific™ Dionex™ IonPac™ UTAC-ULP1 Ultratrace Anion Concentrator Ultra Low Pressure column followed by elimination of the MeOH matrix and pharmaceutical with 1 mL of deionized water to permit the determination of the target inorganic anions without matrix interferences. The linearity, detection limits, precision, and accuracy of the method are described.”

Water Qualification in Pharmaceutical Industry

26 April 2023. Journal of coastal life medicine “**Water for Injection (WFI)** is the highest-purity water used in the pharmaceutical industry. It is produced by distillation and must meet even stricter microbiological and chemical specifications than purified water. WFI is required to be sterile and pyrogen-free, and **it must have a conductivity of less**

than 1.3 $\mu\text{S/cm}$.” As known Conductivity is measured in Siemens (S) per distance (microsiemens per centimeter ($\mu\text{S/cm}$)) From <https://www.pharmaguideline.com/2011/03/specification-for-water-for-injection.html>

Between The quality parameter required by FDA for WFI water : TOC : no more than 500 ppb

Conductivity <1.3 $\mu\text{S/cm}$ at 25° C USP <1.3 $\mu\text{S/cm}$ at 25° C P EUR <1.3 $\mu\text{S/cm}$ at 25° C

Residue on evaporation : less than 0,001%

Related the Conductivity test :Conductivity measures the conductivity of the sample before and after oxidation; this difference yields the amount of TOC.

The sample in the oxidation phase forms dissolved CO2 which acts as a weak acid, strengthening the conductivity of the sample. The

amount of conductivity is proportional to the quantity of TOC in the sample. Hydrophobic gas permeation membranes are used to improve the accuracy of the conductivity method, allowing greater discrimination for dissolved CO2 over other chemical compounds.

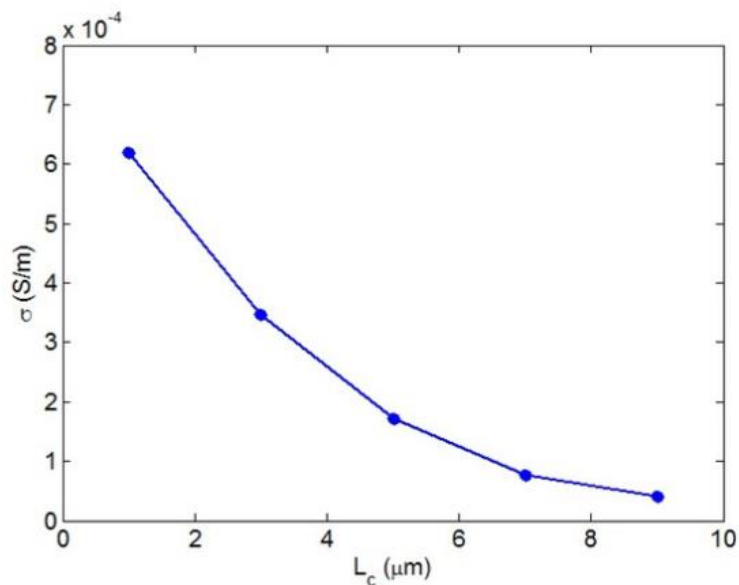


Fig n 14 the figure exhibits the roles of “u” and “τ” in the effective conductivity. The most effective conductivity was attained at the smallest “u” and the highest “τ” .

Lc = interfacial conductivity ; from Effective Conductivity of Carbon-Nanotube-Filled Systems by Interfacial Conductivity to Optimize Breast Cancer Cell Sensors by Yasser Zare ,Kyong-Yop Rhee ,Soo-Jin Park

Nanomaterials 2022, 12(14), 2383; <https://doi.org/10.3390/nano12142383>

Related the effect played on conductivity by different liposome it si possible to verify figure n 15

	Average size (d nm)	PDI	Zeta potential (mV)	Mobility (μmcm/Vs)	Conductivity (MS/cm)
DOX-L	32.67	0.221	−55.6	−4.36	0.0272
DOX-PEL	37.84	0.264	−50.2	−3.937	0.0356

Abbreviations: DOX-L, doxorubicin-loaded liposomes; DOX-PEL, PE-conjugated doxorubicin-loaded liposomes; PDI, polydispersity index.

Fig n 15 size distribution, PDI, and zeta potential of various liposomes; Phosphatidylethanolamine (PE)-conjugated nanoliposomes; Doxorubicin-loaded liposomes (DOX-L) and PE-conjugated doxorubicin-loaded liposomes (DOX-PEL), from DOI: 10.2147/IJN.S13031

Formulations	Zeta Potential (mV) *	PDI *	Mobility (μmcm/Vs) *	Conductivity (MS/cm) *
TNL-PE	−65.2 ± 0.77	0.086 ± 0.06	−5.112 ± 0.06	1.201 ± 0.17
TNL-PE-Ab	−66.8 ± 2.61	0.150 ± 0.07	−5.238 ± 0.20	1.466 ± 0.31

/alues represent mean ± SD (n = 3). Statistical significance evaluations represented on the table by ast
* n < 0.08 at 95% confidence interval).

Fig n 16 Zeta potential, poly dispersity index (PDI), mobility and conductivity of nanolipid vesi- cles.From DOI: 10.3390/polym14122321

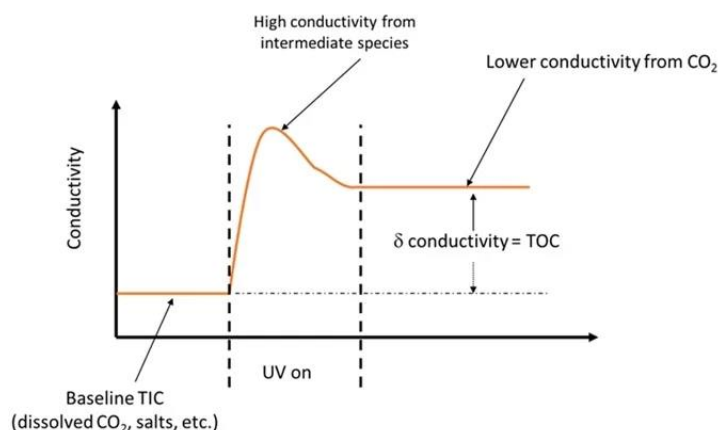


Fig.n. 17 Intermediate species created during partial oxidation can be the cause of false TOC results. Image Credit: Beckman Coulter Life Sciences From <https://www.news-medical.net/whitepaper/20200514/Pharmaceutical-Water-System-Investigations.aspx>

In march 2024 it was asked to a laboratory testing provider what technology they can use to detect activated charcoal and graphene on biopharmaceutical vial : they use GB/T 32198-2015 General Rules for Quantitative Analysis Techniques of Infrared Spectroscopy - Quotation 986 USD price .

Response to a specific inquiry to a producers of activated carbon AC filter about the assurance for not release of carbon particles:

“Our activated carbon AC -impregnated cellulose filter cartridge is made of activated carbon and fiber material. **There is no 100% guarantee that there will be no carbon leakage**, but you can try. Because each customer's use process and the things used for filtering are different, there is no way to say absolutely. As for the use period, it depends on what you have used to filter, the size and amount of the impurity particle inside, if there are many impurities, the filter cartridge is easy to be blocked, and the use time is short. If the impurities are less, the filter cartridge is not easy to be blocked, and the use time is long.”

MATERIALS AND METHODS

For the scope of this work With an observational point of view are reported and analyzed some relevant scientific article and regulatory rules .Various figure (1-21) and table are submitted to clarify some concepts. After this an experimental project hypotesys is reported in order to submit a methods to check final drugs products testing also under the threshods actually requested or suggested by the regulatory agency. In discussion and conclusion are then reported the final consideration.

RESULTS

From literature Physicochemical properties of lipid nanoparticles: Effect of lipid and surfactant composition, July 2011Drug Development and Industrial Pharmacy

“The samples were diluted with MilliQ-water having a conductivity adjusted to 50 μ S/cm by dropwise addition of 0.9% (m/v) saline NaCl solution.” (1)

Nanomaterials (Basel). 2021 Oct. Measurements of the Electrical Conductivity of Monolayer Graphene Flakes Using Conductive Atomic Force Microscopy

Soomook Lim et al, “Monolayer graphene flakes synthesized by chemical vapor deposition on copper exhibited electrical conductivity of $1.46 \pm 0.82 \times 10^6$ S/m.” (2)

Public Assessment Report MHRA, Authorisation for Temporary Supply COVID-19 mRNA Vaccine BNT162b2 “The Public Assessment Report summarises the initial assessment at the time of approval in December 2020. Characterisation of impurities The impurity profile of the BNT162b2 drug product is based primarily on the impurity profile of the materials used for its manufacture.”

Osong Public Health Res Perspect. 2022 Feb; Points to consider for COVID-19 vaccine quality control and national lot release in Republic of Korea: focus on a viral vector platform

Jung Hun Ju et al, “The lot release test items of AstraZeneca AZ vaccine include **appearance, pH, sterility, endotoxin, identity, potency (infectivity), virus particle concentration, purity (ratio of DNA to protein, ratio of virus particle to infectious virus), and container content for injections**. The test items for Janssen vaccine lot release include appearance, pH, sterility, endotoxin, identity (virus identity, virus protein identity), potency (transgene expression, infectious titer, and ratio of virus particle to infectious virus), concentration, purity, and container content for injections. The test items for both vaccines are included in the revised content of the Regulation for Designation, Approval Process, and Method of Pharmaceuticals for National Lot Release. Bulk

A bulk refers to the 1 containing active ingredient prior to formulation. The purification process could be applied to a pool of single harvests.

The purification process should be validated for removal of **impurities**. The following test items may be considered for controlling the bulk: identification, vector concentration, infectious vector titer, ratio of vector particle concentration to infectious vector titer, ratio of infectious vector titer to total protein concentration, transgene expression, host-cell protein, host-cell DNA, vector aggregation, **residual reagents**, residual antibiotics, the absence of replication-competent viral vectors, microbial control (microbial limit or sterility), and endotoxin.”(3)

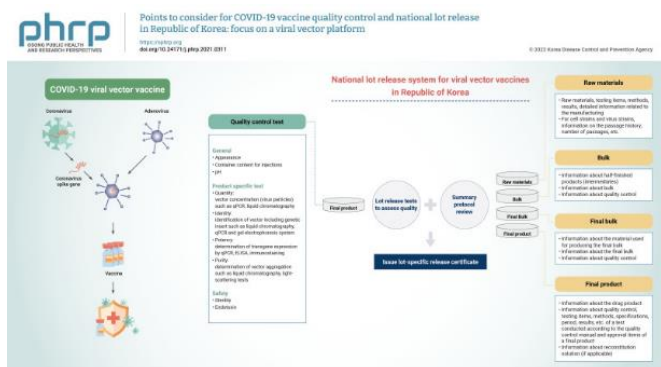


Fig n 18

Gatti AM et al . New quality-control investigations on vaccines: micro- and nanocontamination. *Int J Vaccines Vaccin*. 2017

“Vaccines are being under investigation for the possible side effects they can cause. In order to supply new information, an electron-microscopy EM investigation method was applied to the study of vaccines, aimed at verifying the presence of solid contaminants by means of an Environmental **Scanning Electron Microscope equipped with an X-ray microprobe**.

The results of this new investigation work show the presence of micro- and nanosized particulate matter composed of **inorganic elements in vaccines’ samples which is not declared** among the components and whose unduly presence is, for the time being, inexplicable. A considerable

part of those particulate contaminants have already been verified in other matrices and reported in literature as non biodegradable and non biocompatible. The evidence collected is suggestive of some hypotheses correlated to diseases that are mentioned and briefly discussed. The analyses carried out show that in all samples checked vaccines contain non biocompatible and bio-persistent foreign bodies which

are not declared by Producers, against which the body reacts in any case. **This new investigation represents a new quality control that can be adopted to assess the safety of a vaccine. Our hypothesis is that this contamination is un-intentional, it is probably due to polluted components or procedures of industrial processes (filtrations) used to produce vaccines, not investigated and not detected by the**

Producers. If our hypothesis is actually the case, a close inspection of the working places and the full knowledge of the whole procedure of vaccine preparation would probably allow to eliminate the problem” (4)

Research News

Many vaccines have tiny amounts of inorganic matter, investigation finds *BMJ* ; Feb. 2017. Jacqui Wise, “A wide sample of vaccines contained microscopic amounts of non-biodegradable inorganic matter such as lead, stainless steel, and iron, researchers have found. The quality control investigation, published in the *Int. Journal of Vaccines and Vaccination*, examined 44 vaccines produced by several manufacturers in France and Italy.”

MARCH 2017 Gatti A : Rapid response To doi: <https://doi.org/10.1136/bmj.j596>, "It is a fact, also confirmed by EMA (“The presence of minuscule trace amounts of certain

inorganic particles in vaccines is not unexpected”), that vaccines contain inorganic particles not listed in the ingredients. It is also a fact that those particles, foreign bodies in all respects, are not supposed to be there and, they are not declared among the components of vaccines. And it is a further fact that, as far as nanopathology is concerned, quantity has a meaning quite different from that of classical toxicology, more complex and, in a way, definitely less important.”

<https://www.gov.uk/government/publications/regulatory-approval-of-pfizer-biontech-vaccine-for-covid-19/summary-public-assessment-report-for-pfizerbiontech-covid-19-vaccine>

Decision

Summary of the Public Assessment Report for COVID-19 Vaccine Pfizer/BioNTech

22 Dec 2023, “Characterisation of impurities, The impurity profile of the BNT162b2 drug product is based primarily on the impurity profile of the materials used for its manufacture. The manufacturer has described 4 identified drug product manufacturing process-related impurities. A safety risk assessment for each of these 4 potential impurities has been performed and they are below the safety threshold given the intended product administration schedule. Process-impurities from the sucrose, phosphate and chloride salts used in the final drug product formulation are controlled through testing and specifications ensuring compliance to relevant compendial monographs. This is acceptable. The lipid impurities are controlled through the acceptance criteria used for their manufacture. No critical issues have been identified with respect to the lipids that would preclude the emergency use of the vaccine. Genotoxicity No genotoxicity studies are planned for BNT162b2, as the components of all vaccine constructs are lipids and RNA that are not expected to have genotoxic potential (WHO, 2005).

Carcinogenicity

Carcinogenicity studies with BNT162b2 have not been conducted as the components of all vaccine constructs are lipids and RNA that are not expected to have carcinogenic or tumorigenic potential. Carcinogenicity testing is generally not considered necessary to support the development and licensure of vaccine products for infectious diseases (WHO, 2005). Reproductive and developmental toxicity Fertility and early embryonic development and embryofetal development In the general toxicity studies, macroscopic and microscopic evaluation of male and female reproductive tissues showed no evidence of toxicity. A combined fertility and developmental study (including teratogenicity and postnatal investigations) in rats is ongoing. Prenatal and postnatal development, including maternal function No such studies have been done.”(5)

Content and Format of Chemistry, Manufacturing and Controls Information and Establishment Description Information for a Vaccine or Related Product Office of Communication, Training and Manufacturers Assistance (HFM-40) <http://www.fda.gov/cber/guidelines.htm> 1999

“ **Purification: For each method or combination of methods used, a tabulation of yields, purity, and biological activity should be provided. Verification of the removal or dilution of product related and non-product related impurities, processing reagents, endotoxin, contaminating cell proteins or**

nucleic acids, and other residual contaminants should be included. A standard denominator (international units) should be used to facilitate comparison through processing, concentration, or dilution”(6)

WHO Technical Report Series, No. 937, 2006, Annex 4 Supplementary guidelines on good manufacturing practices: validation “The most common analytical procedures include identification tests, assay of drug substances and pharmaceutical products, quantitative tests for **content of impurities and limit tests for impurities**. Other analytical procedures include dissolution testing and determination of particle size.” (7)

Lee, Y. M. et al 2022. Foreign materials in blood samples of recipients of COVID-19 vaccines. *International Journal of Vaccine Theory, Practice, and Research* “Pablo Campa, a full professor of chemical sciences at the University of Almeida in Madrid reported finding nanostructures of graphene oxide (GO) and other materials later confirmed by Robert O. Young (2021) and other doctors (Wilson, 2021). Campa used Electron Scanning Microscopy (SEM) and Transmission Electron Microscopy (TEM) to find graphene nanoparticles and nanosheets ” (8)

19 February 2021, EMA/707383/2020 Committee for Medicinal Products for Human Use (CHMP) Assessment report Comirnaty, “Dossier should be updated to provide more details on increase batch size including range number of AS

bag and batches used, configuration of filters filter surfaces used and process controls (REC14).

The absence of a test for residuals is considered acceptable. The worst-case levels of residual raw materials and reagents from the BNT162b2 active substance manufacturing process were calculated to be significantly below the pre-determined safety limits. This is found acceptable.

Detailed method validation reports for assay, impurities, and residual solvents for ALC-0315 should be provided. Due date: July 2021

Specified impurities should be further evaluated and appropriate specification limits for individual impurities should be included when more data are available. Acceptance criteria for specified and un-specified impurities should be added to the specification for ALC-0315 and should also be evaluated during stability studies. April 2021”

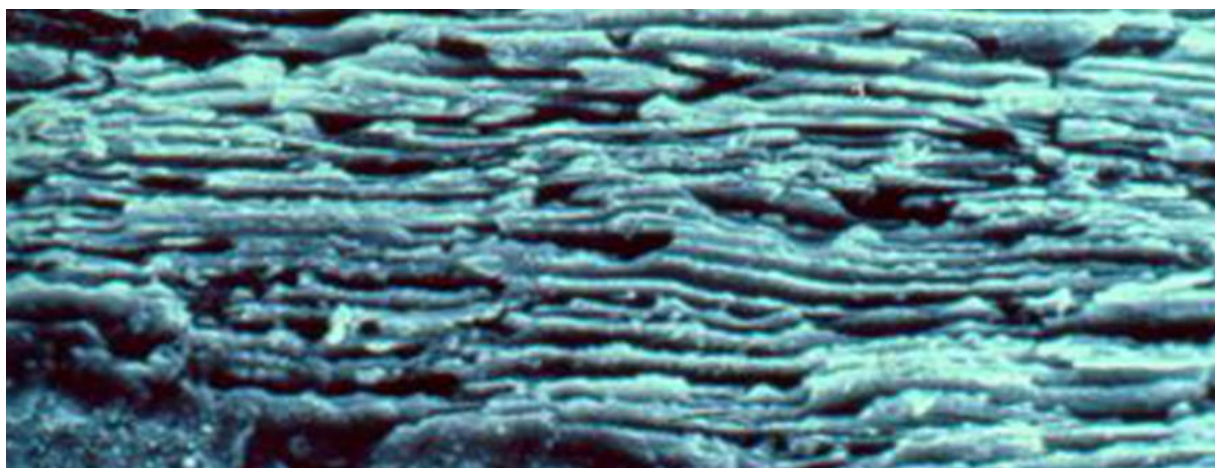


Fig n 19 A photomicrograph of activated carbon AC showing graphitic layers From

<https://www.nanotree.com.my/activated-carbon>

[EDITORIAL FEATURE The Best Sources of Graphene Akanksha Urade .](#)

“Graphene CR and Bio Graphene Solutions are sustainable manufacturers and distributors of graphene that **produce high-quality graphene using biochar (vegetal charcoal) from wood waste rather than graphite.**”

Applied Surface Science May 2019. Graphene-like porous carbon nanostructure from Bengal gram bean husk and its application for fast and efficient adsorption of organic dyes Kanika Gupta et al. “the Bengal gram bean husk is converted into a carbonaceous material and then chemically activated with KOH to obtain a high surface area activated carbon AC (BGBH-C-K). The graphene-like lamellar structure along with the abundance of micropores in the BGBH-C-K provides immense potential for adsorption of organic dyes.” (18)

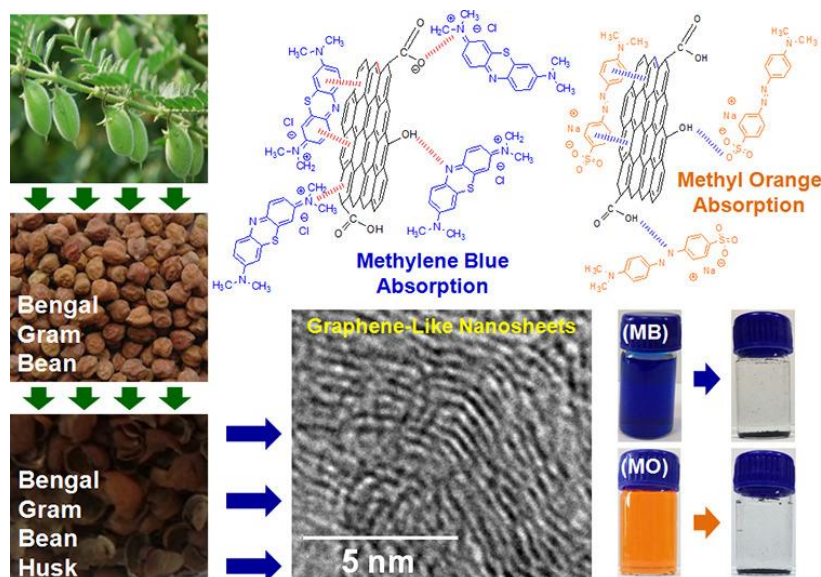


Fig n 20 <https://doi.org/10.1016/j.apsusc.2019.01.138>

Research 19 January 2021. Few-layer graphene induces both primary and secondary genotoxicity in epithelial barrier models in vitro.

Michael J. Burgum et al Journal of Nanobiotechnology, “In monocultures, TT1 and d.THP-1 macrophages showed a statistically significant ($p < 0.05$) cytotoxic response with each ENM following 24-h exposures. Monoculture genotoxicity measured by the in vitro cytokinesis blocked micronucleus assay revealed significant ($p < 0.05$) micronuclei induction at 8 $\mu\text{g}/\text{ml}$ for amine- and carboxyl-FLG. Transmission electron microscopy revealed ENMs were internalised by TT1 cells within membrane-bound vesicles. In the co-cultures, ENMs induced genotoxicity in the absence of cytotoxic effects. Co-cultures pre-exposed to 1.5 mM N-acetylcysteine, showed baseline levels of micronuclei induction, indicating that the genotoxicity observed was driven by oxidative stress.” (19)

Sci Rep. 2013 Dec, Graphene oxide can induce in vitro and in vivo mutagenesis, Yuanyuan Liu et al

“GO treatments at concentrations of 10 and 100 $\mu\text{g}/\text{mL}$ altered gene expression patterns at cellular level, and 101 differentially expressed genes mediated DNA-damage control, cell apoptosis, cell cycle, and metabolism. IV injection of GO at 4 mg/kg for 5 consecutive days clearly induced formation of micronucleated polychromatic erythrocytes in mice, and its mutagenesis potential appeared to be comparable to cyclophosphamide, a classic mutagen” (20)

REVIEW ON ANALYTICAL METHODS FOR THE CHARACTERIZATION OF GRAPHENE STRUCTURES AND TOXICITY PROFILES

Amedeo Cinosi et al Preprint april 2023. “Graphene, graphene oxide, and other derivatives are obtained from graphite through **exfoliation** and Oxidation. Several analytical techniques are employed to characterize the physicochemical properties of graphene nanopowders or derived structures in solid phase, suspension, water, or solvent, but not on complex matrices associated with other compounds where graphene structures are

present in lower concentrations or as traces. The primary investigation techniques are Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), **Raman Spectroscopy** and Micro-Raman. Atomic Force Microscopy (AFM) solid-state Nuclear Magnetic Resonance (ss-NMR), Fourier Transform Infrared Spectroscopy (FT-IR), X-ray, Photoelectron Spectroscopy (XPS), X-ray Diffraction (XRD) and Thermogravimetric Analysis. In some other cases, optical microscopy is mentioned.)

A complex matrix analyzed in Raman spectroscopy RS generates a spectrum equal to the sum of the contributions of individual compounds. Therefore, injectable preparations against SARS-CoV-2 should also yield a spectrum formed by the sum of the spectral fingerprints of the declared ingredients, namely PEG2000 44, 45, liposomes, and present salts, with intensities proportional to their relative concentrations.

The presence of undeclared compounds in the preparations is conceivable after identifying signals in the spectrum that cannot be attributed to the expected compounds.

This is a necessary condition but not sufficient to identify undeclared phases among the ingredients due to **spectral interferences and high background signals that could interfere with and obscure diagnostic spectral fingerprints. The recognition of graphene or other components in a complex matrix might be possible if separative, enrichment, or extraction methods are applied to limit the effects of interferents.**

The EDS analyses for the search of graphene, at a low concentrations, in anti-SARS-CoV-2 preparations, blood, or biological matrices through carbon imprint, present critical issues due to massive contributions from other carbonaceous structures present that emit X-ray fluorescence. Matrix effects, in the search for graphene structures, are also present in Raman spectroscopy. The Raman spectra RS reported in the literature on SARS-CoV-2 preparations do not show the presence of spectral fingerprints of other ingredients present in these formulations; Micro-Raman investigations in anti-SARS-CoV-2 preparations at in the spectral range between 1200-

1800 cm⁻¹ have highlighted only the diagnostic D, G, and 2D signals for the identification of graphene. In the investigated spectral range, there is no evidence of signals attributable to the presence of PEG2000 at excipients or signals interfering with the D and G lines of graphene (KH 2PO 4 at ~1600 cm⁻¹, 1391 cm⁻¹).

Matrix effects, in the search for graphene structures, are also present in Raman spectroscopy. The Raman spectra RS reported in the literature on SARS-CoV-2 preparations do not show the presence of spectral fingerprints of other ingredients present in these formulations; in other words, the reported spectra are consistent with samples predominantly composed of graphene alone. PEG2000 exhibits Raman bands interfering with the D and G bands of graphene. (21)

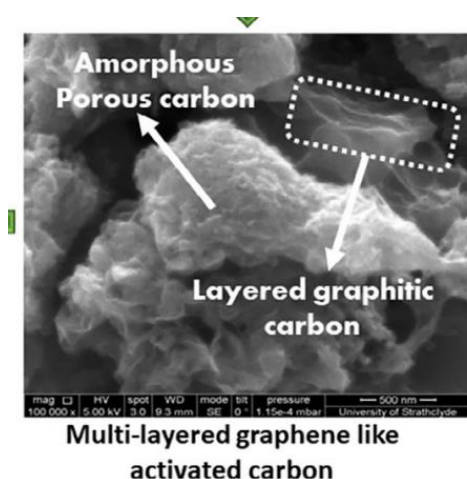


Fig. n 21 Sono-exfoliated graphene-like activated carbon AC derived from waste hazelnut shells has been used as an electroactive material and cellulose derivative based bio-polymer electrolyte as the ionic transport medium to fabricate flexible supercapacitors SC exhibiting excellent electrochemical performance with high specific capacitance. From DOI: 10.1002/er.8314

Liposome Drug Products Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation Guidance for Industry **FDA 2018**

“Information about impurities, including synthetic by-products, where applicable, should be

provided. Impurities may warrant identification and qualification, depending on the following:

- i. The amount of the impurity in the final liposome drug product
- ii. Known toxicities of the impurity
- iii. Structural alerts

For synthetic lipids and semi-synthetic lipids, compare the lipid under test with the reference standard or material using an analytical procedure that is capable of distinguishing the desired lipids from their impurities (HPLC, TLC).

Impurity testing:

1. Trans-fatty acid

2. Free-fatty acid

3. Peroxides (associated with unsaturated fatty acids)

4. Lysophospholipids

5. Solvents and catalysts used in the synthesis or purification Processes”

Evaluation of the Robustness Verification of Downstream Production Process for Inactivated SARS-CoV-2 Vaccine and Different Chromatography Medium Purification Effects by Jia-Hui Pang ,Chang-Fu Guo ,Peng-Liang Hao ,Sheng-Li Meng ,Jing Guo ,Dou Zhang ,Ya-Qi Ji Ping-Gang Ming Vaccines 2024, 12(1), 56; <https://doi.org/10.3390/vaccines12010056>

“The collection of samples must be completed at 0.56 CV, even if there is still substance flowing out at this point during UV 280 nm monitoring. **Following each experiment, the column was regenerated and washed with a 1M NaOH solution until both the conductivity and UV absorbance at 280 nm stabilized, and then washed with PBS solution until both the conductivity and UV absorbance at 280 nm stabilized.**” (22)

Form ZETA PLUS Product Brochure - Filter Cartridges and Capsules “3M Pharmaceutical Grade Zeta Plus Activated Carbon AC filter products meet the market requirements where USP <88> Class VI Biological Reactivity Tests compliance is required, with the necessary traceability and documentation. Identified uses: Manufacturing of pharmaceutical (drug) products, including active pharmaceutical ingredients and vaccines Pharmaceutical APPLICATIONS:

- Decolorization in production of vitamins, antibodies, dextrose, gelatine, enzymes
- Parenterals
- Blood fractionation

ADVANTAGES :After initial flush of the cartridge, there is minimal release of carbon fines in the filtered solution”

Toxicol Rep. 2021;

2021 Apr 20. Safety of COVID-19 vaccines administered in the EU: Should we be concerned?

Antonio F. Hernández et al. “No genotoxicity studies were performed as the components of the vaccine formulation (LNPs , mRNA) are not expected to elicit genotoxic effects.” (23) https://www.appliedmembranes.com/media/wysiwyg/pdf/filters/kx_filters_benefits_and_technology.pdf

“ No release of carbon fines: Extruded filter elements do not release activated carbon AC particles ("gray water") during startup or operation. Extensive flushing or "activation" of extruded filter elements are not required. Some GAC carbon filters will release carbon fines even after the filter elements have been in service for an extended period of time, resulting in contaminated sumps and plumbing and a non-sanitary condition. All components used in filter assembly are manufactured from FDA-compliant material and are routinely registered as NSF/ANSI components as part of their development process.”

EXPERIMENTAL PROJECT HYPOTESYS

In order to verify the absence of graphene or residuals of activated carbon in vials of biopharmaceuticals it is suggested to test 100

sample of a biotechnological products (like a C19 vaccine) using analytical method able to detect this substantia below the suggested thresholds for inorganic impurity. (no more then 0,1%)

To avoid interference in RAMAN spectra it is necessary to pretreat the sample with solvent (to destroy nanolipids). The test is considered negative if all sample show no any level of graphene .

DISCUSSION

Inorganic impurity are listed in the global term impurities by ICH rules. Various independent researcher reported inorganic impurities (graphene similar particles) not declared by producers in some vials of c19 vaccine (P CAMBRA , reference n 6 and other) and detected with specific analitic TESTS. Literature (AUTHORS) reported impurity suspected of graphene similar particle in blood of vaccinated C19. (11)

Activated charcoal is classified as INORGANIC impurity by normative rules of FDA and EMA .

(ICH Topic Q 3 A (R2) 2006 Impurities in new Drug Substances produced by chemical syntesys : According the regulatory agency there are specific thresholds.)

(For biopharmaceutucials there are other specific regulatory rules). In various production steps for drug purification are used : filtering, cartridge , membrane , chromatographic column, composita material monoliths based also on activate charcoal for classic chemical drugs and biopharmaceuticals. (10)

Activated charcoal is produced using chemico physical procedure (using really high temperature) and in literature is reported that it can exfoliate graphite and from this last graphene (in mechanical way) see Turkish Journal of Chemistry -2021 Graphene preparation and graphite exfoliation A. MOOSA et al.

Amorphous carbon is a carbon product without a crystalline structure. Graphene is an single layer of graphite ,and it can be produced by exfoliation of graphite. Activated charcoal , classified as inorganic Impurity : IMPURITIES IN NEW DRUG SUBSTANCES Q3A(R2):From carbon filter used for purification of biopharmaceutical). USP1086 impurities in drug substantie and drug product.

In order to verify the quality of the WIFI water produced are required parameter like TOC total organic carbon and conductivity for also inorganic impurity. In the TOC parameter required by regulatory agency the calculation is : TOC = TOTAL CARBON – INORGANIC

(Graphene according the classic chemical definition is an inorganic compound) Graphene show great conducibility property and the same the profile of nanolipids show peculiar profile for this parameter. Conductivity testing : this measure can also detect graphene if present (due by its properties).

As knowed conductivity, obviously, are not requested in a vial of final mRNA vaccine due by the matrix effect played by nanolipids : In technical sheet of C19 vaccine mRNA the word CONDUCTIVITY not appears USP 644 : it is involved in GENERAL CHAPTER CONDUCTIVITY OF SOLUTIONS

This measure is instead required for WIFI water production (conductivity) and TOC measure for impurities and cleaning procedure.

The TOC MEASURE IS AN INDIRECT PARAMETER = TOTAL CARBON-IC (inorganic carbon)

The inorganic graphene is out of this measure. For chemical drugs According the regulatory agency if the impurity level is below the fixed threshold is not mandatory to test To identify the molecule : non more action is requested.

Instead for the bio technological product : ” **The use of internal action limits by the manufacturer to assess the consistency of the process and These limits, which are the responsibility of the manufacturer, may be used to initiate investigation or further action” as reported in ICH Topic Q 6 B**

at less critical steps is also important. Data obtained during development and validation runs should provide the basis for provisional action limits to be set for the manufacturing process. **These limits, which are the responsibility of the manufacturer, may be used to initiate investigation or further action” And according ICH Q6 A: for new drugs substantie - chemical “ Limited data available at filing It is recognized that only a limited amount of data may be available at the time of filing, which can influence the process of setting acceptance criteria. As a result it may be necessary to propose revised acceptance criteria as additional experience is gained with the manufacture of a particular drug substance or drug product (acceptance limits for a specific impurity). The basis for the acceptance criteria at the time of filing should necessarily focus on safety and efficacy. When only limited data are available, the initially approved tests and acceptance criteria should be reviewed as more information is collected, with a view towards possible modification. This could involve loosening, as well as tightening, acceptance criteria as appropriate.”**

In FDA Guidance for Industry: "Q11 Development and Manufacture of Drug Substances (Chemical Entities and Biotechnological/Biological Entities)." Is reported only a possible example of control strategy (total impurity no more then 0,5%) According ICH Q3D and USP 232 there are limits for elemental impurity also for biotech product, and ICHQ3C for residual solvents .

In 18 July 2023 EMA/CHMP/ICH/83812/2013 Committee for Medicinal Products for Human Use ICH M7(R2) Guideline on assessment and control of DNA reactive (**mutagenic**) **impurities in pharmaceuticals to limit potential carcinogenic risk** : “The purpose of this guideline is to provide a practical framework that is applicable to the identification, categorization, qualification, and control of these mutagenic impurities to limit potential carcinogenic risk. This guideline is intended to complement ICH Q3A(R2), Q3B(R2) (Note 1), and ICH M3(R2)” not applicable to biotechnological.

June 2011 EMA/CHMP/ICH/731268/1998 Committee for medicinal products for human use (CHMP) ICH guideline S6 (R1) – **preclinical** safety evaluation of biotechnology-derived pharmaceuticals “This guidance is intended primarily to recommend a basic framework for the preclinical safety evaluation of biotechnology-derived pharmaceuticals. It applies to products

derived from characterised cells through the use of a variety of expression systems including bacteria, yeast, insect, plant, and mammalian cells. The intended indications may include in vivo diagnostic, therapeutic, or prophylactic uses.”

But if it is not possible and required measure the conductivity test for final vials of C19 vaccine how is possible to exclude the graphene presence if below the fixed (or suggested) thresholds or to exclude toxicological effect if used direct raman spettroscopy without the use of solvent pretreatment?

Regulatory agency allow use of direct (non sample destructive) RAMAN spettroscopy in PAT : but according literature this method without pretreat the sample with solvent is not adequate to measure nanolipids payload. (13) A producer of activated carbon filter writed to a specific inquiry : “There is no 100% guarantee that there will be no carbon leakage” But is to be considered that In the case of exhaust filter also adsorbed material should to be to be finded. (elemental impurities and other)

CONCLUSION

According the decision three of ICH Topic Q 3 A (R2) : for new drug substantie produced by chemical synthesis :it Is impurity greater than identification threshold? If no : no action is required by regulatory agency. (For biotech product there is not a fixed threshold by normative rules for all the inorganic impurity only suggested), there are fixed limits for various elemental impurity , for residual solvents and preclinical study study of genotoxicity. Various purification steps for drugs or biopharmaceutical use also activated charcoal composite monoliths or cartridge or other system. AC produced using really high temperature can Exfoliate graphite(fig 5) and then also graphene in mechanical way : see A. MOOSA et al (so what can happen in exhausted phases ?) .

But what amount in % ?

For This reason It is submitted suggested to the regulatory agency to request in continous analisys of all batch produced of biopharmaceutical to identify impurity also under the threshold actually in use or suggested. Necessary to be used analitical method that are able to detect this kind of impurity(in example using solvent to pretreat sample before RAMAN). If In production of WIFI water the test of conductivity make possible to avoid this impurity for final biopharmaceutical products it is crucial to verify also under the fixed thresholds .

Related biotechnological products :

it is needed that regulatory agency set thresholds (not onlu suggested) for all inorganic impurities a part from The responsibility of the industry. **(ICH Q6 B); It is possible that the limits for acceptation of impurities are a producer responsibility only?**

And related new drug substancies and products : chemical : “ Limited data available at filing It is recognized that only a limited amount of data may be available at the time of filing, which can influence the process of setting acceptance criteria. As a result it may be necessary to propose revised acceptance criteria as additional experience is gained with the manufacture of a particular drug substance or drug product (example: acceptance limits for a specific impurity). **The basis for the acceptance criteria at the time of filing should necessarily focus on safety and efficacy.**

When only limited data are available, the initially approved tests and acceptance criteria should be reviewed as more information is collected, with a view towards possible modification. This could involve loosening, as well as tightening, acceptance criteria as appropriate “: according **ICH Q6 B**

Observig all reported in this work the authors suggest to test for graphene and activated charcoal also below thresholds fixed for chemical drugs and also to test biopharmaceutical under the suggested limits to exclude this impurities to prevent toxicological effects: it is mandatory that the regulatory agency fix specific thresholds Also for the new biopharmaceutical products .

For some biotech products like C19 Vaccine the profile of toxicity at medium and long term can not to be evaluated because new product of last years.(authorized in dec 2020 in EU).

CONFLICT OF INTEREST: NO

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